Proceedings

SIXTY-FIFTH
ANNUAL MEETING

of the
UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

CURTIS HOTEL
Minneapolis, Minnesota
October 30, 31, Nov. 1, 2, 3, 1961
CONTENTS

Officers for 1961-1962 .......................................................... VII
Committees 1961-1962 .......................................................... IX
Record of Previous Meetings .................................................. XIV
Welcome to Minneapolis. Hon. A. Naftalin ............................... 1
Welcome to Minnesota. Hon. E. L. Anderson ............................ 3
Response to Welcome. W. L. Bendix ........................................ 5
Our Common Interest: The Public. M. L. Morris ......................... 6
President's Address. A. P. Schneider ....................................... 14
Presentation of Key to President. R. A. Hendershott ................... 19
Report of the Secretary-Treasurer. R. A. Hendershott .................. 20
Proposed Amendment to the Constitution. R. A. Hendershott .......... 25
Memorial Service. M. N. Riemenschneider ................................ 26
Report of the Nominating Committee ..................................... 29
Election and Induction of Officers for 1962 ............................... 30
The Diagnosis of Canine Diseases. J. H. Sautter, et al. ................. 499
The National Animal Disease Laboratory. E. E. Wedman ................ 518
The Organization and Function of the Veterinary Medical Laboratory. V. B. Robinson 493
Hard Tissue Lesions Associated with Malnutrition. W. S. Monlux .......... 535
Report of Representatives to Annual Meeting of National Association of State
Departments of Agriculture. T. J. Grennan, et al. ....................... 41
Report of the Committee on Programs and Policy. W. L. Bendix, et al. 46

BIOLOGICS AND PHARMACEUTICALS

Biologics Products Activity at the National Animal Disease Laboratory. C. D. Van Houweling .................................................. 64
Standardization of Quantitative Serological Tests. D. S. Robson, et al. 74

CATTLE

Anaplasmosis

Anaplasmosis Immunization Studies. K. L. Kuttler .......................... 79

Brucellosis

Immunologic Response of Calves Vaccinated at Different Ages with B. Abortus
Strain 19. G. Lambert, et al. ................................................. 93
Swine Brucellosis as a Public Health Problem. S. L. Hendricks ............. 100
Swine Brucellosis Eradication. E. D. Hubbard and S. H. McNutt ............. 120
CONTENTS

Leptospirosis
Comments on the Laboratory Diagnosis of Leptospirosis. E. E. Roth .......................... 520
Leptospirosis in Swine. Review and Comments. E. H. Bohl ........................................... 133
Leptospirosis in Pennsylvania. L. C. Clark ................................................................. 140

Mastitis
A Six Months Survey of Staphylococcal Flora in the Milk from a Large Dairy Herd. M. M. Galton, et al. ................................................................. 251
An Evaluation of Existing and Proposed Mastitis Control Programs and Proposals of the National Mastitis Council. R. W. Metzger ........................................ 149
Mastitis Control in Demonstration Herds. W. H. Thompson ........................................ 552

Miscellaneous Cattle Diseases
Salmonellosis in Florida Cattle. E. M. Ellis ................................................................. 161

Tuberculosis
Tuberculosis in New York. G. S. Kaley ................................................................. 166
A Progress Report of Tuberculosis Research at Michigan State University. W. L. Mallman ................................................................. 174
Status of State-Federal Cooperative Tuberculosis Eradication. A. F. Ranney and O. D. Corson ................................................................. 179

PARASITIC DISEASES
Eosinophilic Myositis in Cattle. G. Migaki and P. J. Brandly ........................................ 190
The Role of the Diagnostic Laboratory in Establishing Diagnosis of Insecticide Poisoning. R. D. Radeleff ................................................................. 202

POULTRY DISEASES
The Nature of the Serological Reactions with Complement-Fixation Test for Ornithosis in Avian Sera. Y. Shimizu and R. A. Bankowski ........................................ 542
Report of the Committee on Transmissible Diseases of Poultry. H. E. Goldstein, et al. ..... 221

PUBLIC HEALTH
A Six Months Survey of Staphylococcal Flora in the Milk from a Large Dairy Herd. M. M. Galton, et al. ................................................................. 251

RABIES
Pre-Exposure Immunophylactic Protection of Laboratory Personnel Against Rabies. E. S. Tierkel ................................................................. 269
Rabies in Skunks in the North-Central States. R. L. Parker ........................................ 273
Report of the Committee on Rabies. E. S. Tierkel, et al. ............................................ 281

SHEEP DISEASES
CONTENTS

SALMONELLA SYMPOSIUM

Salmonellosis in Florida Cattle. E. M. Ellis .......................................................... 161
Further Studies on Salmonellae in Human and Animal Foods and in the Environment of Food Processing Plants. E. B. Shotts, et al. ........................................... 309
Laboratory Procedures for Isolating Salmonellae from Human and Animal Food Products. M. M. Galton .................................................................................. 434
Occurrence and Distribution of Salmonellae in Animals in the United States. A. B. Moran .............................................................................................. 442
Salmonella Organisms Isolated from Feed Ingredients. B. S. Pomeroy and M. K. Grady ................................................................. 449
Industries Role in Reducing Incidence of Salmonella in Animal Feeds. C. F. Niven, Jr. ......................................................................................... 453
Finding and Recommendations of USDA on Salmonella in Animal By-Products and Feeds. E. E. Wedman ................................................................. 458
Methods of Investigating the International Spread of Salmonellae. K. W. Newell ............................................................................. 464
Discussion ...................................................................................................... 470
Summary of Salmonella Symposium. P. R. Edwards ........................................ 475

SWINE

The Diagnosis of Hog Cholera. H. W. Dunne ...................................................... 478
Problems Encountered in the Interstate Movement of Swine. K. Myers ........ 319
Investigations into Serum Block. H. W. Dunne and D. C. Kradel ................ 323
A Serological Neutralization Test for Hog Cholera. L. Coggins and B. E. Sheffy ............................................................... 333
Survival of Rinderpest Virus in Experimentally Infected Swine. P. D. DeLay, et al. ................................................................. 376
Report of the Committee on Transmissible Diseases of Swine. L. A. Rosner, et al. ........................................................................ 343
Protection of Pigs Against Hog Cholera with Virus Diarrhea Virus of Cattle. B. E. Sheffy, et al. ........................................................................ 347
Implications of Newer Knowledge in the Eradication of Hog Cohlera. J. A. Baker ................................................................. 354

VESICULAR DISEASES

1960 Outbreak of Vesicular Stomatitis in Cattle in Texas. L. E. Seay ............ 365

VIRUS DISEASES

Differential Diagnosis of Bovine Virus Diseases. C. J. York ....................... 485
Studies on Natural and Experimental Infections of Animals with Encephalomyocarditis Virus. J. H. Gainer ................................................................. 556
The Survival of Rinderpest Virus in Experimentally Infected Swine. P. D. DeLay, et al. .................................................................................. 376
The Relationship of Infectious Bovine Rhinotracheitis Virus to Equine Rhinopneumonitis Virus. L. E. Carmichael and F. D. Barnes ........ 384
Characterization of Virus Isolates from Cattle. P. H. Langer and K. McEntee ................................................................. 389
Constitution and By-Laws ........................................................................... 427
Officers Conference of Veterinary Laboratory Diagnosticians 1962 ............. 432

Fourth Annual Meeting Conference Veterinary Laboratory Diagnosticians 433
OFFICERS 1961-1962

W. L. BENDIX
President

T. J. GRENNAN
President-Elect

R. A. HENDERSHOTT
Secretary-Treasurer

DR. L. A. ROSNER
1st Vice-President

DR. J. W. SAFFORD
2nd Vice-President
OFFICERS AND COMMITTEES FOR 1961-62

PRESIDENT

W. L. Bendix ......................................................................................... Richmond, Virginia

PRESIDENT-ELECT

T. J. Grennan ......................................................................................... Providence, Rhode Island

VICE PRESIDENTS

L. A. Rosner, 1st Vice President .......................................................... Jefferson City, Missouri
J. W. Safford, 2nd Vice President ......................................................... Helena, Montana

SECRETARY-TREASURER

R. A. Hendershott ................................................................................... Trenton, New Jersey

COMMITTEE ON ANAPLASMOSIS

M. N. Riemenschneider, Chairman, Oklahoma City, Oklahoma

W. E. Brock, Stillwater, Oklahoma                                   W. T. Ogleby, Baton Rouge, Louisiana
T. E. Franklin, College Station, Texas                              T. O. Roby, Clarksville, Maryland
R. G. Garrett, Austin, Texas                                         W. L. Sippel, Kissimmee, Florida
K. L. Kuttler, Reno, Nevada                                          F. B. Wheeler, Baton Rouge, Louisiana
O. H. Muth, Corvallis, Oregon                                       E. H. Willers, Honolulu, Hawaii

COMMITTEE ON BIOLOGICS

N. H. Casselberry, Chairman, Berkeley, California

G. D. Cloyd, Ashland, Ohio                                           L. M. Jones, Ames, Iowa
C. G. Durbin, Washington, D. C.                                       C. J. Norden, Lincoln, Nebraska
J. Frank, Hull, Quebec                                               R. S. Radeleff, Kerrville, Texas

COMMITTEE ON BRUCELLOSIS

R. W. Smith, Chairman, Concord, New Hampshire

G. Apple, McKinney, Texas                                            C. A. Manthei, Ames, Iowa
J. B. Armstrong, Selma, Alabama                                       J. L. McAuliff, Cortland, New York
J. R. Bishop, Atlanta, Indiana                                        R. J. McClanahan, Ottawa, Canada
J. S. Brenner, Grant, Montana                                         S. H. McNutt, Madison, Wisconsin
J. B. Finley, Encinal, Texas                                          C. K. Mingle, Washington, D. C.
R. G. Garrett, Fort Worth, Texas                                     J. V. Smith, Hartford, Connecticut
A. E. Janawicz, Montpelier, Vermont                                  J. E. Stuart, Sacramento, California
W. D. Knox, Fort Atkinson, Wisconsin                                  W. C. Tobin, Denver, Colorado
R. Laramore, Gillette, Wyoming                                        G. Van Vleck, Sloughhouse, California
A. O. Wilson, St. Xavier, Montana
OFFICERS AND COMMITTEES

COMMITTEE ON FOREIGN ANIMAL DISEASES

F. A. Todd, Chairman, Washington, D. C.

C. L. Campbell, Tallahassee, Florida
D. E. De Tray, Beltsville, Maryland
F. P. Gluckstein, Washington, D. C.
N. M. Konnerup, Washington, D. C.
F. D. Maurer, Beltsville, Maryland
L. C. Murphy, Bethesda, Maryland
G. C. Poppensiek, Ithaca, New York
R. C. Reisinger, Washington, D. C.
J. E. Stuart, Sacramento, California

COMMITTEE ON INFECTIOUS DISEASES OF CATTLE

A. H. Frank, Chairman, Ames, Iowa

C. G. Bradt, Ithaca, New York
D. E. Bartlett, Arlington Heights, Illinois
W. H. Dreher, Shawano, Wisconsin
E. M. Ellis, Ames, Iowa
H. G. Geyer, Columbus, Ohio
W. D. Knox, Fort Atkinson, Wisconsin
C. A. Manthei, Ames, Iowa
J. G. Milligan, Montgomery, Alabama
F. J. Mulhern, Annandale, Virginia

COMMITTEE ON THE NATIONWIDE ERADICATION OF HOG CHOLERA

C. L. Campbell, Chairman, Tallahassee, Florida

J. A. Baker, Ithaca, New York
A. G. Beagle, Salem, Oregon
H. W. Dunne, University Park, Pennsylvania
W. A. Hagan, Ames, Iowa
R. A. Hendershott, Trenton, New Jersey
H. C. H. Kernkamp, Minneapolis, Minnesota
M. E. Pomeroy, Des Moines, Iowa
W. Powell, Tallahassee, Florida
J. D. Ray, Whitehall, Illinois
J. B. Taylor, Elba, Alabama
G. H. Wise, Washington, D. C.
F. E. Ziegenbein, Lincoln, Nebraska

COMMITTEE ON LAWS AND REGULATIONS

J. W. Safford, Chairman, Helena, Montana

R. J. Anderson, Washington, D. C.
R. L. Elsea, Harrisburg, Pennsylvania
D. Flagg, Bismarck, North Dakota
H. G. Geyer, Columbus, Ohio
F. L. Herchenroeder, Alexandria, Virginia
J. F. Quinn, Lansing, Michigan
H. J. Rollins, Raleigh, North Carolina
W. M. Thompson, Phoenix, Arizona

COMMITTEE ON LEPTOSPIROSIS

E. E. Roth, Chairman, Baton Rouge, Louisiana

E. H. Bohl, Columbus, Ohio
R. J. Byrne, Silver Spring, Maryland
E. A. Carbrey, Ames, Iowa
L. G. Clark, West Grove, Pennsylvania
J. A. Howarth, Davis, California
D. E. Hughes, Ames, Iowa
S. G. Kenzy, Pullman, Washington
A. W. Monlux, Stillwater, Oklahoma
R. L. Morter, Lafayette, Indiana
C. S. Roberts, Auburn, Alabama
L. W. Turner, Nashville, Tennessee
M. J. Twiehaus, Manhattan, Kansas
OFFICERS AND COMMITTEES

COMMITTEE ON NOMINATIONS AND RESOLUTIONS

J. G. Milligan, Chairman, Montgomery, Alabama

A. L. Brueckner, Baltimore, Maryland  F. G. Buzzell, Augusta, Maine
A. P. Schneider, Boise, Idaho

COMMITTEE ON PARASITIC DISEASES

F. R. Koutz, Chairman, Columbus, Ohio

R. L. Cuff, Kansas City, Missouri  L. E. Swanson, Gainesville, Florida
F. D. Enzie, Beltsville, Maryland  W. C. Tobin, Denver, Colorado
F. B. Wheeler, Baton Rouge, Louisiana

COMMITTEE ON PHARMACEUTICALS

S. F. Scheidy, Chairman, Philadelphia, Pennsylvania

C. D. Cloyd, Ashland, Ohio  O. D. Grace, Lincoln, Nebraska
P. C. Enge, Davis, California  H. E. Schaden, Frederick, Maryland
H. Ricker, Dover, Delaware

COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY

H. E. Goldstein, Chairman, Columbus, Ohio

R. A. Bankowski, Davis, California  R. H. Singer, Ames, Iowa
R. Hogue, Lafayette, Indiana  H. Van Roekel, Amherst, Massachusetts
B. S. Pomeroy, St. Paul, Minnesota  C. L. Vickers, Columbia, South Carolina

COMMITTEE ON PROGRAM AND POLICY

T. J. Grennan, Chairman, Providence, Rhode Island

W. L. Bendix, Richmond, Virginia  J. A. McCallam, Washington, D. C.
H. G. Geyer, Columbus, Ohio  L. A. Rosner, Jefferson City, Missouri
R. A. Hendershott, Trenton, New Jersey  J. W. Safford, Helena, Montana

COMMITTEE ON PUBLIC HEALTH AND RADIOLOGICAL FALLOUT

R. J. Schroeder, Chairman, South Gate, California

R. D. Courter, Atlanta, Georgia  J. H. Steele, Atlanta, Georgia
L. H. Frederickson, Nashville, Tenn.  F. A. Todd, Arlington, Virginia
R. H. Huffaker, Ann Arbor, Michigan  E. E. Wedman, Ames, Iowa
R. D. Wenger, Alexandria, Virginia
OFFICERS AND COMMITTEES

COMMITTEE ON RABIES

A. L. Brueckner, Chairman, Baltimore, Maryland
E. S. Tierkel, Vice-Chairman, Atlanta, Georgia

R. L. Elsea, Harrisburg, Pennsylvania
J. G. Flint, St. Paul, Minnesota
D. Ibsen, Little Rock, Arkansas
L. E. Starr, Atlanta, Georgia

E. S. Tierkel, Vice-chairman, Atlanta, Georgia
R. L. Elsea, Harrisburg, Pennsylvania
J. G. Flint, St. Paul, Minnesota
D. Ibsen, Little Rock, Arkansas

COMMITTEE ON DISEASES OF SHEEP AND GOATS

J. E. Stuart, Chairman, Sacramento, California

P. C. Bennett, Ames, Iowa
L. E. Bodenweiser, Albuquerque, New Mexico
A. L. Delez, Lafayette, Indiana
W. F. Fisher, Reno, Nevada
R. G. Garrett, Austin, Texas
J. L. Hourrigan, Washington, D. C.
E. A. Tunnicliff, Bozeman, Montana

L. E. Starr, Atlanta, Georgia

COMMITTEE ON STOCKYARDS, MARKETS AND TRANSPORTATION

F. B. Wheeler, Chairman, Baton Rouge, Louisiana

G. B. Estes, Richmond, Virginia
L. C. Heemstra, Beltsville, Maryland
D. A. McGill, Olympia, Washington
C. T. Sanders, Kansas City, Missouri

COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE

L. A. Rosner, Chairman, Jefferson City, Missouri

E. M. Dwyer, Boston, Massachusetts
A. A. Erdman, Madison, Wisconsin
H. C. Geyer, Columbus, Ohio
D. P. Gustafson, Lafayette, Indiana
R. V. Johnson, Indianapolis, Indiana
S. H. McNutt, Madison, Wisconsin

COMMITTEE ON TUBERCULOSIS

R. W. Carter, Chairman, Columbia, South Carolina

D. DeCamp, Fairfax, Virginia
J. G. Flint, St. Paul, Minnesota
D. Ibsen, Little Rock, Arkansas
C. E. Kord, Nashville, Tennessee
A. B. Larsen, Auburn, Alabama
A. E. Lewis, Calgary, Alberta
R. H. Singer, Ames, Iowa

W. L. Mallman, East Lansing, Michigan
E. A. Murphy, Washington, D. C.
J. F. Quinn, Lansing, Michigan
A. F. Ranney, Washington, D. C.
J. Whitely, Salt Lake City, Utah
E. J. Wilson, Springfield, Illinois
OFFICERS AND COMMITTEES

COMMITTEE ON VESICULAR DISEASES
F. J. Mulhern, Chairman, Annandale, Virginia
J. J. Callis, Greenport, Long Island
A. A. Erdmann, Madison, Wisconsin
R. P. Hanson, Madison, Wisconsin
S. H. Madin, Berkeley, California
N. L. Meyer, Washington, D. C.
L. O. Mott, Ames, Iowa
J. Traum, Berkeley, California

COMMITTEE ON ANIMAL VIRUS CLASSIFICATION
W. R. Hinshaw, Chairman, Fort Detrick, Maryland
C. J. York, Co-chairman, Indianapolis, Indiana
S. H. Madin, Co-chairman, Berkeley, California

F. R. Abinanti, Bethesda, Maryland
R. A. Bankowski, Davis, California
A. O. Betts, Cambridge, England
R. A. Crandell, San Antonio, Texas
H. W. Dunne, University Park, Pennsylvania
J. H. Gainer, Kissimmee, Florida
H. G. Geyer, Columbus, Ohio
J. H. Gillespie, Ithaca, New York
W. A. Hagan, Ames, Iowa
L. E. Hanson, Urbana, Illinois
A. J. Knaazoff, Gainesville, Florida
D. G. McKercher, Davis, California
T. Moll, Pullman, Washington
R. C. Reisinger, Washington, D. C.
W. P. Switzer, Ames, Iowa
J. Traum, Berkeley, California

REPRESENTATIVES TO THE ANNUAL MEETING OF THE NATIONAL ASSOCIATION OF STATE DEPARTMENTS OF AGRICULTURE
W. L. Bendix, Richmond, Virginia
T. J. Grennan, Providence, Rhode Island
R. A. Hendershott, Trenton, New Jersey
L. A. Rosner, Jefferson City, Missouri
J. W. Safford, Helena, Montana
J. F. Quinn, Lansing, Michigan

REGIONAL REPRESENTATIVES ON EXECUTIVE COMMITTEE

Eastern Region
J. McKenny Willis, Jr., Easton, Maryland
P. R. Anthony, Strassburg, Pennsylvania

Southern Region
J. B. Finley, Encinal, Texas
J. B. Nance, Alamo, Tennessee

North Central Region
R. L. Hogue, Lafayette, Indiana
M. Steddom, Granger, Iowa

Western Region
J. S. Brenner, Grant, Montana
O. H. Timm, Dixon, California
<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>2. Oct. 11-12, 1898</td>
<td>Omaha, Nebraska</td>
<td>Mr. C. P. Johnson, Springfield, Ill.</td>
<td>Mr. Taylor Riddle, Kansas</td>
</tr>
<tr>
<td>5. Oct. 8-9, 1901</td>
<td>Buffalo, New York</td>
<td>Dr. E. P. Niles, Virginia</td>
<td>Dr. F. T. Eisenman, Louisville, Ky.</td>
</tr>
<tr>
<td>6. Sept. 22-24, 1902</td>
<td>Wichita, Kansas</td>
<td>Mr. W. H. Dunn, Tennessee</td>
<td>Mr. Wm. P. Smith, Monticello, Illinois</td>
</tr>
<tr>
<td>10. Aug. 15-16, 1906</td>
<td>Springfield, Ill.</td>
<td>Mr. M. M. Hankins, Quanah, Texas</td>
<td>*Dr. C. E. Cotton, St. Paul, Minn.</td>
</tr>
<tr>
<td>15. Dec. 5-6, 1911</td>
<td>Chicago, Ill.</td>
<td>Dr. John F. Devine, Goshen, N. Y.</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>17. Dec. 3-4-5, 1913</td>
<td>Chicago, Ill.</td>
<td>Dr. Peter F. Bahnssen, Atlanta, Ga.</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>22. Dec. 2-3-4, 1918</td>
<td>Chicago, Ill.</td>
<td>Dr. M. Jacob, Knoxville, Tenn.</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>27. Dec. 5-6-7, 1923</td>
<td>Chicago, Ill.</td>
<td>Dr. W. J. Butler, Helena, Montana</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>31. Nov. 30-Dec. 1, 1927</td>
<td>Chicago, Ill.</td>
<td>Dr. L. Van Ee, Lincoln, Nebraska</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>32. Dec. 5-6-7, 1928</td>
<td>Chicago, Ill.</td>
<td>Dr. C. A. Cary, Auburn, Alabama</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>33. Dec. 4-5-6, 1929</td>
<td>Chicago, Ill.</td>
<td>Dr. Chas. G. Lamb, Denver, Colo.</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>Year</td>
<td>Month</td>
<td>Date</td>
<td>Location</td>
</tr>
<tr>
<td>------</td>
<td>-------</td>
<td>------------</td>
<td>----------------</td>
</tr>
<tr>
<td>1930</td>
<td>Dec.</td>
<td>3-4-5</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td>1932</td>
<td>Nov.</td>
<td>30-Dec. 1-2</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>6-7-8</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>5-6-7</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>4-5-6</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>3-4-3</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>1-2-3</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>6-7-8</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>4-5-6</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>3-4-5</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>2-3-4</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>6-7-8</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>5-6-7</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>4-5-6</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>3-4-3</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>13-14-15</td>
<td>Denver, Colo.</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>12-13-14</td>
<td>Columbus, Ohio</td>
</tr>
<tr>
<td></td>
<td>Sept.</td>
<td>23-24-25</td>
<td>Atlantic City, N. J.</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>10-11-12</td>
<td>Omaha, Neb.</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>16-17-18</td>
<td>New Orleans, La.</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>28-29-30</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>13-14-15</td>
<td>St. Louis, Mo.</td>
</tr>
<tr>
<td></td>
<td>Nov.</td>
<td>4-5-6-7</td>
<td>Miami Beach, Florida</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>15-16-17-18</td>
<td>San Francisco, Cal.</td>
</tr>
<tr>
<td></td>
<td>Oct.</td>
<td>3-Nov. 1-2-3</td>
<td>Minneapolis, Minn.</td>
</tr>
</tbody>
</table>

* Deceased.
† This was the last meeting of the Interstate Association of Livestock Sanitary Boards.
‡ Reprinted in 54th Annual Report.
WELCOME TO MINNEAPOLIS
HONORABLE A. NAFTALIN

Mayor Naftalin: Mr. Chairman, Governor Anderson, and delegates to the Sixty-fifth Annual Meeting of the United States Livestock Sanitary Association. That is quite a mouthful, I guess. We are honored in the City of Minneapolis to have you meet for the first time in our city for your annual convention. I hope that this will not be the last time, and that you will like it so well you will come back many times.

I am just a trifle hesitant in this, and unsure of myself, when I talk to a group of veterinarians because I remember that experience Senator Humphrey had some years ago, many many years ago when he was Mayor of Minneapolis. He was invited to address a farm group down in a community not far from here. I accompanied him on that occasion. We went down there and the mayor, Mr. Humphrey, at that time mayor, was very eager to make clear to the farmers that he was their friend, and he had had some farm experience.

He was a little sensitive of the fact he was regarded as a city person and a city lad, and he wanted to make it clear he understood some of the problems of the farm. He explained that as a druggist, he had occasion during an epidemic of cholera, I believe, during the depression days, to vaccinate I don't know how many hundreds or thousands of pigs. He said at one point, dramatically, "I bet I vaccinated more pigs than any man in this room." Whereupon, there were teeters and whispers because sitting in the front row was the county veterinarian.

I come before you to make no boast about how many pigs I vaccinated or how much I know about the profession of the livestock sanitary activities. I am further embarrassed to speak in the presence of Governor Anderson. I have had association with the livestock sanitary industry, and it has been largely on the State level during a previous administration, which I enjoyed, and the present administration does now. It was a very friendly relationship with the State Livestock Sanitary Board. I am sure that all the extra and excellent work done by Doctor West on the State Livestock Sanitary Board is being continued by Doctor Flynn. I know about this from my association over the six years I was associated with the State Government. The members of the board, particularly Doctor West, fought vigorously to defend and protect the professional standards and the activities of the livestock board against any encroachment, such as myself might make. We had tried repeatedly to reorganize this State board, which would incorporate the livestock sanitary board into the Department of Agriculture. We had many a friendly battle over this, and in the course of which, I got a rather substantial education in the matter of livestock sanitary control.
We did enjoy very good relations and I came to have a very high regard for the excellent work that our livestock sanitary board does in this State. We pride ourselves of being clearly in the forefront among the states with respect to brucellosis control and other programs. This is due to the very intensive vigilant attention the people have given the entire livestock industry.

So, I speak proudly, as I am sure, Governor Anderson does, about the accomplishments we have made in the field. I can't claim them on behalf of the City of Minneapolis. Our livestock in the City of Minneapolis has not been very large in number in the city limits. I wanted to say this brief word in the light of the importance of your activities. I know you are all aware of this. It is perfectly apparent when we look at the livestock industry, the application of science, the application of our growing knowledge to a most important industry. It reaches beyond the industrial implication. It reaches into the health of the entire community. Very close association with the livestock sanitary control and public health generally, is apparent to you. The entire field involved here is one of growing importance. It has many lessons to us in other areas of government. It points up the necessity of inter-governmental relations: Federal, State and county. It has been the background of growth for you.

We have many lessons to learn in this, in your many control programs. It is, I feel a fine illustration of what can be accomplished when our scientific knowledge is carefully and efficiently applied through people having high training and high professional standards. So, it is in that spirit I welcome you to the City of Minneapolis. I hope you will honor the axiom, that all work and no play make Jack a dull boy, because I am sure our merchants will appreciate you spending some time in the street, otherwise relaxing and enjoying the wonderful recreation entertainment facilities we have in this community. I know your program will be successful and I am sure you will enjoy the City of Minneapolis and I hope you will come back many times in your convention. Thanks for inviting me to give greetings to you. Thank you.
Governor Anderson: Thank you very much. Mayor Naftalin, Ladies and Gentlemen. I too am glad to welcome you all here. My province is a little larger in inviting you to enjoy yourself. You can certainly have a good time in many ways in Minneapolis, but there are many other ways in addition that you can have a good time in Minnesota. The idea is that you are supposed to. That is a good part of Minnesota, where good business is combined with good living, as one community, where one now looks at you and is referred to in Minnesota, as the land of good living, or the land of good life.

So, really, I will just cite a few things: Michigan State is playing the University of Minnesota Saturday here. I don’t suppose anyone could guarantee the time of the dramatic game, such as we had last Saturday. There just aren’t many like that one ever, but it will be a good game between the two great teams.

Fishing is going on all over Minnesota at a great rate. Some of the best fish are still to be caught. Hunting—you know this is the hunting season: pheasants, small game, ducks, really I think it would be a crime for anybody, particularly anyone who is in Minnesota for the first time to limit yourself to this important and hardworking session. I looked over the 1960 report of your meeting last night and I can certainly see the reason for the description of it as a working conference.

I would just be interested to know how many of you are here in Minnesota for the first time? How many are here?

... Thereupon, many raised their hands. ...

Governor Anderson: Well, just look at that. Well, with the livestock industry what it is in Minnesota, I don’t know how you could have missed us so long. I am aware, of course, and have been for a great many years, Chicago was the annual meeting place. But, we do take pride in Minnesota, and our place in these things. Minnesota is third among all the states in the amount of acreage classified as good and excellent soil. Only Iowa and Illinois exceed Minnesota in this respect. So, it is obvious then that agricultural activities would be very strong in this State and I am sure, as you know, that Minnesota ranks up above the top half dozen states in beef, pork and lamb. Minnesota is first among all of the states now in turkey breeding and likewise in all aspects of livestock, Minnesota is a very important State.

Your activities, as has already been pointed out, is extremely important because of the economic relationships but also because of the very definite public health relationship. I would like to commend you because as I got the thing, your record nationally is very similar to the record here in Minnesota: that there are few agencies in government over the years in
Minnesota like it. I think it would be true of all of you, that it has indicated the courage of conviction in controversial matters, that our livestock sanitary board in Minnesota has, and you show over the country.

You deal in controversy all the time, but I think you have established as an association an amazing record of courage and fact facing when it isn't always popular or publicly easy to do. I think in Minnesota, the courageous leadership we have had, has enjoyed strong legislative leadership. You would know and this makes it unnecessary for me to recall, although I have it here and could do so, the position that Minnesota has taken in many areas—the programs we have, and the progress we have made in brucellosis, being modified certified. Now we are gaining and have a few counties completely certified brucellosis free. It was good in the map of last year's conference to see the whole nation with these white counties standing out pretty uniquely, almost in the map of the whole country. I think it is not without significance that over the years Minnesota's livestock sanitary board has had only four executive secretaries. I think that is an indication of non-partisan support, non-political activity and that the first three of those executive secretaries of the Minnesota Livestock Sanitary Board served as President of this very organization meeting here today. So, Minnesota, is certainly part and parcel and closely involved in all you do and all that you represent, and is earnestly striving to be out in front. It takes constant effort because some of the diseases have been made extinct from very close votes. Before becoming the Governor of Minnesota, I was a member of our State Senate for 10 years.

I can remember many of the decisions, but I think the one I will remember the most in this connection was the one related to the cooking of garbage, and what a controversy that became, and the passage of necessary legislation, so fundamentally important. It passed in our State by one vote, just one vote, made the difference to getting that milestone established. I was so pleased to learn the symposium on hog cholera was being held the other day, and is generally felt to be the finest prepared set of papers on that subject that there had ever been anywhere. You had, probably the best talent relating to the problem that had been gathered from anywhere. So, I think it is splendid you are having such a fine meeting.

But, again, may I say, enjoy yourselves while you are here also. You are supposed to. Thank you very much.

... Applause, everyone arose. ...
RESPONSE TO WELCOMING ADDRESSES

W. L. BENDIX, D.V.M.

Richmond, Virginia

Your Excellency and Mayor Naftalin: We who are not residents of your fine State wish to thank you for the welcome you so graciously extend us on the first official sojourn of our Association in Minnesota. We want you to know that we are pleased to be here in the heart of the great Midwest and that we are enjoying ourselves in Minneapolis. We congratulate you on the progressive and enterprising spirit of your people which has made your city the flour-milling capital of the world, and we have viewed with appreciation in passing through your countryside the splendid gifts of Nature which doubtless have inspired this spirit. I venture to hope that we of this Association may also be inspired to the fruitful accomplishment of those tasks which we have gathered here to perform. I hope, too, that the good will and invigorating climate which prevail in your city may not combine to stimulate so great an exuberance on the part of our membership as to discourage your inviting us back again.

I seem to recall that yours is a State of ten thousand lakes and that the Mississippi River has its source here. I have noted recently, and with some surprise, that the Minnesota Territory was not opened to settlement until the year 1851 and that it became a State seven years later. Being from Virginia, which had its beginnings way back when, I am a little awed by your vast industrial and agricultural accomplishments of one short century. The abundant vigor and productivity apparent here in virtually every field of endeavor lead me to suspect that, like your Ole Man River, you "must know somethin'". It has been said that there is no man from whom we cannot learn something, unless it be one's identical twin. Subscribing to this philosophy in a broader sense, it could well be that some of us attending this convention may take home to our respective parts of the nation a few useful lessons.

And so, Gentlemen, the United States Livestock Sanitary Association today salutes your city and your State. We take pride with you, Governor Anderson, in your great North Woods and Red River Valley, so famed in song and legend, in your fertile plains and sky-tinted lakes and mighty river falls. We contemplate with a profound esteem your world-renowned Mayo Clinic, casting its beacon of hope across land and ocean to the far points of the globe. And, Mr. Mayor, we like your town and will remember warmly in the future the hospitality you have accorded us while here. In closing, I wish to extend to you both the kind wishes of our Association and thank you very much for favoring us with your presence here today.
OUR COMMON INTEREST: THE PUBLIC

DR. MARK L. MORRIS, President, A.V.M.A.

Allenspark, Colorado

THE COMMON INTEREST

Our major common interest is the public welfare. Decision must be based on that which is most desirable in the protection of the public health. Next, consideration must be given to the farmer, rancher, and owner of the livestock. It is essential that these owners derive a livelihood from their efforts and receive an equitable return on the investment. Servicing these owners are many interests. One is the feed industry. Increasing quantities of commercially prepared feeds, containing as many as 30 different feed additives, are sold in this country; thus, the feed industry has an interest in the financial stability of the livestock owner. Another, closely allied, is the chemical industry. Tonnages of various chemicals are used as fertilizers, feed additives, pesticides, vermicides, antiseptics, pharmaceuticals, and biological products. Thus, the chemical industry is another large stockholder in the livestock business. The veterinary profession has a large interest. It renders professional services designed to prevent, eradicate, and control diseases common among animals. Our national, State, and local governments have a very real concern for the welfare of the livestock industry, not only for the animals but for the people who own them. Then there are research institutions, foundations, and others, which find much work to be done, not for the animals alone, but for human welfare. With interests of these magnitudes, it is essential that effective communication be further implemented, that each understand the essentiality and the function of the other. Effective liaison between the various interests must be improved and expanded. Misunderstandings usually are the result of inadequate communication or inability to appreciate the other person's point of view.

ERADICATION OF HOG CHOLERA

On October 20, an announcement appeared in the press that President Kennedy had signed into law a bill which will strike hard at one of the most notorious of livestock killers—hog cholera. This release pointed out that the toll of this disease is currently estimated at $50 million a year. This is the green light for the implementation of a well planned and carefully conceived program of eradication. The eradication program shall be aimed at the continued heat treating of garbage fed to hogs, further control of animal shipment, and increased vaccination. The total cost of eradicating hog cholera is estimated at $100 million—equal to the cost of the disease in just two years. Appropriately, the question has been asked, “Why wasn’t it done sooner?”
This is another great challenge for all those concerned with research, differential diagnoses, and application of eradication techniques, in control of infectious diseases of animals.

**Rabies—Foreign Diseases**

Rabies control programs will receive continuing study and the preparation of a special report which is scheduled for publication in the Journal of A.V.M.A.

The Council on Public Health and Regulatory Veterinary Medicine of the A.V.M.A. is developing thumbnail sketches of foreign and domestic diseases. Of the foreign diseases, African Horse Sickness appeared in the January issue of the Journal. African Swine Fever is in process and others will follow. Leptospirosis and Q fever have received similar treatment and have been published and tuberculosis is in process as is rabies.

**Legislation**

There are currently bills before the Congress of distinct interest to veterinary medicine. The Andrews and Humphrey bills, for instance, would authorize a ten-year program of grants for construction of veterinary educational facilities and for other purposes. Additional educational facilities to provide more veterinarians, continuing education, post graduate study and research, are urgently needed. Your assistance in aiding this legislation will be in the best interest of the public health, the livestock industry, and the veterinary profession.

The Griffith-Moulder Bills introduced in the 1961 Congressional session would create unnecessary red tape in medical experiment programs and would punish the many in the hopes of catching the negligence of the few. This legislation is not needed and is opposed by the A.V.M.A. and many other professional and scientific organizations.

There are a number of developments from the 98th Annual Meeting of the A.V.M.A. to which your attention is directed. A separate section on Regulatory Veterinary Medicine was created. This section will have as its first chairman Dr. C. L. Campbell of Florida and as its secretary Dr. F. J. Mulhern of Washington, D. C. The A.V.M.A. is pleased to announce this section as it will be of interest to many of the members of the U.S.L.S.A.

This should do much to implement and improve liaison between these two great organizations.

**Resolutions**

A number of resolutions were considered by the House of Delegates and given favorable action.

One (No. nine) dealt with Animal Inspection and Quarantine Program, U.S.D.A., recognizing the unprecedented spread of animal diseases throughout the world in recent months. Some examples are African Horse Sickness, African Swine Fever, the appearance of bluetongue in Japan, extensive occurrence of foot-and-mouth disease in England and Scotland, a severe outbreak of hog cholera in Canada, discovery of the African red tick.
in Florida and New York, and diagnosis of equine piroplasmosis in a zebra imported from Africa. Therefore, Agricultural Research Service has recommended that the staff and facilities for the conduct of International Quarantine Activities be greatly augmented. Also, the A.V.M.A. Council on Public Health and Regulatory Veterinary Medicine has recommended that U.S.D.A. redouble its efforts to adopt the most effective possible quarantine measures to safeguard the nation's livestock against exotic diseases. Thus, the A.V.M.A. has been instructed to actively support the securing of sufficient appropriations to staff the International Animal Inspection and Quarantine Program of U.S.D.A.; also, to provide modern facilities adequate for quarantine and inspection, including laboratory procedures. These regulations, it is hoped, will provide maximum protection to North American livestock and poultry.

Another resolution (No. 12) points out that the most urgent problem in the field of veterinary biological products is the speedy development of additional satisfactory techniques for the evaluation of the immunization properties of veterinary biological agents now in common use. It directs that we approve the evaluation program about to be inaugurated in the National Animal Disease Laboratory at Ames, Iowa, and urge sufficient funds be allotted for the speedy and complete evaluation of the program.

Another A.V.M.A. resolution (No. 19) dealt with the International Cooperation Administration. This administration, as of May 15, 1961, had a total of 60 food and agricultural offices in the United States. Also, about 20 U.S. colleges and universities have contracts supported by I.C.A. with universities in foreign countries and many of these contracts include veterinary programs. The International Cooperation Administration has special veterinary programs, with funds for the support of many disease control activities. Furthermore, U.S. Overseas Mission has local veterinary projects such as diagnostic laboratories, slaughterhouse improvements, vaccination programs, artificial insemination, and various animal husbandry projects, which must be carried on in conjunction with animal disease control and eradication measures. In addition, I.C.A. has many training programs involving foreign students who come to the United States to study veterinary medicine. The Food and Agricultural Organization of the United Nations has veterinary sections at administrative and planning levels. Thus, the A.V.M.A. has been requested to report on a previous resolution which dealt with the establishment of a veterinary branch within the I.C.A. The President of the A.V.M.A. has been instructed to appoint a committee to consult with appropriate officials of the Department of State and the I.C.A. to clearly state the position of the A.V.M.A. in regard to establishing a veterinary branch in the organization and to explain the benefits that will be derived therefrom.

On a recent visit to Washington, I had an opportunity to be advised of some of the problems confronting veterinarians currently assigned to I.C.A. and similar programs. Thus, I can fully appreciate the intent and the necessity for the implementation of this resolution.
Another resolution (No. 21) dealt with Federal Regulation of Animal Experimentation and Humane Treatment of Experimental Animals, referring to restrictive legislation now before the Congress. The proposed legislation would permit the unwarranted encroachment upon the research worker’s freedom in biomedical research and would nullify and set back medical research in all categories. It is believed it would delay testing of new concepts, hinder and prevent high quality medical and biological research. The implementation of such legislation would likely require an expensive, massive licensing bureaucracy, establishing a totally unproductive regulatory mechanism and would not provide further development of better animal facilities related to medical and biological research. The A.V.M.A. resolution strongly recommends that proposed legislation requiring licensing of every scientist and certification of all laboratories, as represented by the Griffith-Moulder bills, do not become law.

The Board of Governors has met and discussed this situation with the Trustees of the American Medical Association. The central office staff is currently working with editorial personnel in the A.M.A., in an effort to make available as much authoritative information as possible. It should be pointed out that the sponsors of the Griffith-Moulder bills are well financed and intelligently directed. It is reasonable to predict that some type of legislation in this area may be anticipated in the near future.

**FOOD HYGIENE PROGRAMS**

An action program by the A.V.M.A. was recommended to assist State and local groups in developing and maintaining adequate food hygiene programs with particular emphasis on meat and poultry which is not currently receiving Federal or State inspection. Current practices involving the widespread use of feed additives and the indiscriminate application of antibiotics in the prevention and control of animal diseases necessitate implementation of this action. The initial step will consist of the production of a brochure for distribution to consumers and industry.

**FEED ADDITIVES**

The Council on Public Health and Regulatory Veterinary Medicine endorsed and supported the action of the Council on Biologics and Therapeutics recommending that F.D.A. provide for the exercising of scientific judgment in interpretation of laws relating to feed additives. This action will be an aid to veterinarians employed in the Veterinary Division of the Food and Drug Administration, Department of Health, Education, and Welfare.

**EDUCATION**

Post graduate education and training for veterinarians engaged in regulatory veterinary medicine have been considered cooperatively between the Council on Public Health and Regulatory Medicine and the Council on Education. Recommendations have been developed to encourage such training and education and to provide recommendations which may be of
assistance to regulatory agencies and educational institutions. The report is now in the hands of the Council on Education. The Board of Governors, Council on Education, and central office staff are currently seeking a person to serve as a full-time secretary to the Council on Education to aid in moving forward many of the responsibilities and programs assigned to the Council. Until this Council can be provided with the necessary funds and personnel, it is not in a position to accept these additional responsibilities.

This is but one of the demands of the membership; therefore, to provide the additional services needed to effectively operate the A.V.M.A. means adoption of an expanded budget. In the BLUEPRINT which was presented at the opening session in Detroit, it was indicated that our current budget is $800,000; not less than $1,400,000 is needed.

VETERINARY SPECIALTIES

In this area a forward step was taken at the Detroit Meeting. The Council on Education, through its committee and advisory board on specialties, completed a brochure entitled PROCEDURE FOR ESTABLISHMENT OF VETERINARY SPECIALTY ORGANIZATIONS. This brochure provides information on specialization in veterinary medicine and outlines procedures for establishment of veterinary specialty organizations which have certification of specialists as one of their functions. There is much activity in this direction and the years ahead will witness a great increase toward specialization and organization by groups of veterinarians who have a common interest in a specific area of endeavor.

IMPROVED COMMUNICATIONS

Organized veterinary medicine possesses a ready-made but hitherto largely overlooked instrument for effective intraprofessional communication in the veterinary journals, newsletters, and bulletins now being published on the State, regional, and local levels. The number of these publications has grown considerably in recent years and continues to grow. They deal with issues close to the reader’s home base, thus they enjoy a loyal and interested readership. The potential of these publications as an effective means of intraprofessional communication has been amply demonstrated. An additional A.V.M.A. publication to be known as the A.V.M.A. NEWSLETTER is in process and will go to officers of constituent associations and allied organizations such as United States Livestock Sanitary Association. A layman trained in journalism has been added to the A.V.M.A. Department of Public Information and will assist that department in editing the A.V.M.A. NEWSLETTER. It is suggested that information originating with your association which is of “news” interest to veterinarians be forwarded to the editors of the A.V.M.A. NEWSLETTER for consideration. It is hoped this additional publication will greatly expedite the transmission of news to members of the veterinary profession and allied interests.

In the area of Public Information, a brochure titled ANIMAL DISEASE AND THE FARMER, distributed by the National Safety Council, has been
rewritten to incorporate new information at the request of the National Safety Council.

**AMERICAN JOURNAL OF VETERINARY RESEARCH**

Our research journal is a publication of world-wide prestige, occupying an unchallenged position in the field of animal disease research. It enjoys a large overseas circulation and its contributions to our profession are not limited to this country.

Sometimes success creates problems; however, this Journal has a few difficulties to overcome—such as seeking ways to increase its financial revenues and seeking ways to permit it to publish even more manuscripts. The Council on Research has made a number of suggestions, among them, in addition to revising the subscription rate to non-members, one that a per-page charge be made for publishing manuscripts. This is now the practice of several scientific journals.

**PRACTITIONER RELATIONS**

In your position as participants in regulatory activities, you can do much toward creating good liaison between regulatory personnel and practicing veterinarians. Cooperation is always better accomplished where an attitude of friendly counsel exists. Field regulatory men who are willing to sit down with “so-called problem practitioners” and talk over the situation, can accomplish much towards improved unity. This can best be accomplished by individual visitation. If carefully and diplomatically handled, such a conference can bring about a mutual feeling of trust and confidence. Most practitioners, I am told, want to cooperate, however, there is the occasional individual with whom it is difficult to work. It should always be remembered that the local veterinarian has personal contacts, confidence, and friendships among local people, thus he is in a position to be a worthy servant in the field of regulatory medicine and can often help appreciably with obtaining appropriations and assisting with legislation at the grass-roots level.

**VETERINARY PERSONNEL**

Upon recommendation of the Board of Governors, the Executive Board at its meeting last April approved a tentative plan for a National Recruitment Program for Veterinary Medicine and directed the professional staff to proceed in developing the program. Past President Leasure has pointed out that this is a real step forward because recruitment is everyone’s business, including that of organized veterinary medicine, the colleges of veterinary medicine, the individual veterinarian, and all consumers of foods of animal origin. The need for such a program is obvious as has been reflected over the past few years with a declining number and quality of applicants to our veterinary schools. It is interesting that all branches of agriculture and the medical sciences have experienced a similar trend and recognized its seriousness. Certainly, if we are to meet the growing demands upon the profession for more and better veterinarians, then we must attract more highly qualified and motivated applicants to our veterinary schools.
Those of you who have contacts with or are associated with education in any manner in your State should give this matter your serious attention. The A.V.M.A. office is working on a program to provide updated information to assist in a more effective program of recruitment. Inquiries in this area should be directed to that office.

**ESTABLISHMENT OF FREE DIAGNOSTIC LABORATORIES, OWNED AND OPERATED BY INDUSTRY**

Diagnostic laboratories owned, staffed, and operated by a pharmaceutical firm are now operating in three States, Mississippi, Alabama, Arkansas, and the fourth in Minnesota has been announced. At a meeting of the Board of Governors of the A.V.M.A. with the executives of this company, we were informed that these laboratories were established for three principal reasons: (1) to fulfill a serious need for diagnostic services in the poultry areas; (2) to service their own product line; (3) to serve as bases for field research programs. They claim to offer accurate, fast, and complete diagnoses of diseases of chickens and turkeys on a highly professional level. At the fourth laboratory here in Minnesota they hope to make available, on a limited basis, diagnostic service on livestock diseases. Their goal in offering this type of service is to participate as partners with poultry and livestock producers in increasing the profitability of their production programs. The company believes that more veterinary service begets more veterinary service, and as a result the veterinary profession should also profit. Complete and modern facilities will be provided at each location and the latest in laboratory and diagnostic equipment will be utilized.

A staff of three or four persons will operate the laboratory initially, headed by a veterinarian. Technicians will assist the veterinarian in carrying out daily activities. The company claims that the operation of these facilities will be very similar in all respects to that of any State laboratory. The services are supposed to be only supplementary to the efforts of the State laboratory. The services of these laboratories and the personnel are available **free of charge to anyone** wishing to use them.

At the three installations presently located in Alabama, Mississippi, and Arkansas, only poultry service is offered. At the new facility announced in October, poultry diagnostic service will be offered, but in addition, livestock service will also be performed. All laboratory reports on the poultry service will be made available to the service man, flock owner, or veterinarian, as the case may be. The company asserts that in the case of livestock diagnostic service, no matter who submits the specimen—farmer or veterinarian—reports of findings with appropriate recommendations will be sent only to a veterinarian named by the livestock owner. The company believes that in the livestock field, more so than in the poultry field, the veterinarian is presently the key individual geared to interpretation and performance of the laboratory findings. These details have been provided concerning the functions of these industrial laboratories since one can visualize the need for good liaison between such laboratories and those operated by the State or Federal governments. If these laboratories prove successful for a particular
company, it is reasonable to assume that competing companies may initiate comparative programs, which is now being done. Is a free service offered by commercially owned competing laboratories in the best interest of the owners of livestock and poultry? This is a question to be answered in evaluating such an activity. Furthermore, diagnostic and therapeutic recommendations for the control of diseases of animals originating from industrially owned, free service laboratories, will undoubtedly present an additional area for consideration by the members of the United States Livestock Sanitary Association presently charged with the responsibility for diagnosis, prevention, eradication and control of diseases of animals for the protection of the public health.

SUMMARY

Now and in the future the relationships between members of the American Veterinary Medical Association, members of the United States Livestock Sanitary Association, allied segments of veterinary medicine, agri-business, public health, and the medical sciences, must be approached on a realistic, thoughtful, constructive, long-range basis. We must all work together toward our common interest—the public we serve.
Ladies, Gentlemen, Visitors and Members: It is my pleasure to welcome you to the sixty-fifth Annual Meeting of the United States Livestock Sanitary Association being held here in the beautiful City of Minneapolis, the largest city in Minnesota.

In reviewing the sites of the previous meetings, I note this is the first time the Association has visited this area of the United States and I am sure that we are looking forward to a very important week of education and deliberation on the most vital disease control problems that confront the livestock owners and the veterinary profession at this time.

During the past months I have reviewed the actions and results taken by this Association over a good many years, and it is gratifying to read the previous reports of the Presidents and various committees, and see the definite programs and results that have come about through the functions of this Association.

It makes me glow with a warmth of satisfaction and pride to know that you men and women representing all segments of disease control are willing not only to meet in assembly once a year, but work long hours in Committee meetings, and correspond untiringly throughout the entire year to improve the welfare of the livestock industry.

Practically all of our efforts are donated to this service since all of us have many specific duties other than these meetings to occupy our time.

In reviewing the past year I find that considerable advances have been made on most all of our disease control programs and that we are just about to embark on a new eradication program, namely hog cholera.

During the last several days Federal and State personnel have participated in a symposium on the diagnosis of hog cholera which was sponsored jointly by the United States Department of Agriculture, Agricultural Research Service, Animal Disease Eradication Division and the University of Minnesota, College of Veterinary Medicine, St. Paul, Minnesota and in conjunction with the United States Livestock Sanitary Association meeting. Some of the problems relative to a Hog Cholera Eradication Program present more of a challenge to us than have other programs, in view of the fact that this particular virus disease of swine has no specific diagnostic test, such as the programs on Tuberculosis and Brucellosis.

However, I am sure that with the results of this symposium added to the findings of the Committee on the National Eradication of Hog Cholera, we will be ready to embark on a beginning program for the eradication of this
disease. If we can agree from the start on what must constitute a sound program it will go far in advancing the day when we can add this disease to our list of achievements of diseases eradicated from North America.

**BRUCELLOSIS**

The National Brucellosis Program has progressed in a most satisfactory manner during the last year, and I am informed that the present status is as follows. That one State is now Certified as Brucellosis-free and there are also 84 Brucellosis-free counties in 11 other States. There are 24 States plus Puerto Rico and the Virgin Islands that have achieved Modified Certified Status and there are now 2,191 Brucellosis Modified Certified Counties. This therefore makes a total of both Certified and Modified Counties as being 2,275 or 72.1 percent of all the counties in the Nation. In addition 306 counties are conducting area work leading to certification for a grand total of 2,581 counties or 81.88 percent of all United States counties. These are very impressing figures when you look back a few short years when we were discussing the feasibility of such a program in the same manner as we are reviewing Hog Cholera today.

It is also reassuring to inform you that the Brucellosis program is being adequately financed by the Congress and most of the States and we are pleased to bring these facts to your attention. I am concerned over the request of Congress that not less than a 60 percent—40 percent matching policy be adopted because I do not feel that this would always be in the best interest of disease control.

**TUBERCULOSIS**

This program is one of our earliest programs, and was started in 1917. For more than four decades the livestock industry, the States and the Federal Government have cooperated in an effort to eradicate Tuberculosis from the livestock population of the United States. This disease is still with us and remains a threat to the health of both man and his animals.

While tremendous progress has been made in reducing the incidence of Tuberculosis during these years, we must endeavor to increase our efforts so as to speed-up the day we find our last case. We all know that the closer we approach eradication the more difficult progress becomes.

Increased tracing of all cases and complete epidemiology on all reactors will go far in accomplishing the completion of this program.

The incidence of reactors found for this last fiscal year is .15 of one percent. This corresponds with a similar figure for the proceeding fiscal year, although the number of suspects found has shown an increase. If we can assume that we are looking and tracing animals more diligently each year this then means that we are actually lowering the amount of positive cases because of our closer scrutiny of all reactions and this is being done without the incidence of the disease having increased.
OTHER ERADICATION PROGRAMS

Other active eradication programs such as Scrapie, Screwworm and Scabies, Paratuberculosis, Tick Fever are also of vital importance and the reports of the Committees and the United States Department of Agriculture show that we are winning the battle on these diseases. In some diseases more slowly than others, but we are making progress. The results of the Florida Screwworm Program is one of the most dramatic eradication programs ever to have been undertaken.

We hope that these aforementioned diseases will some day be added to the list of those diseases we have eradicated such as Foot and Mouth Disease, Contagious Pluero Pneumonia, Vesicular Exanthema, Dourine and Glanders.

Recently I had the honor of appearing before the Oregon Veterinary Medical Association where I reviewed the actions of this Association, and the work of the various committees. While referring to the Foreign Animal Disease booklet of the 1954 "Report of the Committee on Exotic Diseases," I found that at least four diseases listed as exotic foreign diseases at that time are now rather taken for granted in this country. These diseases are Bluetongue, Enzootic Abortion, Scrapie of Sheep and Newcastles disease of poultry. This brought to my mind just how many more foreign animal diseases will have appeared here in the North America continent by a like period of time or by 1967.

We are all aware of the fact that African Horse sickness is spreading like wild fire throughout Africa and the Middle East, also that African Swine Fever has spread from Africa to the European continent. These diseases and others are knocking on our door and now in the modern jet age with tourist travel at a peak it will be a miracle if one of these diseases does not gain entry into our country. This coupled with the many new ports of entry on the St. Lawrence Seaway add additional problems to our Federal Animal Inspection and Quarantine Division and those specific States adjoining the seaway.

However, we are encouraged to know that the Canadian officials and the Animal Inspection and Quarantine Division of the United States Department of Agriculture have plans for enlarging the scope of inspection at many of the ports of entry, and that some additional funds have been made available for more thorough inspections. In addition the new multimillion dollar laboratory at Ames, Iowa, has opened its doors, and we look forward to assistance and information from them on many of our disease problems.

You will recall that our Past President Dr. James R. Hay, formerly of Ohio and now of Chicago made several recommendations at last year's meeting. These were reviewed by the Association Executive Committee and given consideration.

His first recommendation concerning the matter of handling the finances of the Association and the liquidation of stock owned by the Association has been accomplished and the moneys placed in Government Bonds and Bank Savings Accounts.
His second recommendation relative to a modification of our future annual programs to develop programs that more appeal to the nation's rancher and farmer has been given consideration by the Secretary and the Committee Chairmen. This year each chairman has been requested to obtain one speaker or paper dealing with the subject matter relative to his Committee. We hope this has been a step in the right direction to obtain compliance with this recommendation. We all realize that it is difficult to discuss and review technical problems and research projects and yet make them appeal to the average livestock man.

His third recommendation dealing with increased membership and the establishment of a membership committee is yet to be activated, but will be reviewed again with the Executive Committee this year.

His fourth recommendation relative to a prepared budget by the Secretary to be presented to the officers and approved was done, however, I feel that this subject should be further pursued and a more complete budget be formulated and that it be presented to the first Executive Committee meeting at each yearly meeting.

During the last year several matters have been brought to my attention and I believe these should be given your attention.

1. The Committee on Federal Programs and Policy is one of the outstanding achievements of this Association and is in general known to you all. However, it appears that some of the efforts and accomplishments of this Committee and also the Executive Committee of this Association could be put to a greater use. If the results and findings of the Committee on Federal Program and Policy and the policies and resolution of this Association could be made more readily available, I believe it would be of tremendous value when meeting with other groups. At present we seem to be a whole year behind ourselves. As an example we meet with the State Plant Board officials of the various States at least once a year and discuss with them various needs of their group, as well as ours, with regards to Federal Programs, and we find them much better prepared and organized with regards to presenting the resolutions of their group. Likewise officers of this Association meet with the heads of the State Departments of Agriculture, and we should have our own resolution on disease control programs ready for presentation at their meeting.

2. The booklet on Foreign Animal Diseases is a "one of its kind booklet" and looked upon with esteem by all groups of people dealing with disease control and since it is now lacking information on some exotic diseases now known to be a problem, I would suggest that this booklet be revised and brought up-to-date and that this Committee be reactivated for this purpose.

3. In view of the fact that our Executive Committee normally meets here or at the meeting site for only two or three days, and in view of the fact that the Executive Committee must approve or reject all reports in a minimum amount of time following each day's session and that since consideration must be given to major changes in the policies and recommendations of this Association dealing with major programs that we must enforce throughout
the year some revision to our procedures seems to be in order so that the
Executive Committee can more thoroughly review Committee recommenda-
tions rather than approving or rejecting them as some material needs con-
siderable deliberation.

4. Set up a committee or give a present Committee the responsibility to
study fallout and radiation as it pertains to the livestock production and the
meat supplies of this country, and furnish information on this subject to the
public and livestock industry.

In conclusion, I wish to take this opportunity to thank the Secretary for
his efforts in organizing this program, and hope that you will all assist us
in maintaining a rigid schedule.

I also wish to thank the Committee Chairmen for their assistance in obtaining
speakers and papers for this meeting. To all the other members I wish
to take this opportunity to thank you, each and everyone, for the opportunity
to have served you as President of this Association. Thank you.
PRESENTATION OF KEY TO RETIRING PRESIDENT

R. A. HENDERSHOTT

Trenton, New Jersey

It is my pleasure at this time, as usual in these meetings to honor our retiring president. This year, of course, it is our good friend, Arthur Phillip (Duke) Schneider, State Veterinarian of Idaho, since 1940. I understand Duke was a graduate of the College of Alabama and took veterinary medicine in Pullman, Washington, and got a B.A. and a D.V.M. degree in 1938. He has been guiding the veterinary medical regulatory phase of animal disease control for the State of Idaho since 1940 and during his tenure up there, the State was declared modified certified brucellosis-free in July, 1960. You will recall that he was elected third vice-president back at the time of our 1958 meeting and progressed steadily until 1960 when at the end of the meeting in Charleston, West Virginia, he was elevated to president. It has been a pleasure to serve with him. Duke, you are one of the long list of well educated, well qualified men, and rate being classed amongst the good ones. It has been a pleasure to have the opportunity to serve with you, and I look forward to working with you in committees in the years ahead.

In appreciation of your service as president during our Sixty-fifth year, on behalf of the Association I wish to present to you at this time, this tie holder and attached to it, you will find the key of the United States Livestock Sanitary Association. May you wear this for many years in memory of your services as president.

President Schneider: Fine, thank you.

... Applause. ...

President Schneider: Thank you, Doctor Hendershott. I also wish to extend my thanks to each and every member of this Association. It has been a pleasure to have been able to serve you gentlemen and I appreciate the help that I have received from the officers, committee, chairmen, committee members and members. Your untiring assistance and guidance has been of great help, and I shall try to conduct the Sixty-fifth meeting in accordance with your wishes. Thank you, Gentlemen.
Mr. President, Members and Visitors: My first duty is to thank the officers that it has been my privilege to serve with and to express my appreciation to the persons who have given so generously of their time and served so well on our Committees and are in the main responsible for the splendid program arranged for this our Sixty-fifth Annual Meeting.

Many of you will recall that immediately following the meeting in Charleston, W. Va., the International Association of Milk Food Sanitarians held a symposium on Mastitis in Chicago. I attended that meeting. One noted speaker expressed the opinion that of the many bacterial causes of Mastitis only in the instance of the condition due to invasion of the udder with Streptococcus Agalactiae had we sufficient knowledge to eradicate the infection. Such authorities as Drs. J. M. Murphy, R. B. Little, O. W. Schalm have covered the subject at former meetings of this Association. We had Doctor McKay discuss the California Mastitis Test at our San Francisco meeting.

We have over the years in several states offered assistance to the dairy industry in the diagnosis of the various causative agents of infections of the udder. Generally I believe all of us have viewed mastitis as a condition that requires that good sanitary measures be applied at each milking. It would seem advisable that our Committee on Disease of Cattle give consideration to the problem of Mastitis and make recommendations for research and a program of sanitary practices designed to reduce the losses due to this condition. There is a great need for an Educational Program amongst dairymen relative to the fundamental sanitary problems involved in milk production.

A Hog Cholera symposium of one and a half days duration has been completed. It was sponsored by Animal Disease Eradication Division of the United States Department of Agriculture and the University of Minnesota, College of Veterinary Medicine.

The program is replete with the names of veterinarians many of whom have long enjoyed a reputation of profound knowledge of Hog Cholera and swine diseases in general.

Those who availed themselves of the opportunity to attend the Symposium were well repaid for the time spent. Certainly this review was timely coming at the time when we are embarking on a program designed to eradicate this needless disease of swine.

I for one feel that we owe those responsible for arranging this symposium a sincere vote of thanks.

The Conference of Veterinary Laboratory Diagnosticians has completed a two-day meeting, THEIR FOURTH. We are pleased to have them meet with
us and through the printing of their discussions in the Annual Proceedings provide a ready reference for workers in the laboratory diagnosis of disease.

MEMBERSHIPS

Again I wish to call to your attention the need for additional members.

PROPOSED BUDGET 1962

Cash Balance, October 17, 1960: 
First Trenton National Bank, Trenton, N. J. $737.21

Increased by Anticipated Cash Receipts:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual Dues</td>
<td>$5,300.00</td>
</tr>
<tr>
<td>Official Dues</td>
<td>2,650.00</td>
</tr>
<tr>
<td>Proceedings</td>
<td>2,400.00</td>
</tr>
<tr>
<td>Foreign Annual Disease Handbook</td>
<td>200.00</td>
</tr>
<tr>
<td>Brucellosis Facts</td>
<td>200.00</td>
</tr>
<tr>
<td>Reprints</td>
<td>2,650.00</td>
</tr>
<tr>
<td>Fees</td>
<td>1,500.00</td>
</tr>
<tr>
<td>Dividend</td>
<td>878.00</td>
</tr>
</tbody>
</table>

| Total                             | 15,778.00|

Estimated Expenditures:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meeting Expense</td>
<td>$900.00</td>
</tr>
<tr>
<td>Printing Stationery</td>
<td>8,400.00</td>
</tr>
<tr>
<td>Salary</td>
<td>6,500.00</td>
</tr>
<tr>
<td>Travel</td>
<td>1,800.00</td>
</tr>
<tr>
<td>Communications</td>
<td>400.00</td>
</tr>
<tr>
<td>Insurance</td>
<td>148.32</td>
</tr>
<tr>
<td>Rent</td>
<td>360.00</td>
</tr>
<tr>
<td>Electric</td>
<td>60.00</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>300.00</td>
</tr>
</tbody>
</table>

| Total                             | 18,868.32|

Estimated Deficit $2,353.11

Following is the report of the Auditor Mr. C. Bergen Groendyke.

In conclusion I wish to again state that it has been a pleasure for me to serve you. Mr. Chairman, I move this report be referred to the Executive Committee.

Thank you.
Dr. Ralph Hendershott,
Secretary-Treasurer,
United States Livestock Sanitary Association,
Trenton, New Jersey.

Dear Sir:

From the books and records of the United States Livestock Sanitary Association I have prepared a statement of cash receipts and disbursements for the period from October 10, 1960 to October 17, 1961.

The cash balance at the end of the period was reconciled to the actual balance in the First Trenton National Bank as confirmed to me directly by the bank. Confirmation was also received direct from the United Savings and Loan Association for the balance on deposit in their savings account.

On separate pages are shown a summarized statement of operations for the period and a statement of net worth as prepared from figures found in your cash books and from other information furnished by you.

The U. S. Treasury Bonds (two bonds at $10,000.00 each) were examined by me on October 20, 1961. You informed me that these bonds were now being kept in a safe deposit box of the Broad Street National Bank in the name of the United States Livestock Sanitary Association.

Respectfully submitted,

C. Bergen Groendyke,
Certified Public Accountant.
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE PERIOD
FROM OCTOBER 10, 1960 TO OCTOBER 17, 1961

Cash Balance, October 10, 1960:
First Trenton National Bank, Trenton, N. J. (Deficit) $(750.59)
United Savings and Loan Association, Trenton, N. J. 1.00

($749.59)

Increased by Cash Receipts:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual Dues</td>
<td>$5,274.00</td>
</tr>
<tr>
<td>Official Dues</td>
<td>2,650.00</td>
</tr>
<tr>
<td>Proceedings</td>
<td>2,464.23</td>
</tr>
<tr>
<td>Foreign Annual Disease Handbook</td>
<td>249.56</td>
</tr>
<tr>
<td>Hog Cholera Pamphlet</td>
<td>368.76</td>
</tr>
<tr>
<td>Reprints</td>
<td>2,763.72</td>
</tr>
<tr>
<td>Brucellosis Facts</td>
<td>250.00</td>
</tr>
<tr>
<td>Registration Fees</td>
<td>1,410.00</td>
</tr>
<tr>
<td>Interest on United States Treasury Bonds</td>
<td>489.67</td>
</tr>
<tr>
<td>Interest on United Savings and Loan Account</td>
<td>140.65</td>
</tr>
<tr>
<td>Columbia Gas System Stock—Proceeds of Sale</td>
<td>27,900.58</td>
</tr>
</tbody>
</table>

Total Cash Receipts 43,961.17

Decreased by Cash Expenditures:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meeting Expense</td>
<td>$895.37</td>
</tr>
<tr>
<td>Printing and Stationery</td>
<td>8,314.10</td>
</tr>
<tr>
<td>Salary</td>
<td>6,499.92</td>
</tr>
<tr>
<td>Travel</td>
<td>1,690.93</td>
</tr>
<tr>
<td>Communication</td>
<td>521.74</td>
</tr>
<tr>
<td>Rent</td>
<td>360.00</td>
</tr>
<tr>
<td>Electric</td>
<td>97.53</td>
</tr>
<tr>
<td>Insurance</td>
<td>151.75</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>208.10</td>
</tr>
<tr>
<td>Purchase of United States Government Bonds</td>
<td>20,000.00</td>
</tr>
</tbody>
</table>

Total Expenditures for 1960-61 $38,739.44

Prior Year's Printing Invoices 1,093.28

Total Cash Expenditures 39,832.72

Cash Balance, October 17, 1961:
First Trenton National Bank, Trenton, N. J. $737.21
United Savings and Loan Association, Trenton, N. J. 2,641.65

$3,378.86
REPORT OF

UNITED STATES LIVESTOCK SANITARY ASSOCIATION

SUMMARY OF OPERATIONS FOR THE PERIOD FROM
OCTOBER 10, 1960 TO OCTOBER 17, 1961

Revenue:
Total Cash Receipts .................................. $43,961.17
Less: Proceeds of Sale of Stock ................. $27,900.58
Accounts Receivable at beginning of
Fiscal Year, October 10, 1960 ............ 800.25
...................... 28,700.83
...................... $15,260.34

Expenditures:
Total Cash Expenditures ......................... $38,739.44
Less: Purchase of United States Government Bonds .... 20,000.00
...................... 18,739.44

Deficit from Operations for Fiscal Period ................................ $(3,479.10)

UNITED STATES LIVESTOCK SANITARY ASSOCIATION

NET WORTH — OCTOBER 17, 1961

Balance, First Trenton National Bank, Trenton, N. J. .................. $737.21
Balance, United Savings and Loan Association, Trenton, N. J. .......... 2,641.65
United States Treasury Bonds, 4%, Due February 15, 1980 .......... 20,000.00
Furniture and Fixtures ........................................... 640.00
...................... $24,018.86

ANALYSIS OF CHANGE IN NET WORTH

Net Worth, October 10, 1960 ........................................ $27,497.96
Reduced by:
Deficit from Operations for Fiscal Period Ended October 17, 1961 .. 3,479.10

Net Worth, October 17, 1961 ...................................... $24,018.86
PROPOSED AMENDMENT TO THE CONSTITUTION

R. A. HENDERSHOTT

Trenton, New Jersey

President Schneider: At this time, we will have our Secretary, Doctor Hendershott read the amendment to the Constitution so it can be voted on next year. It has to be read one year previously.

Secretary Hendershott: Members of the convention. It has been recommended that we amend our Constitution and by-laws as follows: starting with line 89:

“That, with the exception of a change in the name of this Association, upon the dissolution of this corporation or the termination of activities thereof, all remaining net assets thereof shall be contributed for utilization in the advancement and research of diseases of animals, and no part of the net assets shall inure to any person or group of persons for private gain.”

I move it be sent to the Executive Committee for their action next year and in the event they pass upon it favorably, it will be brought out for the vote of the members at the general session or convention in Washington, D. C. at our Sixty-sixth Annual Meeting.

Thank you.
MEMORIAL SERVICE

M. N. RIEMENSCHNEIDER

Oklahoma City, Oklahoma

President Schneider, Ladies and Gentlemen: Each year it is the custom of the Association to pay respect to our departed colleagues. To the best of my information the following members have passed away since our last meeting.

PETER F. BAHNSEN

Peter F. Bahnsen, 90, Americus, Georgia, died July 6, 1961.

Dr. P. F. Bahnsen was Georgia’s first State veterinarian. He was instrumental in the passage of Georgia’s State Practice Act in 1908 and on his 70th birthday he was recognized by a resolution in the State House of Representatives for his pioneer work in cattle tick eradication. Also during his tenure in office as State veterinarian, great strides were made in providing the people of Georgia with quality market milk.

He was the founder and first president of the Georgia V.M.A. in 1908 and he was secretary of the Association for many years. He was president of the United States Livestock Sanitary Association in 1913.

T. O. BRANDENBURG

Dr. T. O. Brandenburg (COR ’13), 71, passed away on August 27, 1961.

Dr. Brandenburg was former State veterinarian of North Dakota where he served in that capacity from 1932 until his retirement.

Doctor Brandenburg was a native of North Dakota, studied Veterinary Medicine at Cornell University and was graduated in 1913. He practiced at Lakota until becoming State Veterinarian, during much of this time he held the title of Assistant State Veterinarian.

Doctor Brandenburg was a past president of this organization, contributing generously of his time and efforts in advancing the principles for which this organization stand.

SCOTT B. BROWN

Scott B. Brown (WSU ’16), 75, Boise, Idaho, died April 8, 1961, in Salt Lake City, Utah.

Doctor Brown was State veterinarian of Idaho from April 8, 1943, till July 15, 1944, prior to that Doctor Brown was a private practitioner at Weiser, Idaho. He also served as deputy state veterinarian and inspector in charge of the Idaho sheep commission.
MEMORIAL SERVICE

EDWARD A. CAHILL

Edward A. Cahill (UP '09), 75, Kansas City, Kansas, died June 13, 1961.

Doctor Cahill was president of Allied Laboratories for 25 years and board chairman for four years before retiring in 1957. He was an active member of this Association for 30 years and served on many of our committees.

REUEL FENSTERMACHER

Dr. Reuel Fenstermacher (OSU '17), 68, professor and head of the Division of Veterinary Diagnostic Laboratories, College of Veterinary Medicine, University of Minnesota, died May 8, 1961.

Doctor Fenstermacher was planning to retire June 30, 1961, after 33 years as a faculty member at the school. He was employed by the Bureau of Animal Industry from 1917 to 1918, was in the Army from 1918 to 1919, and was employed by the Minnesota State Livestock Sanitary Board from May, 1919 to December 31, 1927. On January 1, 1928, he joined the faculty at the University of Minnesota.

Doctor Fenstermacher took very active part in professional organizations, having been president of the Minnesota V.M.A., a member of A.V.M.A., the Minnesota Academy of Science, the Research Workers of North America, and the Wild Life Society.

Doctor Fenstermacher participated actively in committee work of this organization.

AUGUST A. LENERT

August A. Lenert (KCV '17), 69, a faculty member of the A. & M. College of Texas, School of Veterinary Medicine, for 39 years, died May 8, 1961.

Doctor Lenert joined the faculty at Texas A. & M. in 1919 as an associate professor in the Department of Veterinary Medicine and Surgery. In 1937, he was made head of that department, a position he held until going on modified service in 1957. He retired in 1958.

In 1957 Doctor Lenert received the Faculty Achievement Award at the school.

ROBERT W. MERRIMAN

Robert W. Merriman (ISU '23), 59, Los Banos, California, died November 13, 1960.

Doctor Merriman had entered the Illinois Division of Livestock Industry and was employed as the veterinarian in charge of St. Louis National Stockyards until 1933. He joined the Bureau of Animal Industry and the Animal Disease Eradication Division of U.S.D.A. in 1934. After an initial assignment on tuberculosis eradication in Texas, he returned to brucellosis control work in Illinois. In 1952 he transferred to California and was promoted to district veterinarian in 1955.

Doctor Merriman was a recipient of the U.S.D.A. Award of Merit Certificate for outstanding performance.
Dr. T. Childs (O.V.C. '15), Calgary, Alberta, Canada, passed away October 2, 1961.

Doctor Childs served as a colonel in the Royal Veterinary Corps in Europe, India and the Near East.

He became affiliated with the Canadian Government Service in 1925. He became Veterinary Director General in 1947, President of the United States Livestock Sanitary Association in 1953 and retired in 1955.

I respectfully request all present to arise and remain standing in silent prayer for the peaceful repose of the souls of our deceased members.

SILENT PRAYER

Thank you for your respectful participation.

No words which may be said here this day can adequately express the keen sense of personal loss all of us feel on this occasion. It is comforting to recall the words of Jesus, "Blessed are they that mourn; for they shall be comforted."

All of these men gave unselfishly of their time and energy to serve their fellow man. Through their efforts in their respective fields, they have left behind a monument—not one of bronze or stone, but one of accomplishments indelibly written in the sands of time. They were ever seeking to find truth, to accomplish improvement. As a result of their efforts they have left the earth a better place than they found it; they have accomplished what we all are striving for—to leave for our posterity the fruits of our labors that this will be a better place for those who follow.

Their contribution to the livestock industry, to sanitation, and health have been of great value to countless numbers. Their passing has left vacancies very difficult to adequately fill. Thus we memorialize these departed members resolving to emulate the goodness and grandeur their lives displayed. Let us hold high the ideals they passed to us and never falter in striving that they may be fulfilled.
REPORT OF THE NOMINATION COMMITTEE

DOCTOR MILLIGAN: Thank you. Your Nominations Committee this past year was composed of Dr. A. L. Brueckner, Baltimore, Maryland, as Chairman; Dr. G. H. Good, Cheyenne, Wyoming; Dr. J. R. Hay, Chicago, Illinois; Dr. J. G. Milligan, Montgomery, Alabama; Dr. K. F. Wells, Ottawa, Ontario, Canada.

Due to illness of Doctor Brueckner, he was unable to attend. He expressed his wishes in writing to the Committee and requested that Doctor Hastings be allowed to sit in on the deliberations since he was his assistant. Doctor Good and Doctor Wells have been unable to attend this meeting, and for that reason your president appointed Mr. F. G. Buzzell on the Committee.

Since Doctor Brueckner, was not here, I have been chosen as spokesman of the Committee and I will give you their wishes. The Committee wants to extend to this Association their congratulations for having chosen Dr. W. L. Bendix as your president for the coming year, by making him your president-elect last year.

The Committee wishes to present the following slate of officers for 1962 for your consideration:

For President-Elect: Dr. T. J. Grennan, of Providence, Rhode Island
For First Vice-President: Dr. L. A. Rosner, Jefferson City, Missouri
For Second Vice-President: Dr. J. W. Safford, Helena, Montana
For Industry Representatives on the Executive Board:

Southern Region—
    Mr. J. B. Finley, of Texas
    Mr. James Nance, of Tennessee

North Central Region—
    Mr. Robert Hogue, LaFayette, Indiana
    Mr. M. Steddom, Granger, Iowa

Western Region—
    Mr. J. S. Brenner, Grant, Montana
    Mr. O. H. Timm, Dixon, California

Eastern Region—
    Mr. J. McKenny Willis, Easton, Maryland
    Mr. Paul R. Anthony, Strassburg, Pennsylvania

We request you give these men your careful consideration.
PRESIDENT SCHNEIDER: Are there any further nominations from the floor?  
MR. BUZZELL: I move that the nominations be closed and that the secretary cast the unanimous vote for nominating committee choice.
President Schneider: Mr. Buzzell moves that the nominations be closed. Is there a second?

A Doctor: I second that.

President Schneider: Is there any discussion?

A Doctor: Question.

President Schneider: All in favor of this motion signify by the usual sign. Opposed? The motion is carried and the nominations are closed.

President Schneider: Do we have that motion, at this time?

Mr. Buzzell: I move the secretary cast a unanimous ballot for all of these nominations.

A Doctor: I second that motion.

President Schneider: It has been moved and seconded that the secretary cast the unanimous ballot for these officers. All in favor, signify by the usual sign. Opposed? Carried.

President Schneider: All right, Mr. Secretary.

Secretary Hendershott: I hereby cast a unanimous ballot:

For President: Dr. W. L. Bendix,

For President-Elect: Dr. T. J. Grennan,

For First Vice-President: Dr. L. A. Rosner,

For Second Vice-President, Dr. J. W. Safford.

For Industry Representatives on the Executive Board:

Southern Region: Mr. J. B. Finley and Mr. James Nance;

North Central Region: Mr. Robert Hogue and Mr. M. Steddom;

Western Region: Mr. J. S. Brenner and Mr. O. H. Timm;

Eastern Region: Mr. J. McKenny Willis and Mr. Paul R. Anthony.

President Schneider: Thank you Doctor Hendershott, will the following gentlemen, please escort the new officers to the speaker's stand where they will be introduced:

Dr. J. G. Milligan will bring Doctor Bendix forward, please.

Mr. F. G. Buzzell of Maine will escort Dr. T. J. Grennan of Rhode Island.

Dr. A. L. Sundberg will escort Dr. L. A. Rosner of Missouri.

Dr. J. E. Stuart will bring up Dr. J. W. Safford of Montana.

Members of the United States Livestock Sanitary Association, I offer you your new officers for the coming year.

... Applause...

President Schneider: Doctor Safford, if you would care to say a word to the Association. Doctor Safford is your Second Vice-President.

Doctor Safford: Thank you. This is truly an honor. I hope that I will be able to be worthy of this honor. Thank you.

President Schneider: Thank you, and congratulations. Your First Vice-President, Dr. L. J. Rosner of Jefferson City, Missouri.
DOCTOR ROSNER: I am, indeed, appreciative of this honor you have extended my way and you may be sure I will do my very best to advance the objectives of this Association. Thank you.

PRESIDENT SCHNEIDER: Thank you, and congratulations, Doctor Rosner. Next, is your President-Elect, Dr. T. J. Grennan of Providence, Rhode Island. Doctor Grennan.

DOCTOR GRENNAN: I accept this with deep and sincere thanks.

PRESIDENT SCHNEIDER: Congratulations. The next one we have is our new President, Dr. W. L. Bendix, of Richmond, Virginia. Bill?

DOCTOR BENDIX: Thank you, Doc', gentlemen. I am deeply aware of the very high degree of dedication and service that my predecessors have brought to this office and I want to assure the Association that I will do my very level best throughout the coming year to continue in the same vein. Thank you.

... Applause...
REPORT OF THE COMMITTEE ON LAWS AND REGULATIONS


The history of the Committee on Laws and Regulations of the United States Livestock Sanitary Association has been a long one. The dropping of the words "Uniform" and "Unification" from the title of this Committee a number of years ago must have been done for a good reason. A review of the reports and recommendations, and the urging of past Committees, have brought more uniformity of regulations among the States in some livestock disease areas, but in other areas very little has been accomplished.

The Committee, in 1939, made a very detailed report outlining variations in the livestock import regulations of the 48 States, yet, over many years, the demand for more uniform import regulations remains. To attempt to achieve uniformity of State import regulations, your Committee the past few years has urged the States within distinct geographical areas to attempt to bring their import regulations, within each area, to a more uniform status. This resulted in some small degree of success.

Your Committee, in order to graphically illustrate variations in import regulations on a regional basis, has selected a series of requirements for several species of livestock that are, perhaps, of most general concern. The five geographical regions in the United States are shown below and the variations among these regions are tabulated:

I. Northeast region—composed of 12 States:
   Connecticut  New Hampshire  Pennsylvania
   Delaware      New Jersey     Rhode Island
   Massachusetts New York     Vermont
   Maine         Maryland      West Virginia

II. Northcentral region—composed of nine States:
    Illinois      Michigan     Kentucky
    Iowa          Minnesota    Ohio
    Indiana       Missouri     Wisconsin

III. Southeastern region—composed of 10 States:
    Virginia      Mississippi  Georgia
    North Carolina Arkansas  South Carolina
    Tennessee     Alabama     Florida
    Louisiana
IV. Southwestern region—composed of 10 States:

California    Arizona    Oklahoma
Nevada        Texas       New Mexico
Utah          Colorado   Kansas
Hawaii

V. Northwestern region—composed of nine States:

Washington    Montana    South Dakota
Oregon        Wyoming    Nebraska
Idaho         North Dakota    Alaska

Information on import regulations was obtained from “Health Requirements and Regulations—Governing Interstate and International Movement of Livestock and Poultry—Agricultural Research Service 91-17, December, 1959.”

TABLE 1

Variations in Cattle Import Regulations

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Certified herds</td>
<td>4</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>38</td>
</tr>
<tr>
<td>2. Certified herd + 12 mo. herd test + 30-day animal test</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>3. Certified herd + 30-day animal test</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>4. Certified area</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>5. Certified state + 12 mo. herd test + 30-day animal test</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>6. Modified-certified herds</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>9</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>7. Modified-certified area + 12 mo. herd test + a 30-day test</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>8. Modified-certified area + 12 mo. herd test</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>9. Modified-certified area + 30-day animal test</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>10. Modified-certified area + 6 mo. herd test + 30-day animal test</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>11. 30-day animal test</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>10</td>
<td>9</td>
<td>31</td>
</tr>
<tr>
<td>12. 30-day entry test and 30-day retest</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>13. 90-day herd test + 30-day animal test</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>14. 90-day herd test</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>15. Non modified-certified area—3 mo. herd test + 30-day animal test</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>16. 6-mo. herd test + 30-day animal test</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>17. 12-mo. herd test + 30-day animal test</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>18. Exempt from test under 12 mo. age</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>19. Exempt from test under 9 mo. age</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>20. Exempt from test under 8 mo. age</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>21. Exempt from test under 6 mo. age</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>
REPORT OF THE COMMITTEE

### Brucellosis Import Requirements

<table>
<thead>
<tr>
<th>Brucellosis Import Requirements</th>
<th>I. NE Reg.</th>
<th>II. N Reg.</th>
<th>III. SE Reg.</th>
<th>IV. SW Reg.</th>
<th>V. NW Reg.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>22. Exempt from test under 4 mo. age</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>23. Require animal officially calfhood vac.</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24. Vaccinates under 18 mo. admitted</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>25. Vaccinates under 24 mo. admitted</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>26. Vaccinates under 30 mo. admitted</td>
<td>3</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>8</td>
<td>34</td>
</tr>
<tr>
<td>27. Vaccinates over 30 mo. and 30-day animal test</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>28. Vaccinates over 30 mo. and 12 mo. herd test + 30-day animal test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total Number of Variations</strong></td>
<td><strong>20</strong></td>
<td><strong>15</strong></td>
<td><strong>17</strong></td>
<td><strong>7</strong></td>
<td><strong>7</strong></td>
<td><strong>28</strong></td>
</tr>
</tbody>
</table>

### Tuberculosis Import Requirements

<table>
<thead>
<tr>
<th>Tuberculosis Import Requirements</th>
<th>I. NE Reg.</th>
<th>II. N Reg.</th>
<th>III. SE Reg.</th>
<th>IV. SW Reg.</th>
<th>V. NW Reg.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Accredited-free herd</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>5</td>
<td>6</td>
<td>38</td>
</tr>
<tr>
<td>2. Accredited-free herd + 30-day test</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>3. Modified-accredited area — nonquarantined herd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Modified-accredited area + 30-day test</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>5. Modified-accredited area + 3-yr. herd test</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6. Modified-accredited area + 12 mo. herd test + 30-day test</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>7. Modified-accredited area + 12 mo. herd test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8. 30-day negative animal test</td>
<td>10</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>39</td>
</tr>
<tr>
<td>9. 90-day retest after entry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total Number of Variations</strong></td>
<td><strong>6</strong></td>
<td><strong>3</strong></td>
<td><strong>7</strong></td>
<td><strong>7</strong></td>
<td><strong>7</strong></td>
<td><strong>9</strong></td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>26</strong></td>
<td><strong>18</strong></td>
<td><strong>24</strong></td>
<td><strong>14</strong></td>
<td><strong>14</strong></td>
<td><strong>37</strong></td>
</tr>
</tbody>
</table>

### TABLE 2

**Variations in Swine Import Regulations**

<table>
<thead>
<tr>
<th>A. Hog Cholera Vaccination Requirements</th>
<th>Number of States Specifying the Import Requirement</th>
<th>I. NE Reg.</th>
<th>II. N Reg.</th>
<th>III. SE Reg.</th>
<th>IV. SW Reg.</th>
<th>V. NW Reg.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Serum at time of shipment</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>2. Serum within 5 days of shipment</td>
<td></td>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>3. Serum within 7 days of shipment</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>4. Serum within 10 days of shipment</td>
<td></td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>5. Serum within 14 days of shipment</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6. Serum within 15 days of shipment</td>
<td></td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>7. Serum within 21 days of shipment</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8. Serum within 30 days of shipment</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>9. Serum with mixed inf. bact.—not prior to shipment</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
### LAWS AND REGULATIONS

#### Number of States Specifying the Import Requirement

<table>
<thead>
<tr>
<th>A. Hog Cholera Vaccination Requirements</th>
<th>I. NE Reg.</th>
<th>II. N Reg.</th>
<th>III. SE Reg.</th>
<th>IV. SW Reg.</th>
<th>V. NW Reg.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>10. Virus and serum within 30 days</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Virus and serum not permitted within 30 days</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>12. Virus and serum in not less than 30 days</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>13. Virus and serum in not less than 60 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>14. Virus and serum in not less than 21 days nor more than 6 months</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. Virus and serum in not less than 21 days</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. Virus and serum immediately before entry</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>17. Virus and serum vacc. swine not permitted entry</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. M.L.V. not less than 7 days</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. M.L.V. not less than 14 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>20. M.L.V. not less than 15 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>21. M.L.V. not less than 30 days</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22. M.L.V. not less than 30 days nor more than 120 days</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23. M.L.V. not less than 10 days</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24. M.L.V. prior to entry</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>25. M.L.V. not less than 21 days nor more than 1 year</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26. M.L.V. within 15 days</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>27. M.L.V. within 30 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>28. M.L.V. and serum prior to entry</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29. M.L.V. and serum within 15 days</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30. M.L.V. and serum not less than 7 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>31. M.L.V. and serum not less than 15 days</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>32. M.L.V. and serum not less than 15 days nor more than 36 months</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>33. M.L.V. and serum not less than 10 days nor more than 2 years</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>34. M.L.V. and serum not less than 21 days</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>35. M.L.V. and serum not less than 21 days nor more than 6 months</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>36. M.L.V. and serum not less than 21 days nor more than 1 year</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>37. M.L.V. and serum not less than 21 days nor more than 6 months</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38. M.L.V. and serum not less than 21 days nor more than 6 months</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39. M.L.V. and serum not less than 21 days nor more than 6 months</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40. B.T.V. not less than 30 days nor more than 6 months</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### A. Hog Cholera Vaccination Requirements

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>41. Tissue vaccine at least 45 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>42. C.V.V. not less than 10 days nor more than 6 months</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>43. C.V.V. not less than 14 days nor more than 6 months</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>44. C.V.V. not less than 21 days nor more than 6 months</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>45. C.V.V. not less than 30 days nor more than 6 months</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>46. C.V.V. and B.T.V. not permitted entry</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>47. Statement of vaccine used</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>48. Unvaccinated—vacc. after arrival</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>49. Exempt vacc. if from area free of hog cholera 6 months</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

**Total Number of Variations**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>23</td>
<td>20</td>
<td>11</td>
<td>15</td>
<td>49</td>
</tr>
</tbody>
</table>

### B. Leptospirosis Requirements

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Leptospirosis—free herd</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2. 30-day neg. lepto test</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

### C. Erysipelas Vaccination Requirements

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Swine erysipelas serum more than 30 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2. Swine erysipelas serum and culture not less than 30 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>3. Attenuated erysipelas vaccine not less than 30 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

### D. Brucellosis Requirements

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Certified brucellosis—free herd</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2. Certified brucellosis—free herd + 30-day test</td>
<td>1</td>
<td></td>
<td>3</td>
<td>1</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>3. Negative 30-day brucellosis test</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>4. Negative herd test within 12 months + 30-day animal test</td>
<td>1</td>
<td></td>
<td>3</td>
<td>1</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>5. Exempt from brucellosis test under 4 months</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>6. Exempt from brucellosis test under 6 months</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

### E. Garbage-Feeding Requirements

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No raw-garbage fed swine</td>
<td>8</td>
<td></td>
<td>4</td>
<td>4</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>2. Cooked garbage—fed hogs not permitted within 30 days</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

**Total Variations**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>29</td>
<td>24</td>
<td>15</td>
<td>23</td>
<td>62</td>
</tr>
</tbody>
</table>
### Table 3

**Variations in Sheep Import Regulations**

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Number of States Specifying the Import Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Must dip for scabies</td>
<td>I. NE Reg. 1 II. N Reg. 1 III. SE Reg. 1 IV. SW Reg. 1 V. NW Reg. 1 Total 1</td>
</tr>
<tr>
<td>2. If exposed, must dip for scabies</td>
<td>4 6 1 11</td>
</tr>
<tr>
<td>3. If scabies exists in state of origin, must dip for scabies</td>
<td>2 9 1 6 5 23</td>
</tr>
<tr>
<td>4. 30-day negative tuberculosis test</td>
<td>2</td>
</tr>
<tr>
<td>5. 30-day negative brucellosis test</td>
<td>2</td>
</tr>
<tr>
<td>6. If bluetongue in state of origin, require vaccination and dipping</td>
<td>1 1 2</td>
</tr>
<tr>
<td>Total Variations</td>
<td>4 1 2 4 3 6</td>
</tr>
</tbody>
</table>

### Table 4

**Variations in Dog Import Regulations**

<table>
<thead>
<tr>
<th>Rabies Requirements</th>
<th>Number of States Specifying the Import Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No requirements listed</td>
<td>I. NE Reg. 2 II. N Reg. 1 III. SE Reg. 3 IV. SW Reg. 3 V. NW Reg. 2 Total 6</td>
</tr>
<tr>
<td>2. Prohibited from quarantined areas</td>
<td>7 2 6 3 3 21</td>
</tr>
<tr>
<td>3. Prohibited from area in which rabies reported within 6 months</td>
<td>1 1 4 2 3 11</td>
</tr>
<tr>
<td>4. Not exposed to rabies within 100 days</td>
<td>2 1 1 4</td>
</tr>
<tr>
<td>5. No rabies within 25 miles within 6 months</td>
<td>1 1</td>
</tr>
<tr>
<td>6. From rabies area—vacc. within 6 months</td>
<td>1</td>
</tr>
<tr>
<td>7. From state in which rabies exists—vacc. 60 days prior to entry</td>
<td>1 1</td>
</tr>
<tr>
<td>8. Vaccinate for rabies within 10 months</td>
<td>1 1</td>
</tr>
<tr>
<td>9. Vaccinate for rabies within 12 months</td>
<td>3 2 3 4 3 15</td>
</tr>
<tr>
<td>10. Tissue vaccine not less than 30 days nor more than 6 months</td>
<td>1 1</td>
</tr>
<tr>
<td>11. Tissue vaccine not less than 30 days nor more than 12 months</td>
<td>1 1</td>
</tr>
<tr>
<td>12. Tissue vaccine not more than 1 year</td>
<td>3 2 5</td>
</tr>
<tr>
<td>13. Modified live virus not more than 2 years</td>
<td>1 1</td>
</tr>
<tr>
<td>14. Modified live virus not more than 3 years</td>
<td>3 2 1 6</td>
</tr>
<tr>
<td>15. Exempt from vaccine under 6 weeks</td>
<td>1</td>
</tr>
<tr>
<td>16. Exempt from vaccine under 8 weeks</td>
<td>1</td>
</tr>
<tr>
<td>17. Exempt from vaccine under 3 months</td>
<td>1</td>
</tr>
<tr>
<td>18. Exempt from vaccine under 16 weeks</td>
<td>1</td>
</tr>
<tr>
<td>19. Exempt from vaccine under 4 months</td>
<td>1 2 1 4</td>
</tr>
<tr>
<td>20. Exempt from vaccine under 6 months</td>
<td>2</td>
</tr>
<tr>
<td>Total Variations</td>
<td>10 11 5 7 10 20</td>
</tr>
</tbody>
</table>
TABLE 5
Summary of Variations in Livestock Import Regulations

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle Variations---</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brucellosis</td>
<td>20</td>
<td>15</td>
<td>17</td>
<td>7</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>6</td>
<td>3</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Swine Variations---</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hog cholera vaccine</td>
<td>17</td>
<td>23</td>
<td>20</td>
<td>11</td>
<td>15</td>
<td>49</td>
</tr>
<tr>
<td>Other requirements</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Sheep Variations---</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over-all requirements</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Dogs Variations-----</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabies requirements</td>
<td>10</td>
<td>11</td>
<td>5</td>
<td>7</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Total Variations</td>
<td>62</td>
<td>59</td>
<td>55</td>
<td>40</td>
<td>50</td>
<td>125</td>
</tr>
</tbody>
</table>

DISCUSSION

Table 1 indicates that only 38 States will accept cattle from certified brucellosis-free herds without further tests. Only one State in the Northeast Region of the United States, one in the North Central Region and one in the Southeastern Region will accept cattle from modified-certified brucellosis areas without further restriction. This study indicates there are a total of 28 variations in brucellosis import requirements of the various States in the United States. Northeastern States have imposed 20 of these variations, the North Central States 15, the Southeastern States 17, the Southwestern States and Northwestern States seven, each.

The tuberculosis requirements imposed by the various States fall into nine variations. Six of these various regulations are employed by the Northeastern States, three by the North Central, and seven, each, by the Southeastern, Northwestern, and Southwestern States. Accredited-free herds are recognized by only 38 States and modified-accredited tuberculosis areas by 16 States through their import regulations.

It is quite astounding to note there are 49 variations of hog cholera vaccination requirements imposed in the import regulations of the various States. (See Table 2.) The North Central States have 23 variations among themselves, the Southeastern States contribute 20, the Northeastern employ 17, the Southwestern 11, and the Northwestern States 15. Some of the regulations on the use of hog cholera immunizing product are contradictory.

The variations of sheep import regulations, as shown in Table 3, are only six.

Table 4 indicates 20 variations on rabies import regulations of the States. Ten variations are employed by each of the Northeastern and Northwestern States, 11 by the North Central, five by the Southeastern States, and seven by the Southwestern States.
In summary, the total variations of the import regulations reviewed by the committee show 125. The Northeastern States contribute most, overall, to variations in import regulations with 62, followed by 59 among the North Central States, 55 in the Southeastern States, 50 in the Northwestern, and 40 in the Southwestern States.

It is obvious, from this review, that the area of greatest lack of uniformity of livestock health import requirements between the States are: (1) Hog cholera, (2) brucellosis in cattle and (3) rabies. These three account for 99 variations in the total of 125 variations found.

The three regions in the United States that contribute most to the lack of uniformity in brucellosis requirements, hog cholera vaccination requirements, and rabies requirements, are the Northeastern, North Central, and Southeastern States.

Every State, through State constitutional or legislative directives, must safeguard the health of the livestock within that State. It is the responsibility of each State livestock disease-control agency to establish the best possible means to prevent the introduction of livestock diseases into its State. There can be no doubt that the State boundary line, and the fact that each State must be responsible for livestock health within that State, has been a major contributing factor to the over-all general health of livestock and the prevention of the widespread movement of animals affected with dangerous diseases in this nation. It would be hard to conceive a more effective control of the transmission of livestock diseases in a nation as large as the United States without the use of the State boundary lines. Your Committee urges that each State do everything within its power to prevent the introduction of livestock diseases into that State. This, in turn, will assure continued success in the control of the transmission of livestock diseases in the nation.

RECOMMENDATIONS

It is an established and recognized fact that there is a great variation in livestock disease problems, livestock husbandry, and livestock marketing practices in the various regions of the United States. Therefore, it is imperative that disease-control procedures be employed that are most applicable to that region to safeguard the livestock industry within the region. It is the opinion of this Committee that sufficient recognition has not been given in the past to this fact, and attempts to obtain uniformity of interstate regulations, on a nationwide basis, has not been too successful. Your Committee recommends, again, that the United States Livestock Sanitary Association go on record as urging the States within the various regions of the United States to review their import regulations, making every effort to obtain more uniformity among the States within their respective regions. The Committee finds that the greatest lack of uniformity among the States in a given region is between the States in the Northeastern region, first, next the North Central, and then the Southeastern, followed by the Northwestern areas and Southwestern areas. It is recommended that preference of effort be given where most needed.
Due to the fact that extreme variations, and the largest number of variations in livestock import requirements, are found in the use of hog cholera immunizing agents, and due to the fact that efforts are being made to establish a nationwide hog cholera-eradication program, your Committee feels it is imperative that this area of requirements be clarified as soon as possible. We feel this should be done before a nationwide hog cholera-eradication program can hope to be approached with any degree of success. It is recommended that (1) the Committee on the National Eradication of Hog Cholera of the United States Livestock Association evaluate and present to this Association for its adoption the proper use of the various hog cholera immunizing agents available, (2) recommend procedures of hog cholera vaccination that will assure the various States maximum protection from the introduction of hog Cholera through the importation of hog cholera vaccinated swine, and (3) develop a model swine import regulation that can be recommended for adoption by the various States.

It is recommended that the Committee on Rabies develop rabies vaccination procedures, that can be adopted in the import regulations of the States, that will assure maximum protection against the introduction of rabies.

Only 16 States out of 50 indicate they will accept cattle from non-quarantined herds in modified-accredited tuberculosis areas without further tuberculosis tests. It is recommended that the Tuberculosis Committee of this Association take note of this fact.

It is recommended that the Brucellosis Committee of this Association propose a brucellosis import regulation that the United States Livestock Association can recommend for adoption by the States.

States and regions used in this report were selected only for purposes of illustration. It is recommended that regions, as selected by the United States Livestock Association, be the regions in which efforts be made to obtain a greater degree of uniformity of livestock health import regulations.

The Committee on Laws and Regulations wishes to present the following Resolution for your consideration:

Whereas, the marketing system of livestock in the United States is extremely complex, and
whereas, the tracing of diseased livestock has become most difficult to the detriment of the health and welfare of the livestock of the nation,

Now, therefore, be it resolved, to further aid in the eradication of brucellosis, tuberculosis and other infectious, contagious diseases of livestock, that all animals moving intrastate and interstate be identified either by eartag, tattoo, registered brand, or backtag in such way that each animal can be traced back to the herd of origin.
REPORT OF REPRESENTATIVES TO THE ANNUAL MEETING OF NATIONAL ASSOCIATION OF STATE DEPARTMENTS OF AGRICULTURE

Delegates


The Forty-third Annual Convention of the National Association of State Departments of Agriculture was held at the Hershey Hotel, Hershey, Pennsylvania, October 1-5, 1961. Representatives from the United States Livestock Sanitary Association attending this meeting were: Dr. J. R. Hay, Chicago, Illinois; Dr. A. P. Schneider, Boise, Idaho, and Dr. R. L. Elsea, Harrisburg, Pennsylvania.

The delegates from this Association were invited to attend the committee meetings and were especially urged to attend and participate in the deliberations of the Committee on Animal Industry. Many phases of Animal Industry functions in the State Departments of Agriculture were discussed. These discussions resulted in the preparation of a total of 10 resolutions to be presented to the full assembly.

The last general session of the full assembly was held the last day of the meeting October 5, 1961. The total, 35 in number, of all resolutions submitted from all committees for consideration by the General Assembly were acted upon at this time. A total of nine resolutions having to do with Animal Industry functions were passed at this session.

These resolutions follow:

RESOLUTION NO. 14—WET HAMS

WHEREAS, The Secretary of Agriculture announced recently that he proposes to reinstate the standards for Federally inspected hams that were in effect prior to December 30, 1960,

NOW, THEREFORE, BE IT RESOLVED, That the National Association of State Departments of Agriculture in convention assembled at Hershey, Pennsylvania, October 1-5, 1961, commends the Secretary of Agriculture for his proposal which will reinstate the standards for Federally inspected hams that were in effect prior to December 30, 1960, affecting the amount of water that may be added.

BE IT FURTHER RESOLVED, That the Association encourage each State to initiate immediate control measures to prevent plants doing intra-state business from adding any moisture to hams in excess of the original green weight.

41
REPORT OF RESOLUTION NO. 25—ERADICATION OF SCREWWORM

WHEREAS, The screwworm was eradicated from the Southeastern United States in the cooperative eradication program of 1958-59 at a cost of more than eight million dollars jointly shared by the cooperating states and the Federal Government, and
WHEREAS, The screwworm continues to exist in areas of the Southwest which was the source of original infestation in Southeastern states in 1933, and
WHEREAS, The only protection of Southeastern states against reinestation from the Southwest depends on precautions taken at the Federal inspection stations maintained along the eastern borders of Arkansas and Louisiana, and
WHEREAS, The screwworm has reappeared twice in Florida since it was eradicated there, the most recent discovery being in West Florida near the Alabama border, requiring immediate and costly measures to prevent it from gaining a new foothold in the Southeast;
NOW, THEREFORE, BE IT RESOLVED, That the National Association of State Departments of Agriculture, in annual convention assembled at Hershey, Pennsylvania, October 1-5, 1961, requests the United States Department of Agriculture to take immediate action to (1) increase the effectiveness of inspections of livestock and other animals being transported from screwworm-infested areas of the Southwest into Southeastern states; (2) put into operation immediately a method of enforcing compliance with Federal inspection regulations at the time livestock shipments are crossing the inspection line, and (3) with all possible speed inaugurate a program for complete eradication of screwworms from the Southwest and thus free United States livestock raisers from the burden of costly losses.

RESOLUTION NO. 26—FUNDS FOR FACE FLY ERADICATION PROGRAMS

WHEREAS, Face fly continues to move into more States and become an increasingly annoying and costly pest of livestock, and
WHEREAS, Little progress has been made in developing effective materials and methods for the control of face fly, and
WHEREAS, To further the control and eradication of face fly, an accelerated research program is required;
NOW, THEREFORE, BE IT RESOLVED, That the National Association of State Departments of Agriculture, in annual convention assembled at Hershey, Pennsylvania, October 1-5, 1961, urges the United States Department of Agriculture to request Congress to support the eradication program for face fly by appropriating the necessary funds.

RESOLUTION NO. 27—HOG CHOLERA ERADICATION PROGRAM

WHEREAS, Congress has passed legislation directing the United States Department of Agriculture to cooperate with the states in a national program to eradicate hog cholera, and
WHEREAS, Adequate State and Federal funds will be needed, and

WHEREAS, The contagious nature of this disease requires that the Department’s resources be completely flexible and available to effectively eradicate the disease and prevent reinfection of states having active eradication programs;

NOW, THEREFORE, BE IT RESOLVED, That the National Association of State Departments of Agriculture in annual convention assembled at Hershey, Pennsylvania, October 1-5, 1961, supports the United States Department of Agriculture in its requests for funds for this program, and it further urges each State to provide ample funds and other resources in its cooperative program with the United States Department of Agriculture.

RESOLUTION NO. 28—SHEEP SCABIES ERADICATION PROGRAM

WHEREAS, We wish to recognize the progress made in the sheep scabies eradication program during the 12 months since our last meeting when only eastern South Dakota was designated a sheep scabies eradication area, and

WHEREAS, During the past year North Dakota, Nebraska, Arkansas, Tennessee, New York, Illinois, and Western Kansas have properly qualified themselves as eradication areas and are carrying out active eradication programs and 11 counties in Western Kansas have achieved eradication of the disease, and the States of Wisconsin, New Jersey and Hawaii are desirous of establishing themselves as eradication areas, and

WHEREAS, This is the most encouraging evidence seen in many years of the desire of the States concerned, the United States Department of Agriculture and the livestock industry to eradicate this disease from the entire United States;

NOW, THEREFORE, BE IT RESOLVED, That the National Association of State Departments of Agriculture, in annual convention assembled at Hershey, Pennsylvania, October 1-5, 1961, requests the United States Department of Agriculture, in cooperation with the States, to accelerate the eradication programs, to the maximum extent,

BE IT FURTHER RESOLVED, That the United States Department of Agriculture request the necessary funds from Congress so that the Federal contribution along with that of the States will be sufficient to achieve the national goal of the eradication of sheep scabies.

RESOLUTION NO. 29—REMODELING OF BELTSVILLE PARASITE RESEARCH LABORATORY

WHEREAS, The present parasite research laboratory at Beltsville, Maryland is inadequate to develop effective materials and methods for the control and eradication of livestock parasites, and

WHEREAS, The United States Department of Agriculture has plans to remodel this laboratory in order to accelerate research for the control and eradication of livestock parasites;
REPORT OF

Now, Therefore, Be It Resolved, That the National Association of State Departments of Agriculture in annual convention assembled at Hershey, Pennsylvania, October 1-5, 1961, cooperate with the United States Department of Agriculture in requesting Congress to appropriate $500,000 for the remodeling of the Beltsville Parasite Research Laboratory.

RESOLUTION NO. 30—SUPPORT OF SENATE BILL 860

Whereas, Senate Bill 860 of the 87th Congress, First Session, entitled "An Act to provide greater protection against the introduction and dissemination of diseases of livestock and poultry, and for other purposes," has passed the Senate of the United States of America in Congress assembled, and

Whereas, The said Act is now in the House of Representatives of the United States in Congress where it has been referred to the subcommittee on Livestock and Feed Grains of the Committee on Agriculture, and

Whereas, The authority provided in this bill is essential for the United States Department of Agriculture to cope with emergency outbreaks of animal diseases and to discharge its responsibilities in the prevention and spread of such diseases;

Now, Therefore, Be It Resolved, That the National Association of State Departments of Agriculture in annual convention assembled in Hershey, Pennsylvania, October 1-5, 1961, instructs its Executive Committee to work actively with and reaffirm to the House of Representatives of the United States Congress and the various members of the United States Senate its support of Senate Bill 860.

RESOLUTION NO. 31—SWINE BRUCELLOSIS ERADICATION

Whereas, Brucellosis is a serious disease of swine causing approximately ten million dollars in losses to the swine industry each year, and

Whereas, Brucellosis in swine is now the most serious remaining reservoir of human brucellosis (Undulant fever) in the United States, and

Whereas, Brucellosis in cattle has been reduced to a remarkable extent during the past seven years through the cooperative efforts of State and Federal governments, and

Whereas, Brucellosis cannot be completely eradicated as long as it exists unchecked in swine and other domestic animals;

Now, Therefore, Be It Resolved, That the National Association of State Departments of Agriculture in annual convention assembled at Hershey, Pennsylvania, October 1-5, 1961, recommends:

That the individual States and the United States Department of Agriculture cooperatively conduct an intensified swine brucellosis eradication program based upon procedures recommended by the United States Livestock Sanitary Association;

That swine herds which are free of brucellosis be designated "Validated Brucellosis-Free Herds," and
Be It Further Resolved, That this program shall not affect the present bovine brucellosis eradication program.

RESOLUTION NO. 32—FEDERAL STATE AGREEMENT ON INSPECTION OF RED MEATS

WHEREAS, Some states maintain and perform ante-mortem and post-mortem inspection of red meats and meat food products for wholesomeness comparable to Federal red meat inspection standards, and
WHEREAS, Present Federal law prohibits the movement of such State inspected red meat and meat food products in interstate commerce, and
WHEREAS, Most Federal installations refuse to purchase such State inspected products, thereby limiting the market for livestock slaughtered in State inspected plants, and
WHEREAS, A proven system of Federal-State cooperative inspection for other products is operating successfully with full safeguards of public interests;

NOW, THEREFORE, BE IT RESOLVED, That the National Association of State Departments of Agriculture in annual convention assembled at Hershey, Pennsylvania, October 1-5, 1961, requests its Executive Committee to conduct a thorough study of this situation.
REPORT OF THE 1961 COMMITTEE ON FEDERAL PROGRAMS AND POLICY

W. L. BENDIX, Chairman, Richmond, Virginia; W. F. FISHER, Reno, Nevada; H. G. GEYER, Columbus, Ohio; T. J. GRENNAN, JR., Providence, Rhode Island; R. A. HENDERSHOTT, Trenton, New Jersey; J. A. McCALLAM, Washington, D. C.

The Committee on Federal Programs and Policy of the United States Livestock Sanitary Association for 1961 held three meetings during the year. The first meeting was held in Washington, D. C., on February 1 and 2, 1961. The second meeting also was in Washington, April 19 and 20. The third meeting was held in Detroit, Michigan, on Tuesday, August 22. The responsibilities of this Committee call for considerable travel on the part of its members, as well as a great deal of work. Most of the travel involved is done at the expense of organizations other than the United States Livestock Sanitary Association. The chairman wishes to acknowledge this and to express his thanks to those organizations which have permitted the various Committee members to use their time and funds as necessary to carry on the Committee’s work. And he wishes to express his thanks for the cooperation he has received from the Committee. The chairman wishes also to acknowledge with gratitude the splendid cooperation of the various staff members of the Agricultural Research Service and its several Divisions, not only for the frankness with which they have discussed their various problems with the Committee but for the time and effort that went into the preparation and presentation of this material. The chairman knows that he speaks for the entire Committee in hoping that through our combined efforts we have achieved some worth-while results.

As the work of this Committee covers such a wide range of subjects, all of them of continuing interest to the livestock sanitary officials of the nation, it was the Committee’s feeling that as wide a distribution as possible among such officials should be obtained for the minutes of Committee meetings. While the Committee does not deal specifically with procedures, methods, or policy involving the handling of specific problems and while it seeks always to avoid any trespass upon the work or area of responsibility of any of the fine standing Committees of this Association, it does concern itself with new problems as they arise, with resolving differences of opinion regarding existing problems, particularly as they may refer to Federal participation with the States, with adequate financing, both State and Federal, for agreed problems, and with suggesting and supporting legislation which would accomplish the purposes of this Association. It was agreed at the Committee meeting in April in Washington that in the future the minutes of all Committee meetings would be furnished to each member of the Executive Committee as soon after such meetings as this could be accomplished. The
chairman attempted to do this for the first two meetings held this year, but the Committee felt that the Secretary of the United States Livestock Sanitary Association, who is a member ex officio of this Committee, would be the proper person to do this and requested he handle the matter. Your Committee wishes to recommend to the Association that this be done on a continuing basis by the Secretary of the United States Livestock Sanitary Association.

ANIMAL DISEASE ERADICATION DIVISION

TUBERCULOSIS. Your Committee is very much concerned about the tuberculosis situation. We had a full and free discussion with members of the Animal Disease Eradication Division, and the matter was presented in some detail to the Agricultural Appropriations Subcommittees of both the House and the Senate. As a result of this work and of course the work of a great many others, an additional one million dollars was provided in the fiscal 1962 budget of the Department of tuberculosis eradication. For fiscal 1963, your Committee has urged that the Department seek yet an additional two million dollars for this work. Your Committee feels that while an increased rate of testing is undoubtedly necessary, there are other factors which need developing in our continuing battle against this disease. We therefore recommend that these additional funds be used in the following manner:

1. To provide for field and laboratory studies of factors other than tuberculosis that cause response to the tuberculin test.

2. Provide for field and laboratory studies to establish the relationship between bovine, avian, human, and paratuberculosis organisms and their effect on the eradication effort.

3. The development of more effective case finding through slaughterhouse tracebacks. Seventy-five percent of reactors are now found as a result of testing for reaccreditation, and 25 percent are located through tracebacks. Nationally, the traceback effort is only about 85 percent accurate, and this should be greatly improved.

4. Increasing the annual rate of testing for tuberculosis by two to three million head.

The Animal Disease Eradication Division was requested to give consideration to regulations which would authorize depopulation proceedings in problem herds. Your Committee recommends that this matter be sent to the Committee on Tuberculosis of this Association for further study, that they pursue the matter with the Animal Disease Eradication Division, and that recommendations for specific procedures be developed. In an effort to stimulate additional testing, particularly in the dairying areas of the nation, the Committee recommends that this Association refer to its Committee on Tuberculosis the matter of the frequency of testing in dairy herds. Some States require an annual test for tuberculosis in all dairy herds; other States
have no such requirement but follow the United States Public Health Service Model Ordinance, which accepts county-accredited status as acceptable compliance. This provision was made by the United States Public Health Service upon recommendation of the Department of Agriculture. Your Committee feels that this matter should be reviewed by our Committee on Tuberculosis with the Department of Agriculture and the United States Public Health Service, with the thought in mind that such a review would reveal the need for possible amendment to the Model Milk Ordinance and Code requiring more frequent tuberculin tests of all dairy herds.

**BRUCELLOSIS.** The Brucellosis-Eradication Program appears to be moving at an acceptable pace, and the current financing, both State and Federal, is most gratifying. Continued industry support at an extremely high level also is highly commendable. Based on current figures and rate of progress, it appears that we have a very good chance of reaching our goal for 1961, with only 373 of the nation's counties not yet in the accelerated program.

The matter of calf vaccination is causing concern. In fiscal 1960, there were 6.4 million calves vaccinated, which is 300,000 less than the previous year. About the minimum acceptable vaccination rate that will permit us to maintain the gains we have made and the goal we seek would be eight to 10 million calves annually. This would provide approximately a 60-percent coverage, which should be considered minimum. This slump in calf vaccination starts in the certified States, and it is an all too familiar pattern. County testing is complete, the State is certified, and in the minds of far too many people the disease is eradicated and can be forgotten. We are reaping the harvest of this type of thinking in tuberculosis right now, and your Committee is very concerned to see the same pattern developing in brucellosis. Your Committee recommends that this be called to the attention of the Committee on Brucellosis of this Association in the hope that they will give it very serious thought.

The Animal Disease Eradication Division was urged by your Committee to develop a program for the control and eventual eradication of swine brucellosis and to set up the machinery to engage in cooperative projects with the various States in this field. Your Committee recommends that the Association approve this proposal and that the work of developing such a cooperative program be assigned to the proper Committee of this Association.

**SHEEP SCABIES.** Sheep scabies continues to plague the nation, though six States currently considered infected have plans for joint State-Federal eradication programs that only await Federal financing to implement. Your Committee urgently recommended that the additional $200,000 needed to implement the programs in these States be sought from the Congress and also urged the Congress to provide this sum during fiscal 1962. We are very happy to report to the Association that this sum has been provided, and we hope for an early implementation of the program in the States involved.
Scabies, either in sheep or cattle, is known to exist in about 1,700 counties in the United States. It is estimated that $6,000 to $8,000 per county would achieve total eradication. This will involve about 11 million dollars total to eradicate sheep and cattle scabies over a five-year period. Your Committee urged the Animal Disease Eradication Division to develop cooperative programs designed to accomplish this and to begin financing this operation, at least in part, for fiscal 1963. Your Committee recommends that the Association endorse this proposal and that every means at our command be exercised to implement it.

**Hog Cholera.** Reliable sources indicate that the cost of eradicating hog cholera once it is seriously undertaken as a State-Federal cooperative program would involve the expenditure in Federal funds of approximately four million dollars during the first full year of operation and eight to 10 million dollars annually thereafter in Federal funds for a period up to eight years. The cooperative program would of course require State funds for matching purposes of approximately this sum.

Your Committee is pleased to report the enactment into law of the bill before the last Congress directing the Secretary of Agriculture to develop a national hog-cholera eradication program and to prohibit the interstate movement of virulent virus to whatever extent it was necessary to accomplish this purpose. Your Committee on behalf of this Association actively supported this legislation, along with virtually all the livestock and farm organizations in the country.

Your Committee recommends that this Association endorse this proposed program and, through its Committee on the Nationwide Eradication of Hog Cholera, use every means at its command to develop and support it. This Association should not forget that whereas today the eradication of hog cholera is being promoted by virtually every national organization or agency having any interest in the subject, the first voice raised nationally in the matter was the voice of the United States Livestock Sanitary Association many years ago. The nation will look to us for leadership in this field only if we provide that leadership. This is a challenge that we should meet head on.

**Animal Inspection and Quarantine Division**

Your Committee has been recommending to the Department of Agriculture for several years that it seek increased authority, increased personnel, and increased facilities to handle the inspection and quarantine work load at all air, ocean, and land ports of entry in the United States. Your Committee has considered this a "must" item, because in no area are we more vulnerable than we are in the possibility of foreign animal plagues being introduced into this country. During 1960, the Agricultural Research Service established a review Committee which traveled all over the United States and in Canada studying this whole question. The final report of this study Committee has been released. Your Committee reviewed this report and endorsed its recommendations.
Your Committee recommends that this Association endorse the recommenda-
tions contained in this study Committee report and that it put its weight
and influence behind whatever effort is necessary to implement it. It is not
going to be cheap or easy to do. To get anything like an acceptable job
done, it will take a minimum of 67 new people, six new facilities along the
Canadian border, modernization of the Clifton, New Jersey, Quarantine
Station, and the construction and operation of two more similar quarantine
stations, one preferably in southern Florida and the other on the west coast.
New authority is needed from the Congress to get this job done, which will
involve amendment to existing laws and enactment of new ones. But this is
a potentially dangerous situation in which are the seeds of actual disaster.

POULTRY DISEASES

There is definite evidence of increased interest throughout the country in
the field of an organized program for the eradication of some of the major
poultry diseases. Your Committee discussed this matter very fully with the
Agricultural Research Service staff people. There seems to be a great deal
more interest developing within the industry currently than perhaps among
some of the livestock regulatory officials across the nation. Part of the
evidence that led your Committee to this conclusion was the recent proposed
rule-making publication by the Department of Agriculture involving the
interstate movement of birds to prevent the dissemination of fowl typhoid.

This proposed interstate regulation was drawn up and published by the
Department upon request; but when notice was sent of the proposed regula-
tion, along with a copy and a request for comment, a considerable response
was received from industry, most of it opposed to certain provisions of the
proposed regulation, it is true, but not one State livestock sanitary official
responded, either for or against. In the major poultry-producing areas of
this country, there is real need for regulatory veterinary service to this
industry. Your Committee has recommended that a thorough epidemiological
study of poultry diseases be undertaken, and we have urged the Animal
Disease Eradication Division to add to its staff at least five specialists to do
this work. It is expected that the cost would be approximately $15,000 each,
or a total of $75,000 for the first year. We recommended that the Depart-
ment seek these funds and begin operating during fiscal 1963. Your Com-
mittee hopes that the Association will approve this proposal and will support
this effort to whatever extent appears necessary or desirable.

ANIMAL DISEASE AND PARASITE RESEARCH

Your Committee is quite naturally pleased, as will be this Association, that
the Department of Agriculture's National Animal Disease Laboratory at
Ames, Iowa, is now in operation. This, in the Committee's opinion, is a very
great step forward in our disease-control work and should pay for itself
many times over in the years to come. Your Committee is also pleased that
the Congress has seen fit to provide for two additional laboratories, designed
primarily to do research work in poultry diseases, one at Athens, Georgia, and another at State College, Mississippi. These two additional laboratories are under construction and should be available during the 1963 fiscal year.

Laboratories such as these are not built, completed, staffed, and forgotten if they are going to accomplish the task assigned them. On a continuing basis, staffs will have to be enlarged as new problems appear or new solutions are sought for old ones. New facilities in the way of buildings and equipment will have to be provided from time to time to take care of increasing work loads. Perhaps even new locations will have to be sought and new facilities provided from scratch as it were. While by no means was ours the only voice raised in support of the development of the Department of Agriculture's laboratory program, we certainly were in the forefront of this effort from the beginning, and we have a real obligation to stay there. Your Committee wishes to call this to the attention of the Association, because it realizes the ease with which we could have our attention diverted to other problems while these laboratories are left to shift for themselves. This must not happen; we must keep a close watch on these laboratories and their operation and support them actively whenever the need arises. This, too, your Committee recommends as the continuing policy of this Association.

We have one further step to recommend in this matter, and that is the conversion of the Beltsville center to a parasite-research facility. Your Committee has urged this upon the Department repeatedly, beginning at the time that funds for the Ames Laboratory were first approved. At that time, approximately three million dollars was recommended; but at today's prices, the cost probably would exceed this amount. The Department has repeatedly assured your Committee that this was a part of their long-term planning, but we have not yet been told just how long "long-term" is. Your Committee recommends that this Association pursue this project with vigor and determination to its completion. There are several places in this country one could go to get reliable estimates of what livestock parasites, both internal and external, are costing the United States. When you look at some of these figures, the estimated operating budget increase of about $650,000 annually to operate an extensive parasite-research facility at Beltsville seems very small indeed.

Your Committee appreciates the opportunity afforded us for serving you this year, and we sincerely hope that our efforts will meet with your general approval.
COMMITTEE ON RESOLUTIONS


MR. F. G. BUZZELL: The Committee on Resolutions offer the following resolutions:

RESOLUTION NO. 1

WHEREAS, The United States Livestock Sanitary Association at its Sixty-fourth Annual Meeting in Charleston, West Virginia, October 18-21, 1960, has passed a resolution supporting the American Veterinary Medical Association in recommending the establishment of a Veterinary Branch within the International Cooperation Administration (now agency for international development), and

WHEREAS, the conditions described in this resolution are still prevailing, and

WHEREAS, the A.V.M.A. at its last annual convention passed a resolution reiterating its recommendation that a Veterinary Branch be established within the agency for International Development (formerly ICA);

THEREFORE BE IT RESOLVED, that the United States Livestock Sanitary Association does in Convention assembled at Minneapolis, Minnesota, October 30 to November 3, 1961, endorse the aforementioned resolution of the A.V.M.A. and again recommend to the Department of State that it establish a Veterinary branch within the Agency for International Development, and

BE IT FURTHER RESOLVED, that a copy of this resolution be sent to the Secretary of State, to the Director of the Agency for International Development; to the Director of the Office of Food and Agriculture, Agency for International Development; to the Director, Office of Public Health, Agency for International Development and to the Director, Office of Training Programs, Agency for International Development.

RESOLUTION NO. 2

WHEREAS, laboratory diagnostic assistance is an essential part of all animal disease prevention, control and eradication programs, and

WHEREAS, the National Conference of Veterinary Laboratory Diagnosticians recognizes the need for diagnostic consultant and reference assistance to State diagnostic laboratories in carrying out their responsibilities in the prevention, control and eradication of animal diseases not included in National programs and for which assistance is not now available.

BE IT RESOLVED, that the United States Livestock Sanitary Association appeal to the United States Department of Agriculture and the Congress
to provide the Agriculture Research Service with the necessary means to carry out reference and consultant assistance to State diagnostic laboratories.

It is requested that this resolution also be brought to the attention of the National Association of State Departments of Agriculture.

RESOLUTION NO. 3

Resolved, that the work of this Committee (Virus Research Committee) as reported be implemented:

1. By establishing a repository of virus information at a recognized impartial institution.
2. That an executive secretary be appointed by the committee to supervise the work necessary to maintain the repository. By necessity the secretary must be a man qualified in the field and associated with the institution providing the housing.
3. That funds to carry out this work be obtained from an impartial non-profit organization of greatest prestige on a national and international level and having previous experience in this sort of endeavor.
4. That the Executive Committee of the United States Livestock Sanitary Association direct the Committee on Virus Research to represent the Association in this work and take the steps necessary to carry out the objectives outlined above.
5. That the name of this Committee be changed from Virus Research to "The Committee for Animal Virus Classification."

RESOLUTION NO. 4

Whereas, the Marketing System of Livestock in the United States is extremely complex, and

Whereas, the tracing of diseased livestock has become most difficult to the detriment of the health and welfare of the livestock of the nation,

Now, Therefore, Be It RESOLVED, to further aid in the eradication of brucellosis, tuberculosis and other infectious contagious diseases of livestock, that all animals moving interstate and intrastate be identified either by eartag, tattoo, registered branch, or backtag in such a way that each brand, or backtag in such a way, that each animal can be traced back to the head of origin.

RESOLUTION NO. 5

Whereas, the poultry industry in the United States has made such outstanding growth in the last 20 years that its value is approximated at four billion dollars, and

Whereas, through this great expansion in activities and poultry population, the disease problems have likewise developed to an alarming extent and are reflected by disease losses on the farm, in the hatchery, and in poultry processing plants, and
WHEREAS, professional veterinary services, specialized for poultry diseases, have also increased in late years but are regarded still as greatly inadequate, and

WHEREAS, many of the disease problems are infectious in nature which require not only adequate diagnostic service, but also competent and effective regulatory service to cope with their control, and

WHEREAS, many states have no specialized regulatory service for the control and eradication of infectious poultry diseases due to lack of regulatory measures and professional personnel, and

WHEREAS, to cope with these important inadequacies in the control and eradication of infectious poultry diseases for the present as well as the future,

THEREFORE BE IT RESOLVED, that each State provide for specific adequate regulatory measures to cope with the control and eradication of infectious poultry diseases, and

BE IT FURTHER RESOLVED, that these regulatory measures in each and every State be placed under the jurisdiction of the official State livestock disease regulatory agency and that a competent veterinarian trained in poultry diseases be delegated for the control and eradication of infectious poultry diseases, and

BE IT FURTHER RESOLVED, that copies of this resolution be sent to the proper offices of the American Veterinary Medical Association, State livestock disease regulatory agencies, the American Poultry and Hatchery Federation, and the National Turkey Federation.

RESOLUTION NO. 6

WHEREAS, the Sixty-fifth Annual Meeting of the United States Livestock Sanitary Association in convention assembled at Minneapolis, Minnesota, October 31-November 3, 1961, has proven to be enjoyable and profitable to all members of the Association. The papers given and Committee reports with their recommendations will greatly assist in the Control and Eradication of animal diseases,

THEREFORE BE IT RESOLVED, that the members of this Association express their sincere appreciation for courtesies and assistance extended by the College of Veterinary Medicine, University of Minnesota, and Animal Disease Eradication Division of Agricultural Research Service, United States Department of Agriculture, for their part in a very successful symposium on hog cholera, by Dr. J. C. Flint, and Dr. Allen Morrow, and their staff; by the Chamber of Commerce of Minneapolis; by the National Band and Tag Company; by the Hotel Curtis, and by the Press, Radio and others who have contributed to the success of this meeting.

BE IT FURTHER RESOLVED, that copies of this resolution be sent to the appropriate individual concerned.
REPORT OF COMMITTEE ON STOCKYARDS, MARKETS AND TRANSPORTATION


This Committee met and the following recommendations were discussed and approved by the members present:

1. It is recognized that progress is being made by the A.I.Q. Division of the Agricultural Research Service in effecting improved sanitary facilities at Ports of entry along the Canadian border. It is recommended that these efforts be continued. These facilities should include shelter from inclement weather, inspection chutes, satisfactory pens, and cleaning and disinfection equipment. The installation should compare favorably with public stockyards and specifically approved livestock markets. If these facilities cannot be furnished by private ownership, then it is recommended that Congress appropriate funds for this purpose.

2. The problem of Canadian livestock shipments, released at the border, which fail to arrive at consigned destination in the United States was again reviewed. In such instances the State livestock sanitary officials at destination are urged to inform the A.I.Q. Division at port of entry of the non-receipt of such shipments so that they may be traced and recurrence of diversions prevented.

3. The question of issuing State Permits for livestock shipments was reviewed. While it is recognized that the shipper should be informed as to the requirements of the State of destination yet the problem of paying for long telegraphic permits continues to exist. It is recommended that telegrams be limited to the fewest words needed to serve the purpose.

4. As it is apparent that there is a continued need for reducing death losses in stocker and feeder pigs it is urged that the handling of such swine be more strictly controlled. It is recommended that the movement of stocker and feeder pigs from market to market be prohibited in order to prevent loss of identification and duplication of vaccination.

5. The handling of official vaccinates and cattle from certified areas continues to be a problem in some stockyards and auction markets. It is recommended that acceptable and uniform procedures be estab-
lished whereby these animals may be identified and certified with the least possible delay.

(6) The problem of handling livestock affected with certain disease conditions continues to be a problem at stockyards and auction markets. It is recommended that if the necessary legal authority becomes available, the A.D.E. Division and the appropriate State livestock sanitary officials develop acceptable procedures for prompt handling of such livestock.

(7) The question of permitting some auction markets to be specifically approved for the handling of livestock, under certain provisions of the Federal regulations, in a manner somewhat similar to that set up for specific approval under the Federal brucellosis regulations, was discussed. It is recommended that the A.D.E. Division review the need for such approval under the recent scabies regulation and the proposed hog cholera regulation.
PROPAGATION AND ATTENUATION OF HOG CHOLERA
VIRUS IN TISSUE CULTURE

JAMES H. GILLESPIE, * BEN E. SHEFFY, LEROY COGGINS,
STEWART H. MADIN† AND JAMES A. BAKER

Ithaca, New York

A strain of hog cholera (HC) virus, designated persistent strain A (1),
has been found to produce cytopathic effects in tissue-cultured pig kidney
cells (2). Additional information is presented here concerning propagation
of this cytopathogenic strain in tissue culture and its modification in virulence
for pigs after continued serial passage.

METHODS

Tissue-cultured cell types. Primary monolayer test tube cultures were
prepared from kidneys of disease-free pigs that were less than 10 weeks of
age and from kidneys of pig fetuses. One or two secondary cell transfers
were made from fetal kidney cells. Primary monolayer cultures of skin
and muscle from pig fetuses also were prepared. In addition, primary cultures
of embryonic bovine kidney cells and of dog kidney cells were used.

Many stable cell lines were used that had been derived by cloning from
pig kidney cells and maintained at the Naval Biological Laboratory, University
of California, Berkeley, California. Included in this group were cell clones
33, 36, 39, 54, 61, 62, and 64. Stable cell lines PK-H 13, a pig kidney cell
strain, Madin's bovine kidney cell and Lu7, a hamster lung cell strain, also
were used.

Media. Growth of primary cell cultures from young pigs was initiated
with Earle's balanced salt solution (80 percent), 0.5 percent lactalbumin
hydrolysate (10 percent), bovine serum (10 percent), penicillin (500 units
per ml) and streptomycin (100 mg per ml).

At the time of virus inoculation, the tubes were washed with buffered salt
solution; for cell maintenance a medium was used consisting of Parker 199
(86 percent), lamb serum (four percent), 0.5 percent lactalbumin hydro-
lysate (10 percent) plus antibiotics.

* Part of this investigation was performed at the Naval Biological Laboratory, School
of Public Health, University of California, Berkeley, California during the tenure of a
Special Fellowship from the National Institute of Allergy and Infectious Diseases, United
States Public Health Service.

† Naval Biological Laboratory, School of Public Health, University of California, Berkeley, California.
Growth of the other cell cultures was initiated with Hanks' balanced salt solution (80 percent), 0.5 percent lactalbumin hydrolysate (10 percent), and fetal calf serum, calf serum or lamb serum (10 percent) plus antibiotics. Just prior to virus inoculation, tubes were washed two times with a maintenance medium consisting of H-M medium (3) (90 percent), fetal calf serum (10 percent) and antibiotics. When fetal calf serum was used in the growth medium, washing of cells was not necessary.

**Titration of virus.** Tenfold dilutions of tissue culture fluid from infected tubes were made in maintenance medium. Each of five tubes that contained tissue-cultured pig cells, either of fetal or immature pig origin was inoculated with 0.1 ml of a virus dilution. Comparative titrations were made in pigs. Endpoints for tissue-cultured virus were calculated by the method of Reed and Muench.

**Tests in pigs.** All pigs used in these tests were obtained from a disease-free swine herd maintained by Cornell's Virus Institute. Experimental pigs were placed in isolation units before inoculation. Each test pig was given one ml intramuscularly of undiluted tissue-cultured HC virus from the 4th, 8th, 16th, 29th, 40th, 46th, 50th and 72nd transfers. Uninoculated pigs were placed in contact with those given the 46th, 50th and 72nd transfers. Observations and temperatures of each pig were recorded daily. Any pig that died was autopsied and examined for lesions.

Each pig was bled at the time of inoculation or when exposure began and again 21-28 days later, if it survived.

**TABLE 1**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Designation</th>
<th>Cytopathic Effects*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary pig fetal kidney</td>
<td>None</td>
<td>+1</td>
</tr>
<tr>
<td>Primary pig fetal skin-muscle</td>
<td>None</td>
<td>+</td>
</tr>
<tr>
<td>Primary pig immature kidney</td>
<td>None</td>
<td>+2</td>
</tr>
<tr>
<td>Primary bovine fetal kidney</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Primary dog immature kidney</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Stable pig mature kidney</td>
<td>PK36, PK39 and PK54 clones</td>
<td>±</td>
</tr>
<tr>
<td>Stable pig mature kidney</td>
<td>PK33, PK61, PK62 and 64 clones</td>
<td>—</td>
</tr>
<tr>
<td>Stable pig mature kidney</td>
<td>PK-H13</td>
<td>—</td>
</tr>
<tr>
<td>Stable bovine calf kidney</td>
<td>Madin's</td>
<td>—</td>
</tr>
<tr>
<td>Stable hamster adult lung</td>
<td>LU7</td>
<td>—</td>
</tr>
</tbody>
</table>

* + means CPE regularly produced; ± means not always produced; — means CPE never observed.
1, titer 3.5—4.5 per ml; 2, titer 2.5—3.5 per ml.

In addition, antiserums were obtained from pigs vaccinated with rabbit origin vaccine (kindly furnished by Dr. Victor Cabasso of Lederle Labora-
constitutions and Dr. Charles York of Pitman-Moore Co.) and by the simultaneous
method of virulent virus and antiserum (kindly furnished by Dr. Jack Ray of
Affiliated Laboratories). Pigs were bled before vaccination and 21 days
afterwards. Neutralization tests of all collected serums were made (4). Each
pig that survived tissue-cultured virus was given one ml intramuscularly of
a 10 percent suspension of spleen from a pig infected with virulent strain A
virus 21 or 28 days after initial inoculation. All vaccinated pigs were reported
to be immune when tested for immunity.

RESULTS

Tissue culture effects. As shown in Table 1, persistent strain A HC virus
produced cytopathic effects in monolayer cultures of fetal pig kidney cells,
in pig kidney cells from immature pigs and in fetal pig skin-muscle cells.
Cell changes were seen on the second or third day and either marked or
complete degeneration had occurred by the fifth or sixth day. The cloned
stable cell lines 36, 39 and 54 that had been derived from a pig kidney showed
cytopathic effects in some experiments, but in two trials no effect was seen
in cloned stable cell lines 33, 61, 62 and 64. No cytopathic effects were
observed in primary monolayer cultures of dog kidney cells, in fetal bovine
kidney cells or in stable cell monolayer cultures of bovine kidney cells and in
hamster lung cells.

Titration in tissue culture. HC virus from the 27th passage was titrated
in kidney cells from immature pigs maintained in medium containing Parker
199, 0.5 percent lactalbumin, four percent lamb serum and antibiotics. End-
points varied from CPID\textsubscript{50} 10^{2.5} per ml to CPID\textsubscript{50} 10^{3.5} per ml. Eight

![Graph](image)

**Figure 1.** Response of pigs to various passage levels of tissue-cultured hog cholera virus.
different passages of cytopathic HC virus beyond the 40th transfer were
titrated in fetal pig kidney cells bathed with H-M medium + 10 percent
fetal calf serum. Endpoints varied from CPID_{50} 10^{3.5} per ml to CPID_{50} 10^{4.5}
per ml, with an average of CPID_{50} 10^{3.8} per ml. Cytopathogenicity in tissue
culture showed correlation with infectivity for pigs. Inoculation of pigs
showed virus to be present within tenfold dilution indicated by tissue culture.

*Pig response.* Results are summarized in figure 1. Pigs inoculated with
the 4th, 8th, 16th and 29th transfers of tissue-culture virus showed signs
of illness typical of hog cholera, followed by death nine to 13 days after
inoculation. Autopsy revealed lesions characteristic of hog cholera.

The pig given 40th passage virus showed an elevated temperature two days
after inoculation that lasted for four days. No other signs of illness were
observed. The two pigs given the 46th passage virus each had an elevated
temperature for three days that began upon the second day after inoculation.
Other signs of illness were not observed. One contact pig showed a tempera-
ture rise 12 days after exposure for two days, while the other showed a
rise after 15 days for two days. Of two pigs inoculated with the 50th passage,
only one had a temperature elevation for two days that began eight days
after inoculation. One contact pig showed a slight temperature rise, while
the other showed none. Two pigs given the 72nd passage had a rise in
temperature of 1\degree F for one day on the fifth day after inoculation. No other
signs of illness were observed. Contact pigs remained normal.

Serums from all pigs that recovered after inoculation with tissue-cultured
virus neutralized virus while serum obtained at the time of inoculation did
not neutralize. Only those contact pigs that showed elevated temperatures
developed neutralizing antibodies. Pigs that had neutralizing antibodies
showed no signs of illness when given virulent virus; those without neutral-
izing antibodies died. Serums from pigs given rabbit-adapted virus, as
well as those inoculated with virulent virus and serum, neutralized after
vaccination, but they did not do so at the time the vaccines were given.

**DISCUSSION**

The conclusion can be made that persistent strain A HC virus grows in
tissue culture because inoculums from early passages of this virus killed pigs
with signs and lesions characteristic of hog cholera. Inoculums from sub-
sequent passages did not kill pigs, but immunized them against virulent
hog cholera virus. Because others have grown hog cholera virus in tissue
culture without observing similar cytopathic effects (5-16), however, there
was some doubt as to whether these cytopathic effects that had been noted
with persistent strain A were specific for hog cholera virus. The following
information indicates that a cytopathogenic HC strain of virus has been found:

1. Bacteria and PPLOs have not been isolated from infective cultures.
2. Titers in cell cultures and in pigs showed good correlation.
3. Cytopathic effects were neutralized by serums from pigs after immuniza-
tion against hog cholera but not before immunization. This finding
seems conclusive, especially in view of the diversity of serums that neutralized, which may briefly be summarized as follows:

a. Serums of pigs immunized with rabbit-adapted HC virus neutralized.

b. Serums from pigs that were immunized by simultaneous inoculation of virulent HC virus and antiserum neutralized.

c. Susceptible pigs that became immune to virulent HC virus after exposure to pigs given either the 40th, 46th or 50th serial passage of cytopathogenic HC virus developed neutralizing antibodies, whereas serums from exposed pigs that did not become immune failed to neutralize.

After 40 passages, modification of virus occurred, and it changed from a killer to a non-killer of pigs, although virus still could spread from inoculated pigs to others exposed by contact. After 70 serial passages had been made, however, the virus no longer spread and no signs of illness were observed other than a slight increase in temperature. As yet, the total numbers of pigs that have been tested are insufficient to recommend this virus for a vaccine, but its promise as an immunizing agent has led to field tests of safety, efficacy, and spread of virus according to figure 2 (17).

If this modified cytopathogenic HC virus meets all of the standards outlined above, it should be an improvement over the present modified virus vaccine because:

1. Virus content can be standardized for presence of immunizing virus, because it can be titrated in tissue cultures.

2. Efficacy of vaccine readily can be determined without the use of virulent virus as required in present pig tests, providing the neutralization test continues to prove an accurate indicator of immunity (18, 19).
A strain of hog cholera virus has been procured which produces a cytopathic effect in monolayer cultures of pig kidney cells and in embryonic pig skin-muscle cells. After sufficient serial transfer, this virus lost virulence, produced only a slight elevation in temperature and did not spread from inoculated to exposed pigs. Virus content can be titrated in tissue-cultures and cytopathic effects can be neutralized by hog cholera antiserum. These findings may lead to a vaccine that will have the desirable attributes of uniformity and easy standardization.

ACKNOWLEDGMENT

We are indebted to Norman Darby, Naval Biological Laboratory, School of Public Health, University of California, Berkeley, California, for invaluable technical services in preparation of tissue cultures.

REFERENCES


The National Animal Disease Laboratory at Ames, Iowa, has been designed to perform two basic functions. They are: (1) basic and applied research on the principal infectious animal diseases of the United States, and (2) laboratory services required by the regulatory divisions of the Agricultural Research Service, charged with animal disease prevention, control and eradication responsibility.

The director of the laboratory is Dr. William A. Hagan. Doctor Hagan served with distinction for many years as Dean of the New York State Veterinary College at Cornell University. Doctor Hagan has the responsibility for the total direction of the laboratory within the Animal Disease and Parasite Research Division. Personnel of the regulatory laboratories are administratively responsible to Doctor Hagan, but program direction for the regulatory activities rests with the directors of the Animal Disease Eradication and Animal Inspection and Quarantine Divisions.

Of the total space in the laboratory, approximately four-fifths will be utilized for research and one-fifth for the regulatory activities. Space available in the main laboratory building for regulatory activities consists of seven modules which are approximately 48 feet x 53 feet each and three wings of building No. three, one of the animal isolation buildings. In addition to this laboratory space, the regulatory laboratories are provided with common services including media preparation, laboratory glassware washing, laundry, animal procurement and administrative services, from the common source provided by the National Animal Disease Laboratory.

Specifically, the regulatory laboratories will: (1) provide animal disease diagnostic and epidemiological services, (2) produce, evaluate, standardize and improve diagnostics for animal diseases, and (3) provide the laboratory services required by the Animal Inspection and Quarantine Division in discharging its veterinary biologics licensing and inspection responsibility.

The basic objectives of the biological products activity at the National Animal Disease Laboratory are to provide information and service to the Animal Inspection and Quarantine Division in connection with its responsibilities under the Virus Serum and Toxin Act. Basically, this act provides authority to prohibit the interstate shipment of veterinary biologics which are worthless, dangerous, harmful or contaminated. The importance of this phase of animal disease prevention is readily accepted as being of great economic significance.

The Agricultural Research Service, and more specifically the Animal Inspection and Quarantine Division, has recognized the need for laboratory
facilities to augment and support the program of veterinary biologics licensing and inspection for at least 10 years. The advisory Committee of this Association, now known as the Committee on Federal Programs and Policy, has urged and actively supported the establishment of laboratory services for the Animal Inspection and Quarantine Division. The American Veterinary Medical Association has also supported providing the Animal Inspection and Quarantine Division with laboratory facilities and staff. Organizations representing the commercial producers of veterinary biologics have also recommended and supported the establishment of such laboratory facilities. The facilities, equipment and personnel which have been assembled for this laboratory service will provide the Animal Inspection and Quarantine Division with program services that have long been needed. No one should think, however, that all of the problems relating to veterinary biologics, which need study in laboratories, can be undertaken at once, or even in the near future.

The specific programs of work, or objectives, for the biologics laboratory at N.A.D.L. come under two major headings. One is to assay commercially produced veterinary biologics and determine their potency, safety and purity. The second is to review and evaluate existing standards and requirements, to develop new standards and requirements where they are needed, and to make improvements in those that can be improved. In both of these areas, the laboratory will recommend to the division actions or decisions based upon the findings of the laboratory work.

In regard to the evaluation and development of standards and requirements, consideration will have to be given to the following questions: (1) Do the results of the current assay procedures establish the ability and methodology so that different laboratories can obtain evaluations which are the same and to what degree can reproducible results be expected? (2) Are the methods currently being used for measuring the factor or factors, which are responsible for producing safe and potent products, valid under various conditions of product use and for the animal species for which the product is recommended? (3) Are the products maintaining satisfactory levels of potency, safety and purity until the end of the expiration date?

We have organized biologics control activities at N.A.D.L. around a classification or grouping for veterinary biologics. Under this classification, we have four principal biological units, two for large animal biologics, one for small animal biologics and one for poultry. Investigations of new biologics and studies outlined above will be assigned to the applicable unit. In addition to these four units, there is also a service unit which will care for and handle the animals and samples for the other units.

Dr. George O. Johnson occupies the position of Chief Veterinarian of Biologics Control. Doctor Johnson has the responsibility for laboratory program establishment and execution, as well as organization. He also has the responsibility to recruit personnel and to develop them for positions within the laboratory, and the training of field personnel in laboratory
methods. He is responsible for the acquisition and assignment of equipment and space in the laboratory.

Doctor Johnson is a native of Nebraska and received his primary education there and at the University of Nebraska. After graduation from Colorado State University in 1948, his first Federal assignment was in the foot-and-mouth disease vaccine production laboratory in Mexico City. Since 1949, he has held various positions with the Biologics Licensing and Inspection Agency—first in Omaha, then in Berkeley, California, and most recently in Washington, D. C.

Dr. Charles E. Phillips is the principal veterinarian in charge of the large animal product unit for viral and aerobic biologics. Products assigned to this unit include those used in the prevention and control of swine erysipelas, brucellosis, leptospirosis and infectious bovine rhinotracheitis. Doctor Phillips graduated from Ontario Veterinary College in 1940 and was employed on the staff there and in the biologics industry until 1958. His first Federal assignment was as a biologics inspector at the Omaha station. In 1960 he transferred to East Lansing, Michigan, where he conducted a veterinary biological products test evaluation and development program at Michigan State University.

Dr. Merlin E. Macheak is the veterinarian responsible for the large animal product unit concerned with the spore-forming bacteria and biologics used for diseases caused by this group of organisms. Doctor Macheak was educated in Iowa and graduated from Iowa State University in 1950. Since graduation, Doctor Macheak has held assignments in biologics inspection at Fort Dodge, Iowa, and Kansas City, Kansas. For two years previous to his Ames assignment, he conducted investigational studies of anaerobes and anaerobic biologics at Montana State College. He received the Master of Science degree there this year. His thesis title was “The Combination of Clostridium Perfringens Epsilon Antitoxin with Toxin and Toxoid.”

The veterinarian in charge of the product unit for small animal biologics is Dr. Donald Croghan. Products for which this unit is responsible are those used to prevent rabies, canine and feline distemper, canine hepatitis and leptospirosis. Doctor Croghan is a graduate of Kansas State University in 1949. He has been employed by the Biologics Licensing and Inspection Agency since 1951 at the St. Joseph, Missouri, field station and at East Lansing, Michigan. During his assignment at Michigan State University, East Lansing, he conducted a veterinary biological products test evaluation and development program. The title of his thesis for the Master of Science degree was “Some Studies of Infectious Laryngotracheitis Virus Cultivated in Chicken Embryo Liver Cells.”

Responsibility for the product unit concerned with poultry biologics is assigned to Dr. Dale Oshel. Doctor Oshel is a native of Kansas and received his veterinary degree at Kansas State University in 1951. Doctor Oshel entered the Federal service through the veterinary training program. His service with the A.I.Q. Division includes assignments in biologics inspection at field stations in Kansas City, Kansas, and Pearl River, New York. His
assignment to Michigan State University in 1958 was for the purpose of conducting investigational studies of methods for the examination of poultry biologics. The title of his thesis for the Master of Science degree there was "A Study of Some Variables in the Neutralization Test as used for Potency Testing Commercially Available, Infectious Bronchitis Vaccines."

The service unit for animals and samples is an important part of the total operation. The responsibility for the work of this unit is assigned to Dr. V. H. Clark and includes operation of the samples repository and management of all animals used for investigations and for assay of veterinary biologics. The purpose of the repository is to provide immediately available samples of all commercially produced veterinary biologics in order that a wide variety of materials may be available either for immediate assay or studies in standards development. Another purpose is to have samples in reserve in the event field problems develop in connection with certain products. Doctor Clark is a native of Kansas and a graduate of Kansas State University in 1932. He has been in Federal service since 1934. His service with the A.I.Q. Division includes assignments in biologics inspection at field stations in Nebraska since 1941.

Priorities have been established for undertaking work in the laboratory on the various products. Factors considered in establishing these priorities were: (1) the reports of field problems or other information which would indicate problems with a particular product, and (2) the economic and public health significance of the products. Our immediate plans are to give attention to the products which have the highest priority rating based upon these factors. Our first effort in regard to the products will be a survey of their potency and the adequacy of the standard requirements. When standard requirements do not seem to be adequate, priority will be given to the development of other procedures which possess potential as improved methods for establishing standards for products in regard to safety, purity and potency. Work is now underway in all product units. The samples repository was the first unit to be activated and the stock of material in it is growing rapidly.

Products which are scheduled for assay and on which actual assay is now underway include infectious bovine rhinotracheitis vaccine, erysipelas bacterin, clostridium hemolyticum bacterin, rabies vaccines and infectious bronchitis vaccine. Much of the work currently being done consists of production and standardization of cultures and viruses to be used as reagents in assay procedures. Isolation units to be used for small animals, including laboratory animals, and poultry are in the process of development. We are attempting to develop out of plexiglass what has been referred to as modified Horsfall-Bauer units. If we are successful in the development of these units, they will provide isolation facilities for potency assay involving the use of aerosol viruses, as well as other animal inoculations. At the present time, however, this type of assay must be based on laboratory procedure. We are hopeful, however, that we will have our isolation units installed and operating in the very near future.
In conclusion then, the objectives of biologics control are to develop, evaluate, improve and apply methods that can be used to insure the safety and efficacy of veterinary biologics. The organization consists of four product units—one for each major class of biologics and a service unit. The work program is based on priorities which have been determined. Work on specific products is now underway. The personnel of the laboratory are dedicated to the job of preventing losses from animal diseases and aiding the industry in producing the best veterinary biologics possible.
REPORT OF THE COMMITTEE ON BIOLOGICS AND PHARMACEUTICALS


We have attempted to keep in touch with information pertaining to field use and evaluation of biological and pharmaceutical products. We have summarized some of this information to inform you of matters that we think will be of definite interest to this group.

Hog Cholera

We are all aware of the great need for safe and highly immunogenic vaccines for prevention of hog cholera. We must keep this disease under control and it can only be accomplished by public confidence in safe immunizing agents to an extent that they will be used extensively to induce a high degree of resistance in as high a percentage of our swine population as possible.

Last year it was reported to this Association by Doctor Zinober that certain modified live hog cholera vaccines used in the pilot hog cholera eradication program in Florida did not induce adequate protection in some pigs for the normal feeding period. On investigation of these failures to protect, it was found in most cases that lack of protection appeared to be associated with the use of aged vaccine even though all the vaccine was used within the labeled expiration date.

This observation indicated need for thorough study of this problem, even though widespread use of all three types of modified virus vaccines were performing very satisfactorily under less regulated and less critically evaluated field conditions.

Prompt and thorough joint action was taken by the Agricultural Research Service and a Committee of the Veterinary Biological Licensees Association representing the producers of hog cholera vaccine. The first step agreed on was a statistical study of the Florida data in minute detail. This study indicated that most failures to protect to market age were encountered in aged vaccine of one type which was used within the labeled expiration date of the product. It was most apparent though that immunizing deficiencies were encountered more dramatically in some individual lots of vaccine than in others of the same type and in general these potency deficiencies were most common in one type of modified vaccine.

Therefore, this study seemed to indicate the problem to be one of stability and indicated the need for changing the testing procedures prior to the
release of vaccine for market. The test needs to be increased in severity to the point of indicating substantial surpluses of virus well beyond a possible minimum dose necessary to immunize and, therefore, to allow for some loss of virus in shipment and storage so the vaccine could continue to induce a protective immunity when used at any time during the labeled dating period. In thorough discussion of this problem by the Agricultural Research Service and a Committee of the Veterinary Biological Licensees Association a mutually acceptable plan for prompt evaluation of the stability of each producer's product was instituted.

This program for critical re-evaluation of the vaccine was aimed first of all at determining that the expiration date used by each manufacturer was in line with the stability of the product produced by that manufacturer. To do this, it was agreed that each manufacturer would run tests on vaccine at its expiration date or within one month of that date and the test would be run on each of five serial lots using \( \frac{1}{10} \) of the recommended field dose to determine whether or not this dose at expiration would immunize pigs. It was generally agreed that this should definitely indicate whether or not the product was being marketed with an expiration date that was properly supported by evidence of the stability of the product.

The study also indicated a fault in the official potency test as routinely applied on each serial lot before it was marketed, namely, the required test which has been in use allowed for the test animals to receive a full field dose of vaccine. It was decided that the test on freshly prepared dried vaccine should be applied using a small fraction of the field dose as the test dose. If a small fraction of the field dose was found to be immunogenic this would give valid evidence that a substantial surplus of live virus was available in the product to allow for the normal losses of live virus that occur during storage for any labeled market period when handled as recommended. The fraction of a field dose which must be used by each manufacturer was not immediately determined but should be based on the stability information developed by each manufacturer for his product since all the vaccines are not of the same type and production methods vary between manufacturers of the same type in some details which conceivably could influence the rate of virus loss on storage. This program is in process and each manufacturer must establish a critical test dose for his product to insure an adequate dose of virus to induce a protective immunity throughout the dating period. Some manufacturers are already testing at a very critical level to determine that these substantial surpluses of virus in their products are sufficient to label them with the expiration date in use.

We feel this problem of more critical testing for hog cholera vaccine potency for the labeled date is under control and you can have confidence in it.

A new approach to hog cholera vaccine evaluation has been suggested by Baker and Robson. This is being discussed in detail on this program. We feel this testing for response to vaccine by measuring virus neutralizing antibody induced may be an important advance. However, at this time we
cannot suggest it be a substitute for direct challenge of vaccinated animals in testing hog cholera vaccine.

We want to again remind this Association that hog cholera vaccine, like any other biological product must be used within certain limitations to properly immunize pigs. One of the limitations of all of the hog cholera modified live virus vaccines is that they do not induce a good immunity if the pigs have recently been passively protected by a dose of anti-hog cholera serum. This problem was discussed in detail by Dr. H. W. Dunne last year and the experience of others was reviewed at that time. Your attention was also directed to this problem by Dr. C. L. Campbell in his report last year. There is no question but what the immune response to vaccination with modified vaccines is severely reduced if vaccination is attempted before the passive immunity induced by a previous dose of serum has expired or nearly expired.

We do not have up-to-date information on the latest regulations in all of the States regarding the immunization of pigs to qualify them for movement through community sales, or Federal regulations for movement of hogs interstate. However, unless your individual State regulations require vaccination of pigs with both serum and vaccine at the same time rather than serum alone, the value of later vaccination may be questionable. If serum alone is permitted then regulations should require that a certificate accompany the pigs emphasizing the fact that serum alone was used and contain a warning to the purchaser indicating that hog cholera protection is only temporary. For durable protection modified vaccines cannot be expected to properly immunize the animals unless vaccination is delayed for approximately two weeks from date of serum injection. This alternate is a poor one as this would be a difficult regulation to administer but if serum alone is used this would serve to bring this matter to the attention of the purchaser by this means as well as by the information provided in directions accompanying the vaccine.

Perhaps you have brought your house in order here but if not, we want to emphasize that you men with regulatory responsibilities have a stake in the proper use of these vaccines and you can do something about it.

**Infectious Bovine Rhinotracheitis Vaccine**

The use of Infectious Bovine Rhinotracheitis Vaccine has been rather spectacularly successful in reducing the incidence of this disease in feedlot animals. This apparently has created interest in attempting to immunize pastured animals where the disease has been diagnosed, suspected, or empirically to attempt control of respiratory infections without a diagnosis. Whether or not vaccination of pastured animals was necessary we don't know but in any case a substantial number of them have been vaccinated and many of them have been pregnant females. Then, when a rather high incidence of abortions began to occur in cows and heifers which had previously been injected with Infectious Bovine Rhinotracheitis Vaccine, the vaccination was suspected as the cause. We have no positive proof that the
vaccine actually did cause abortion but since some virus infections of the pregnant female do adversely affect the developing fetus, the vaccine was incriminated. Therefore, in view of a reasonable doubt, producers of the vaccine were requested to include a warning in their directions accompanying the product against the vaccination of pregnant females.

**Ovine Vibriosis**

Last year a limited license was issued for the production of a vibriosis vaccine to attempt prevention of this cause of abortion in sheep. The license was limited to the calendar year for critical field evaluation of the product. Information developed from extensive trials in our western States was not sufficiently conclusive to warrant a regular license but a limited license was again granted for further evaluation.

In the meantime, information has been published by Miller of Colorado on an improved vibriosis vaccine which utilizes an adjuvant to enhance the immunizing stimulus. This was an extension of the basic work in development of vibriosis immunizing agent at Colorado A. & M. Vibriosis is an important cause of abortion in sheep and there is a definite need for an immunizing agent that will be effective in the prevention of this disease.

**Bovine Virus Diarrhea**

Considerable information has been developed by investigators in working with this disease indicating that the incidence of the disease is widespread. Its clinical appearance is rare compared to its actual incidence which is indicated by the high percentage of animals carrying neutralizing antibody that must have suffered silent infection. It has been shown by York and others that antibody can be induced by vaccination with virus modified in two ways and the immunity induced while not perfect did limit the incidence of the infection significantly.

**Virus Diarrhea Relationship to Hog Cholera**

As reported on this program the virus causing virus diarrhea of cattle, or mucosal disease, has been shown to induce resistance to virulent hog cholera exposure. The relationship between these two diseases is of great interest both academically and practically. The use of this virus as an immunizing agent for reducing incidence of hog cholera has been suggested with the advantage that it could be used in a program for hog cholera eradication without fear of reversion to a virulent state as is feared of the modified hog cholera vaccines.

It is possible that this potential can be realized and modified hog cholera virus vaccines may become obsolete in favor of this virus in prevention of hog cholera. Perhaps we should say probably this will happen. However, before we are in a position to get over-enthusiastic about this, much information will be required to exhaustively evaluate this type of immunizing agent before we scrap our modified hog cholera vaccines presently in use as they too are capable of safely and effectively immunizing pigs against hog cholera.
RECOMMENDATION FOR COMMITTEE RE-ORGANIZATION

Some members of the Committee on Biologicals and Pharmaceuticals, for the past few years, have felt a need for revamping the structure of this Committee to more properly inform this Association. For many years now, the general trend of Committee activity has been to summarize information on new biological products or new pharmaceutical products so that current information as to their effectiveness in disease treatment and prevention could be presented to you and represent the experienced evaluation of many qualified people.

The Committee has also attempted to inform the membership of any information developed from current experience pertaining to re-evaluation of biologicals or pharmaceuticals that may have been available for a varying period of years. It is often true that after continued use of a vaccine or drug the effectiveness or safety of the product may appear better or worse than when first made available. The problem of summarizing this information has in recent years become more complex. There have been opinions expressed, and I share them, that the pharmaceutical products have been neglected by this Committee. This is largely due to the fact that the Committee has usually been predominantly made-up of members more qualified in the biological than in the pharmaceutical field. This is natural since traditionally the interests of this Association have been in biological products for the prevention of disease. However, in recent years it is certainly true that pharmaceutical products have become of much more interest since they are being applied on a herd or flock basis in the prevention of disease and find application in many disease management areas where the biological approach is impractical or less effective. Therefore, pharmaceuticals have become of more interest to those of you with regulatory responsibilities in the control of animal disease, and you are entitled to the best information you can get on these products through your Association. Your Committee feels, therefore, in this situation that attention can be more properly focused on biologicals and pharmaceuticals by having two Committees, one to deal with each group of products and each Committee can be made-up of specialists in those two fields. We would like to make this recommendation to the Executive Committee for consideration and in doing so, I should mention that this is not a unanimous opinion of the Committee. One of us is opposed to dividing it.
STANDARDIZATION OF QUANTITATIVE SEROLOGICAL TESTS*

D. S. Robson, B. P. Hildreth, G. F. Atkinson, L. E. Carmichael, F. D. Barnes, B. Pakkala, and J. A. Baker

Veterinary Virus Research Institute and Biometrics Unit, Department of Plant Breeding, Cornell University, Ithaca, New York.

The continuing development of quantitative serological tests and their increasing importance as a tool in veterinary research and the commercial production of veterinary products necessitates a further standardization of these techniques to ensure an acceptable level of reliability. All existing serum neutralization tests must differ inherently in reliability due to natural differences in the cytopathic effect of the agents involved; standardization to a common level of accuracy, therefore, requires differential modifications of the individual tests. To accomplish this we propose the following procedure.

Dilution levels. Once a standard laboratory technique has been established, a control (immune) serum is tested at not more than twofold dilutions with no less than 15 tests per dilution. The purpose of this experiment is to determine the sensitivity of the test as measured by the range of dilutions between a zero and a 100 percent response to inoculation. If $10^{-A}$ is the greatest serum dilution at which no infection occurs and $10^{-B}$ is the lowest serum dilution giving 100 percent infection then the range $R=B-A$ directly reflects the inherent variation of tolerance to the virus dosage. For the purpose of standardization, this range $R$ is used to determine the dilution rate; in subsequent applications of the test the dilution rate should not exceed $10^{R+0.5}$ and should, preferably, be approximately equal to $10^{R}$. If, for example, $R=0.5$ then the dilution rate should not exceed tenfold and should, preferably, be threefold. The mathematical properties of procedures for computing the 50 percent end point produce biased results when the dilution rate exceeds $10^{R}$, a dilution rate of $10^{10}>10^{R}$ will result in positive and negative biases as large as $(D-R)/2$ in the log ID$_{50}$.

Number per dilution. When a dilution rate of $10^{D}$ has been established in the above manner the reliability of the test is then governed by the number of inoculations made per serum dilution. The number of tests per dilution required for assurance that errors in the log titer will not exceed an amount $E(E>(D-R)/2)$ is given approximately by $D^2/E^2$ when $D$ is close to $R$, and otherwise by $4D^2/(2E-D+R)^2$. For example, using tenfold dilutions ($D=1$) when $R=0.5$ would require 16 tests per dilution in order to assure that the resulting titer will not be in error by more than threefold ($E=0.5$):

$$
\frac{4(1)^2}{[2(0.5)-1+0.5]^2} = 16
$$

* Supported by a grant-in-aid from the American Kennel Club.
Computation of log ID$_{50}$. The above formulation of accuracy requirements is based upon the Spearman-Kärber method of estimating the log ID$_{50}$ and assumes that the serum dilutions used cover the range from zero to 100 percent response. For a dilution series $10^x, 10^{D+x}, 10^{2D+x}, 10^{3D+x}, \ldots, 10^{(n-1)D+x}, 10^{nD+x}$ in which the lowest dilution ($10^x$) completely neutralizes the virus and the highest dilution ($10^{nD+x}$) produces 100 percent infection, the Spearman-Kärber formula gives

$$\log_{10} ID_{50} = x + D \left( n \frac{1}{2} - (p_0 - p_1 - p_2 - p_3 - \ldots - p_{n-1} - p_n) \right)$$

where $p_0, p_1, p_2, p_3, \ldots, p_{n-1}, p_n$ are the proportions infected at the corresponding dilutions, with $p_0 = 0$ and $p_n = 1$. The general procedure is outlined in Table I and illustrated with a numerical example in Table II, where $x = 2, D = 1, n = 4,$ and 10 tests were made at each serum dilution.

**The distemper test in eggs.** An extensive test on a control distemper serum was made at Cornell to determine the sensitivity of the neutralization test in eggs. At each serum dilution level, 20 eggs were inoculated with 0.2 ml of the serum-virus mixture containing a calculated 300 egg-infectious dose of virus. Dilution levels ranged from $10^{-2}$ to $10^{-7}$ by steps of $10^{-0.3}$ or less. The test was conducted with four replicates of five eggs per dilution, but a subsequent analysis of variance revealed no significant differences among the replicates and these were then pooled to give 20 eggs per dilution. Dead embryos reduced this number on the average, to 18 eggs per dilution.

**TABLE I**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Proportion Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^x$</td>
<td>$p_0=0$</td>
</tr>
<tr>
<td>$10^{D+x}$</td>
<td>$p_1$</td>
</tr>
<tr>
<td>$10^{2D+x}$</td>
<td>$p_2$</td>
</tr>
<tr>
<td>$10^{3D+x}$</td>
<td>$p_3$</td>
</tr>
<tr>
<td>$\vdots$</td>
<td>$\vdots$</td>
</tr>
<tr>
<td>$10^{(n-1)D+x}$</td>
<td>$p_{n-1}$</td>
</tr>
<tr>
<td>$10^{nD+x}$</td>
<td>$p_n=1$</td>
</tr>
<tr>
<td>Total</td>
<td>$T$</td>
</tr>
</tbody>
</table>

$$\log_{10} ID_{50} = x + D \left( n \frac{1}{2} - T \right)$$
TABLE II

Numerical Example of the Spearman-Kärber Method

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Proportion Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>$100 = 10^2$</td>
<td>0/10 = 0</td>
</tr>
<tr>
<td>$1000 = 10^3+2$</td>
<td>0/10 = 0</td>
</tr>
<tr>
<td>$10000 = 10^4+2$</td>
<td>5/10 = 0.5</td>
</tr>
<tr>
<td>$100000 = 10^5+2$</td>
<td>10/10 = 1.0</td>
</tr>
<tr>
<td>$1000000 = 10^6+2$</td>
<td>10/10 = 1</td>
</tr>
<tr>
<td>Total</td>
<td>2.5</td>
</tr>
</tbody>
</table>

\[ \log_{10} \text{ID}_{50} = 2 + 1 \left( 4 + 0.5 - 2.5 \right) = 4.0 \]

The results of this experiment are summarized in Figure 1 in terms of the proportion of eggs showing lesions at each serum dilution. Here there is an indication that a small fraction of eggs, on the order of five percent, are either resistant to infection or exhibit symptoms which are undetected by the technician. Because this fraction is so small, however, it does not seriously affect the calculation of a 50 percent end point and so may be disregarded.

As indicated by the straight line graph in Figure 1, the range of dilutions from zero to essentially 100 percent infection is therefore approximately $10^{-4.25}$.
to $10^{-4.75}$; that is, the change from neutralization to essentially complete infectivity takes place over approximately a threefold dilution range.

A second serum was tested in a similar manner. With this serum the results were essentially the same; the change from neutralization to essentially complete infectivity took place over approximately a fourfold dilution range.

The infectious canine hepatitis test in tissue culture. A test on a control infectious canine hepatitis serum was also conducted to determine the sensitivity of the neutralization test in tissue culture. At each serum dilution level five tissue culture tubes were each inoculated with 0.2 ml of a serum-virus mixture containing 50 to 100 TCID$_{50}$ of virus. Final serum dilution levels ranged from $10^{-2.4}$ to $10^{-5}$ by steps of $10^{-0.2}$ or less. The test was replicated three times but analysis of variance revealed no significant differences among replicates and these were pooled to give 15 tubes per dilution.

![Figure 2. Response pattern of a hepatitis serum neutralization test in tissue culture.](image)

The results of this test are summarized in Figure 2 in terms of the proportion of tubes showing lesions at each dilution. As indicated by the straight line graph in Figure 2 the range of dilutions over which infection changes from essentially zero to 100 percent is approximately $10^{-3.1}$ to $10^{-3.7}$; that is, the change from neutralization to complete infection takes place over approximately a fourfold dilution range.

Conclusions. The implication of these results is that threefold serum dilutions should be used in neutralization tests in eggs for distemper and in
tissue culture for infectious canine hepatitis in order to eliminate bias in the calculated 50 percent end point. A two-stage procedure using first tenfold dilutions and then threefold dilutions could be followed, however, to attain the desired precision with less effort. The 50 percent end point may first be determined approximately by a series of tenfold dilutions with five eggs or five tissue culture tubes per dilution and then determined more accurately from a series of five threefold dilutions centered near the approximate 50 percent end point and again using five eggs or five tissue culture tubes per dilution.

Theoretically, this two-stage procedure should result in no more than a twofold error in the final 50 percent end point. Technical errors do occur, however, and as a safeguard in such critical tests as the evaluation of a commercial antiserum the second stage of the procedure should be supplemented with a parallel test run on a reference serum of known titer.

REFERENCES

1. Technical methods in use at the Veterinary Virus Research Institute, Cornell University, Ithaca, New York, are available on request.
ANAPLASMOSIS IMMUNIZATION STUDIES

K. L. KUTTLER, D.V.M., M.S.*

Anaplasmosis is endemic in much of Nevada with incidence ranging as high as 75 percent, based on a statewide survey in which over 3,800 cattle were tested using the complement fixation test. As shown in Figure 1 the incidence diminishes in the southern portion of the State to less than five percent infection.

Recent survey work (1) on yearling steers in northern Nevada over a three-year period, has shown an extremely rapid rate of transmission after animals are turned onto range and meadowland after May. Even though 75 percent of these young animals were carriers after their second summer on range, clinical manifestations were either unnoticed or very mild. The movement of adult cattle, not previously exposed to anaplasmosis, into these areas results in the exposure of a highly susceptible host to the infection with subsequent losses. The introduction of two- or three-year old breeding bulls from anaplasmosis-free areas into range herds is often a desirable management practice, but high losses frequently occur from this practice.

In consideration of this problem the desirability of a vaccine to immunize cattle being brought into such an area becomes obvious. A vaccine which would prevent a carrier state would be ideal but one limited to reducing the severity and preventing death losses would be useful. Our vaccine studies were started with the objective of studying the feasibility of developing a material capable of inducing an immune response in susceptible adult cattle.

This paper presents results of vaccination attempts on splenectomized calves and adult cows followed by needle challenge, and of a field trial depending on natural exposure for challenge.

Materials and Methods

Vaccines have been prepared using different antigenic materials, which were incorporated with mineral oil, and an emulsifying agent to give a water in oil emulsion. The most successful antigenic base has been prepared from blood containing 40 to 60 percent anaplasma infected red blood cells (RBC) with a packed cell volume (PCV) of 20 percent or greater. The antigenic material was prepared in much the same manner as the CO₂ complement fixation antigen (2) and vaccines prepared from this base material have been designated CO₂ adjuvant vaccine. The R.B.C.s were washed four times in physiological saline (.9 percent NaCl) and then hemolyzed in 30 volumes of cold distilled water saturated with CO₂. The CO₂ was added to the water by placing dry ice in the distilled water. The precepsitate which settled out

* University of Nevada, Agricultural Experiment Station, Reno, Nevada.
Incidence of Anaplasmosis

May 1959
overnight at 5° C was collected and washed three times in cold distilled water. The precipitate was suspended in Veronal buffer pH 7.5, in the ratio of one part precipitate to three parts buffered saline. This material was mixed two minutes using a tissue homogenizer* at full speed. The resulting suspension was used as the antigenic base and then mixed with oil adjuvants in a Waring blender to form a smooth emulsion. The final vaccine consisted of the following:

### TABLE 2

*Effects of Vaccination* on Splenectomized Calves and Adult Cows When Challenged With Virulent Blood

<table>
<thead>
<tr>
<th></th>
<th>Number of Animals</th>
<th>Average Low PCV†</th>
<th>Time Interval in Days from Challenge to the Development of Low PCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated Animals</td>
<td>17</td>
<td>20.1%</td>
<td>35.9</td>
</tr>
<tr>
<td>Unvaccinated Controls</td>
<td>13</td>
<td>14.3</td>
<td>30.7</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>5.8</td>
<td>5.2</td>
</tr>
<tr>
<td>Possibility of Error</td>
<td></td>
<td>&lt;.01</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

* Table includes cattle vaccinated with 5 different vaccines. Some were better than others, but all produced a CF response.† Packed Red Cell Volume.

47.5 percent antigenic base
47.5 percent mineral oil —55/65 vis. at 100° F, Drakeol 6, Pennsylvania Refining Company, containing 2 mg. heat killed, lyophilized mycobacterium butyricum per ml. of oil.

five percent emulsiﬁying agent—(Atlas Chemical Co. Arlacel “A”)

Infectivity trials are conducted on the antigenic base prior to mixing with adjuvants. Infectivity trials consist of 20 ml. antigenic base being injected subcutaneously. This material does not produce a C.F. response in animals used to check it for infectivity. The CO₂ treated vaccines have always been noninfective at this point. The antigenic base of one vaccine (not CO₂ treated) was found infective at this point, but not infective following mixing with adjuvants. This may be accounted for by the heating which usually takes place during the mixing procedure.

A number of variations have been tried in both the composition of the antigenic base and the adjuvants, but the above is basically the formula used. This vaccine produced moderate to large swellings when injected subcutaneously. All field trials, and laboratory studies reported in this paper used the above formula except observations recorded in summary Table 2, which includes other vaccine preparations.

Four splenectomized calves were inoculated with 8 ml. CO₂ adjuvant vaccine subcutaneously. A complement fixation response developed in about

* Vir-Tis Macro Tissue Homogenizer.
10 days in all animals. Each of these vaccinated animals, along with three unvaccinated splenectomized calves, were challenged 28 days after vaccination by injecting subcutaneously 10 ml. whole blood taken from an anaplasmosis carrier. Comparisons of the average P.C.V., complement fixation response and percent red blood cells infected with anaplasma bodies were made for vaccinated and unvaccinated animals, and are recorded in figures 2, 3, and 4.

A total of 50 yearlings were injected with five ml. of CO₂ adjuvant vaccine, and along with an equal number of unvaccinated controls, turned out on range known to be in an area of high incidence and rapid transmission. Natural transmission was relied upon to produce challenge in vaccinated and unvaccinated controls. Death losses were not anticipated in these young animals as a result of anaplasmosis infection, but a marked reduction in P.C.V. has been observed (1) in yearling cattle contracting anaplasmosis under similar conditions. By statistical analysis it was hoped to determine if vaccination would reduce the severity of anaplasmosis as reflected by P.C.V. determinations or possibly prevent the occurrence of anaplasmosis. Observations of the C.F. titers were made at the time of vaccination, 15 days after vaccination, and again 101 days later, along with P.C.V. for both the vaccinated and unvaccinated animals.

A third observation was made as to the effects of vaccination on 11 cows and six splenectomized calves when artificially exposed to anaplasmosis. Controls for these experiments consisted of nine cows and six splenectomized calves. Several different types of vaccines which for the most part were minor variations of the CO₂ adjuvant vaccine, are included in these observations. Challenge usually consisted of five or 10 ml. of carrier blood injected subcutaneously. The same challenge was given both controls and vaccinated animals in every experiment.

### TABLE 1

**Summary of Field Trial in Which 50 Yearlings Were Vaccinated for Anaplasmosis**

<table>
<thead>
<tr>
<th>Controls</th>
<th>Total No. of Animals</th>
<th>Reaction to the Complement Fixation Test</th>
<th>PCV*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neg. or ≤1/6 Reaction</td>
<td>1/5</td>
</tr>
<tr>
<td>Day 1+</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Day 15</td>
<td>48</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Day 101</td>
<td>45†</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
<td>11</td>
</tr>
</tbody>
</table>

+ Animals vaccinated on day 1.
† Between day 15 and 101 the cattle were on range. The drop in numbers is due to the mechanics of handling. No known deaths occurred in the experimental groups.
Figure 2
Average Anaplasmosis Reaction in 4 Vaccinated, and 3 Unvaccinated Splenectomized Calves Following Challenge with Anaplasmosis Infected Blood

--- Unvaccinated Controls

--- Vaccinated Animals
In order to partially check the specificity of the complement fixation test in detecting circulating antibodies produced by anaplasma antigens a CO₂ adjuvant vaccine was prepared from normal red blood cells taken from a noninfected bovine. This vaccine was injected into four normal calves; two receiving five ml. subcutaneously and two receiving 10 ml. subcutaneously. Regular bleedings were taken for six weeks to see if a reaction to the anaplasmosis C.F. antigen would occur.

Results

Considering the P.C.V. as a partial indication of the severity of anaplasmosis infection the averages for four vaccinated calves and three unvaccinated calves are recorded following challenge (Figure 2). Unvaccinated control animals developed lower P.C.V.s than did the vaccinated animals. A statistical analysis of P.C.V. values after day 20 showed a highly significant difference, with values in vaccinated calves being higher. Considerably higher C.F. titers occurred among vaccinated animals after challenge than was observed among the unvaccinated controls (figure 3). Possibly the most marked difference occurred in the percent R.B.C. infected with anaplasma bodies (figure 4). Vaccinated animals showed a low level of R.B.C.s infected with anaplasma bodies beginning about day 12 and continuing to day 40. The highest average was recorded on day 24 and was four percent of the R.B.C.s showing marginal bodies. Among the unvaccinated controls the highest average level of infection occurred on day 20 and was 32 percent. Anaplasma bodies were sporadically observed throughout the 80-day observation period in the unvaccinated controls. Results of the field trial in which 50 animals were vaccinated are recorded (Table 1). Based on three years' experience in testing yearling cattle turned out on similar range we expected about a 60 percent challenge rate. Unfortunately only three of 45 controls (seven percent) showed evidence of anaplasmosis at the conclusion of the experiment. For this reason the only value of this trial was to show the uniform C.F. response in vaccinated animals. 15 days after vaccination all 50 of the vaccinated animals showed a positive C.F. test, 20 had titers of \( \frac{1}{6} \), four—\( \frac{1}{10} \), four—\( \frac{1}{20} \), thirteen—\( \frac{1}{40} \), six—\( \frac{1}{80} \), and one—\( \frac{1}{160} \). During this 15 day period the unvaccinated controls remained negative. After day 101, 28 of the 45 vaccinated animals had negative or less than \( \frac{1}{6} \) complement fixation reactions, with 13 showing residual vaccine titers and four showing complement fixation titers of \( \frac{1}{160} \) or over, indicative of anaplasmosis infection. Of the 45 controls, three developed C.F. titers of \( \frac{1}{160} \) or over indicating anaplasmosis infection. No significant difference in P.C.V. values of vaccinated and unvaccinated animals occurred.

The results of several related vaccines, producing C.F. response in vaccinated animals, are recorded in table 2. Several experimental vaccines failed to produce even C.F. response and are not included in this table. Seventeen vaccinated animals again showed lighter infections following challenge to anaplasmosis than did the unvaccinated controls. The average low P.C.V. of vaccinated animals was 20.1 percent as compared to 14.3
Figure 3
Average CF Response in 4 Vaccinated, and 3 Unvaccinated Splenectomized Calves Following Challenge with Anaplasmosis Infected Blood

- CF Response in Vaccinated Animals
- CF Response in Unvaccinated Animals

Days After Challenge
Figure 4
Average Percent Red Cells Infected with Anaplasma Bodies in 4 Vaccinated, and 3 Unvaccinated, Splenectomised Calves Following Challenge with Anaplasmosis Infected Blood

<table>
<thead>
<tr>
<th>Days After Challenge</th>
<th>Unvaccinated</th>
<th>Vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>32</td>
<td></td>
<td>40</td>
</tr>
</tbody>
</table>

Percent Infected RBC

K. L. KUTTNER
percent for the unvaccinated controls. The average time required for the low P.C.V. to occur was 35.9 days for the vaccinated animals and 30.7 days for the unvaccinated animals. The average low P.C.V. and the time required to produce the average low P.C.V. in vaccinated animals, proved to be significantly different from the unvaccinated controls.

In no instance has vaccination prevented the development of anaplasmosis following a known challenge. It has shown evidence of reducing the severity of infection.

The subcutaneous injection of CO₂ adjuvant vaccine, prepared from non-infected cells of normal cattle, failed to induce a C.F. response in four animals so treated.

Discussion

It would appear that vaccination is a feasible method of reducing the severity of the disease in highly susceptible adult animals. Present vaccines must be improved before recommendations could be made for this method of protection. A greater concentration of antigenic material, than now used with the CO₂ method, would be desirable and probably possible. Such a material would undoubtedly increase the immunizing capacity of this vaccine. Improved adjuvants may possibly increase the immunizing potential.

A vaccination program would be useful in areas such as we have in northern Nevada, where the incidence is high, and where cattle frequently run on unfenced ranges. Vaccination, because of the production of positive CF titers, would not be indicated in areas of low incidence or in areas where test and segregation or slaughter is the method of choice in control.

The reason for failure of yearlings to develop anaplasmosis in areas where infection has been readily transmitted in the past is unknown. There may be some connection with the poor range conditions this year. The area has experienced a very dry season with water and available feed being very low. It is possible this may affect the vector population.

Summary

Vaccination of anaplasmosis susceptible calves and cows has produced evidence of increased resistance to the clinical effects of the disease, but has not prevented the disease. Animals vaccinated show significantly higher packed cell volumes, longer incubation periods, and higher complement fixation titers after challenge than do unvaccinated controls. Complement fixation titers developed in practically all vaccinated animals with titers ranging from $\frac{1}{6}$ to $\frac{1}{180}$. Most complement fixation titers due to vaccination do not persist over 101 days.

LITERATURE CITED

COMMITTEE ON ANAPLASMOSIS

M. N. RIEMENSCHNEIDER, Chairman, Oklahoma City, Oklahoma; W. E. BROCK, Stillwater, Oklahoma; T. E. FRANKLIN, College Station, Texas; R. G. GARRETT, Austin, Texas; O. H. MUTH, Corvallis, Oregon; W. T. OGLESBY, Baton Rouge, Louisiana; T. O. ROBY, Silver Spring, Maryland; E. E. SAULMON, Washington, D. C.; W. L. SIPPEL, Kissimmee, Florida; F. W. WHEELER, Baton Rouge, Louisiana; E. H. WILLERS, Honolulu, Hawaii.

Anaplasmosis continues to be a major problem to the livestock industry. It has been estimated that this devastating bovine disease costs the cattleman up to $50 million dollars per year depending on the incidence of the disease, and occurs in 40 states.

Last year's committee report revealed that the production of Anaplasmosis antigen for the Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture was underway at the Texas A. & M. Experiment Station. The Anaplasmosis Research Group at Texas A. & M. College, Dr. T. E. Franklin, Project Leader reports that, "The second phase of anaplasmosis CF antigen production has recently been successfully completed by the Texas Agricultural Experiment Station and the School of Veterinary Medicine at Texas A. & M. A total of over four million test doses of antigen were produced and pooled in two separate pools, TAES 1 and TAES 2 and were delivered to the Animal Disease Eradication Division's (ADED) Anaplasmosis Laboratory at Beltsville, Maryland.

"Some of this antigen was retained by Texas A. & M. and some preliminary studies have been made to determine the feasibility of lyophilizing this antigen. Most research and other interested people agree that the final CF antigen should be a lyophilized product which will give results comparable to those obtained with the standard antigen which is maintained at dry ice temperatures."

The policies developed by the Agricultural Research Service outlining requirements and guide-lines for the use of standardized complement-fixation antigen continues to be followed. The antigen is available without cost for research studies, cooperative control programs and field trials.

The antigen continues to be available from the Animal Disease and Parasite Research Division of the Agricultural Research Service, United States Department of Agriculture for research purposes and from the Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture for diagnostic, control screening procedures, and field trials under a Memorandum of Understanding between the Cooperating Laboratory or State and the respective division of the U.S.D.A.

There are 15 cooperating laboratories that have personnel trained to perform the complement-fixation test. Thirty serologists have been trained
in the use of the Anaplasmosis CF test and another 12 to 15 will receive training during the next year.

It is recognized that the regulatory officials should and do favor the identification and control of the movements of Anaplasmosis infected cattle when such infected cattle originate in a herd under a voluntary cooperative herd agreement. Some states require a specific identification of reactors to the official CF test and quarantine procedures are invoked. This procedure is especially recommended in those states in which anaplasmosis is not presently enzootic. This only conforms to the dictates of good management and disease control.

Experimental field studies on identification and control are being conducted in Texas, Mississippi, Tennessee, Hawaii, Oklahoma, Wyoming, Louisiana, Arkansas, Nevada and other states.

Texas A. & M. College reports the following: "At the present time seven privately owned herds in different areas of the State and the Veterinary Research herd of cattle at College Station are under voluntary experimental CF testing and segregation program. Four of these herds are going through their second season under this program with no additional reactors to date. The other three herds are in their first season with no additional reactors. This experimental segregation and isolation program with periodic spraying seems to be preventing the occurrence of anaplasmosis which formerly was a serious problem in these herds. According to the cooperating livestock owners, they have encountered no serious problems in their management of this type program. A complete report will be made at a later date."

Dr. E. H. Willers, State Veterinarian of Hawaii, submits the following progress report on their anaplasmosis program: "During fiscal 60-61, no clinical cases of anaplasmosis were observed in Hawaii. The only cattle proved to be carriers of anaplasmosis were recent imports. Four detected at entry quarantine and three on the 60-90 day retest. Fourteen other cattle given positive complement-fixation test reactions and 27 giving suspicious reactions were removed from the reactor-suspect classification on the basis of subsequent retests or calf trials. This number is less than ½ percent of the total number of cattle tested.

"One dairy animal and 12 beef cattle stand on our records as reactors for lack of proof to the contrary. The dairy animal was a young native Guernsey in a herd experiencing a storm of abortions. This animal aborted 10 days after being tested and was sent to slaughter with other brucellosis reactors, thus preventing retesting or calf trial. That herd has subsequently had two complete retests without any further suspects or reactors to the anaplasmosis test. Ten of the 12 beef cattle were calves under one year of age (where we find most of our false positive reactions), the other two were a 20-month heifer and a five-year-old cow. Seven of the animals, including the two older ones, were sent to slaughter without opportunity for retest or calf trial. The other five are being held for further testing.

"Because no native animals were found as carriers of anaplasmosis during the year, we are again optimistic that eradication has been accomplished."
However, our records will not prove this until we can convince our ranchers that they must hold reactors and suspects for retesting and calf trial rather than shipping them off for immediate slaughter. We have received assurances that this will be done—thus, anticipate that our next report will be conclusive.

"Further work with cattle vaccinated with L. pomona vaccine indicates that an interference with the complement-fixation test for anaplasmosis does occur following such vaccination and that the response is more pronounced in leptospirosis infected herds than in noninfected ones."

The Anaplasmosis Committee wishes to quote from the 1960 Report of the Committee on Infectious Diseases of Cattle as this information is pertinent.

"Research progress on anaplasmosis has been slowed by the necessity for using cattle in experimental studies. Expanded efforts to cultivate the etiological agent in tissue culture or in some type of small laboratory animal would, if successful, provide means for greater research productivity on the disease. The exact nature of the etiological agent needs elucidation and further study. At present there is no laboratory method for detecting whether ticks contain the infective agent or not. Theoretically, certain species of ticks provide the agent with a suitable medium for survival and possibly propagation. However, it has not been possible to determine whether ticks are infected except by feeding them on a susceptible cow or calf. Laboratory studies using ticks could be a very profitable tool if there were means for recognizing the agent in tick tissues. Application of such a method could be used to survey the extent of the disease in naturally occurring ticks. Although ticks have been incriminated as vectors under controlled conditions, their role in the transmission in nature is more speculative than factual. Research studies to determine the true role of insects and ticks in the transmission and perpetuation of the disease are urgently needed. Investigations on methods for the control of vector species found is needed. Further studies on the extent of anaplasmosis in wildlife, such as deer, antelope, and elk, are needed in those areas where the disease is enzootic. The possibilities of such animals playing a reservoir role of the disease, with arthropods being the means of carrying infection to cattle must be more completely investigated. Research directed towards a means for making cattle resistant to the disease is also needed."

Last year the Committee cited that the characteristics of the causative agents were not well understood. Bringing the information up to date, the biological classification and characterization of the life cycle of the anaplasmata is still controversial.

There has been a renewed interest and effort by a number of research workers to further elucidate the nature of the anaplasmosis agent. Studies have suggested the presence of from one to eight subunits within the classical anaplasma body. It has been suggested that these bodies reproduce by binary fission. The significance of accessory structures associated with and apparently attached to the marginal body remains unclear. Some workers believe these structures are a portion of the organism while others consider them to be artifacts resulting from lysis of the infected red blood cell.
research groups are conducting studies to more clearly define the nature and character of the causative agent of anaplasmosis.

The possibility of a vaccine has been mentioned in other reports of this Committee and today we were fortunate in having Dr. K. L. Kuttler report his work toward the development of an immunizing agent.

There has been gradual, steady, progress in our knowledge of anaplasmosis bringing us ever closer to a goal of knowing more about the actual disease, the causative agent, and controlling it by testing and management.

Some progress has been made as to the vectors and their control and in the treatment of the disease. Progress is very evident in the interest exhibited by cattle owners in the disease and their becoming acutely aware of its inroads on the economy of the industry.

The Committee is firmly convinced that anaplasmosis is a major problem to the cattle industry and should receive all possible attention and concentrated effort.

With this in mind the Committee makes the following recommendations:

1. That adequate funds be provided to carry out the following research work.

2. Study of known vectors and possible unknown vectors as to their life cycles, and the use of insecticides, be expanded by close cooperation between animal disease workers and entomologists.

3. Intensified research be continued and expanded to determine the exact nature of the causative agent. Without this information the production of an immunizing agent, the understanding of the pathology, the successful treatment and the control of the disease are difficult if not impossible.

4. The anaplasmosis problem varies in terms of incidence, transmission and reservoirs in the different geographical regions of the country, therefore, the Committee believes there is a dire need for continued and broadened experimental studies in the various geographical areas of the country and recommends such steps to be taken. By such efforts we may expand our knowledge of the vectors and reservoirs of infection making possible the development of feasible control and eradication procedures.

5. The Committee recommends that research be continued to improve the complement-fixation test including its specificity and further investigate the possibility of a simplified test method for anaplasmosis.

6. That the Agricultural Research Service, United States Department of Agriculture, continue to make available sufficient anaplasmosis antigen to supply the cooperating laboratories, research and other experimental work.

7. That the Agricultural Research Service, United States Department of Agriculture, continue and expand their training program for serologists, that adequate trained personnel be available to all possible cooperating laboratories.

8. That continued and expanded research be conducted to develop an immunizing agent.

9. That every effort be made to develop an effective, inexpensive treatment.
10. That the Secretary of Agriculture provide a means by which properly identified reactors may move interstate for slaughter.

The Committee urges that every possible effort be made to implement these recommendations with the view of lessening the economic loss to the cattle industry brought about by this devastating complex disease. We urge the industry and all State and Federal agencies to put forth every effort to conduct numerous field trial studies in the various geographical areas with these objectives in mind.
IMMUNOGENIC RESPONSE OF CALVES VACCINATED AT DIFFERENT AGES WITH BRUCELLA ABORTUS STRAIN 19

G. Lambert, D.V.M.; T. E. Amerault, B.S.; C. A. Manthei, D.V.M.; and E. R. Goode, Jr., D.V.M.*

Soon after the discovery of Brucella abortus Strain 19, Buck (1), and Cotton et al. (2) showed advantages of its use as a vaccine for calves, rather than adults. Consequently, it was recommended that calves be vaccinated at four to eight months of age (19). Haring and Traum (7) enlarged upon and confirmed the original findings. Moreover, research has shown that most animals develop a serviceable immunity through at least five gestation periods (5) (13) (18), that vaccinal immunity is no greater in adult vaccinated than in calf vaccinated cattle, and that method and dosage have little or no influence on the ultimate immunity (14) (15).

In May, 1956, research was initiated at the National Animal Disease Laboratory, Beltsville, Maryland, to develop conclusive information about the relationship of vaccinal age to the degree of immunity induced with Brucella abortus strain 19 vaccine. The original experiment was duplicated in 1958 to insure adequate numbers of calves vaccinated at exact ages and exposed by a constant method and dosage of virulent Brucella abortus to challenge vaccinal immunity.

Since this work was initiated, Gilman and Wagner (3) have reported that they were unable to demonstrate any marked differences between the degree of immunity established in 14 calves vaccinated at four months of age and 13 calves vaccinated at eight months of age. However, their experimental design differed somewhat from the present study in the method of exposure and the dosage of virulent Brucella abortus employed. Recently King and Frank (10) have reported, also, that the resistance produced in four calves vaccinated at three months of age compared favorably with that of six calves vaccinated at six months of age and four calves vaccinated when nine months old.

MATERIALS AND METHODS

Experiment 1.

Forty-three Holstein calves were selected for the experiment, 25 of which were from the Brucella-free herd at the National Animal Disease Laboratory, Beltsville, Maryland. The remainder were purchased locally when one to

* From Bacteriological Investigations, National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.

The authors acknowledge the technical assistance of Dr. E. L. Love, Animal Disease Eradication Division, National Animal Disease Laboratory, Ames, Iowa, and Mr. R. L. Sealock, Animal Disease and Parasite Research Division, Beltsville, Maryland.
three days of age. The calves were bled at birth or day of purchase and then at monthly intervals for serologic studies. The first group of nine calves was vaccinated with five ml. of *Brucella abortus* strain 19 vaccine, as each calf attained the exact age of four months. Similarly, a second group of 11 calves was vaccinated as each attained the age of six months. The third group of 11 calves was vaccinated at exactly eight months of age. The vaccine was supplied through courtesy of the Animal Disease Eradication Division, Agricultural Research Service, Beltsville, Maryland. The fourth group of 12 calves served as nonvaccinated controls.

Results of the standard sero-agglutination tube test (STT) were negative (titer of 25 or lower) for all animals at the time of vaccination. Post-vaccinal titers were measured at semiweekly, weekly and later monthly intervals by end-titer determinations of the S.T.T. The blood was examined for postvaccinal bacteremia (17) at semiweekly intervals for eight weeks. The extent and persistence of local reactions at the site of vaccination were determined by daily measurements. In addition postvaccinal morning and evening body temperatures were recorded for 21 days. Serologic tests were conducted on the nonvaccinated cattle at monthly intervals until exposure.

Midway through their first gestations (mean gestation 140 days) both vaccinated and nonvaccinated cattle were exposed on the same day to virulent *Brucella abortus* strain 2308. Smooth colonies only were picked from a 40-hour growth on serum potato agar. The stock culture suspension containing \(10^{10}\) cells per ml. was adjusted with sterile one percent peptone broth to contain approximately \(7.5 \times 10^6\) cells per ml. Exactly 0.05 ml. of this suspension was deposited on the conjunctive of the cupped lower lids of each animal's eyes, so that each animal received 0.1 ml. of the exposure material. Each eyelid was held in position for three minutes to prevent escape of the exposure material. Plate counts of the exposure material before and after exposure revealed that the exposure dose per animal was 666,000 viable *Brucella abortus* cells. This was within the range previously reported (15) for *Brucella abortus* strain 2308, as being capable of producing infection in more than 80 percent but less than 100 percent of the controls, which permitted measurement of the degree of vaccinal immunity of the principals.

Each animal was kept in an isolated stall. Attending personnel took precautions to prevent spread of infectious material by disinfecting boots, gloves, and equipment before entering each stall. Following exposure, blood samples were collected from all animals at semiweekly intervals for four months and then weekly for two additional months. The animals were then bled at monthly intervals until the termination of the experiment. An attempt was made to correlate the serologic and clinical responses of the individuals, and of the groups.

One to 12 hours after parturition, blood, uterine contents, and quarter milk samples were collected from each animal for bacteriologic examination (17). At this time, the agglutinin titer of each animal was determined. Each aborted fetus or full term dead calf was necropsied and examined bacteri-
IMMUNOGENIC RESPONSE WITH ABORTUS STRAIN 19

ologically for the presence of Brucella abortus. Milk samples were either centrifuged or allowed to stand over night under refrigeration so that cream and sediment were separated. Samples of cream and sediment were inoculated on plates of modified tryptose medium (12) and special "W" medium (11). The inoculated plates were incubated in an atmosphere of 10 percent CO₂ at 37.5 C. for seven days. A five ml. portion of each quarter milk sample (combined cream and sediment) was inoculated intraperitoneally into each of two guinea pigs. Guinea pigs were held in isolation for 35 days, then necropsied and examined bacteriologically for the presence of Brucella abortus. A blood sample was collected at necropsy from each guinea pig for serologic studies.

Where Brucella abortus was not demonstrated in the cream or sediment at the time of parturition, a second and a third sample were collected at biweekly intervals and procedures similar to those described above were used in an attempt to demonstrate the presence of the organism.

Portions of uterine contents were diluted approximately one to four in one percent peptone broth and then 0.2 ml. was plated on modified tryptose medium or "W" medium, or both. In addition, 0.5 ml. of this suspension in peptone broth was injected subcutaneously into the flank region of each of four guinea pigs. These guinea pigs were necropsied at the end of 35 days and examined serologically and bacteriologically for the presence of Brucella abortus infection.

Proof of infection in the cattle was based upon isolation of Brucella abortus (8) from one or more of the following: milk, uterine contents, blood, fetus, or tissues obtained at necropsy (12) (17).

**Experiment 2.**

Experiment 2 was designed to be a duplicate of Experiment 1, except postvaccinal hemocultures and body temperatures were not obtained.

Forty-eight calves were selected from the Brucella-free herd at the National Animal Disease Laboratory for this experiment. Fifteen were vaccinated at four months of age; 11 were vaccinated at six months of age, and 12 were vaccinated at eight months of age. The remaining 10 calves served as nonvaccinated controls.

The vaccinal, serologic and bacteriologic procedures were identical to those used in Experiment 1. All 48 cattle were exposed in the middle of their first gestation (mean gestation 143 days) to approximately 7 x 10⁵ cells of virulent Brucella abortus strain 2308 via the conjunctiva. Plate counts showed the exposure dose was approximately 6.9 x 10⁵ virulent organisms.

**RESULTS**

In Experiment 1, there was little difference in the average temperature rise after vaccination in the four- and six-month groups (4.6 and 4.7 F. respectively). The average temperature rise was least in the eight-month group (3.3 F.). Average persistence of temperature rise was 3.4 days in all
three groups. Postvaccinal bacteremia was demonstrated in five of nine calves in the four-month group and in one of 11 calves in the six-month group. Postvaccinal bacteremia was not demonstrated in any of the 11 animals of the eight-month group.

In Experiments 1 and 2, anorexia of two-day duration occurred in one of the four-month group. No similar systemic reactions were observed in the six- and eight-month groups. Persistence of local reactions at the site of vaccination ranged from an average of 12.5 days in the eight-month group to 14.6 days in the four-month group. The average size of the local reaction ranged from 96 sq. cm. in the six-month group to 140 sq. cm. in the eight-month group.

TABLE I

<table>
<thead>
<tr>
<th>Postvaccinal Time in Weeks</th>
<th>Average Postvaccinal Sero-Agglutinin Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 mos. Vacc.</td>
</tr>
<tr>
<td>3</td>
<td>619</td>
</tr>
<tr>
<td>6</td>
<td>161</td>
</tr>
<tr>
<td>9</td>
<td>92</td>
</tr>
<tr>
<td>12</td>
<td>73</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>52</td>
<td>8</td>
</tr>
</tbody>
</table>

The relationship of the magnitude and the persistence of the postvaccinal titer to the age of the calf at the time of vaccination is shown in Table I. The average titer patterns shown here were similar to both the geometric mean and the median titer pattern of the groups. Maximum postvaccinal titers were detected 14 to 21 days after vaccination and ranged from a low of no titer (less than incomplete at 25) in one animal to a high of incomplete at 6,400. The geometric mean peak postvaccinal titers of the four-, six- and eight-month groups were 421, 1,096 and 1,030 respectively, while the median peak postvaccinal titers were 600, 1,200, and 1,050 respectively. One year after vaccination, the geometric mean titers of the four-, six- and eight-month groups were 7, 10 and 20 respectively; whereas the median titers at this time were 15, 25 and 45 respectively.

There was a direct relationship between the magnitude and the persistence of the postvaccinal titer. Calves with maximum postvaccinal titers of 400 and 800 required an average of 10 weeks for their titers to recede to a negative status (50 or lower) (4); whereas calves with maximum postvaccinal titers of 1,600 and 3,200 required 14 and 19 weeks respectively to become classified as negative. Postvaccinal titers were higher and persisted longer in the groups vaccinated at six- and eight-months of age. At the time of exposure all vaccinated and nonvaccinated cattle were classified negative by the sero-agglutination tube test.
The combined postexposure immunologic results of both experiments are shown in Table II. After exposure, a rise in titer was observed first in the vaccinated cattle. This preceded a similar rise in titer of the nonvaccinated cattle by about two weeks. The agglutinin titers of the Brucella resistant cattle (vaccinated and nonvaccinated) rose to the suspect or low reactor levels after exposure, then receded rapidly to the preexposure level. In general, the titers of the susceptible cattle rose quickly to the higher reactor levels and remained relatively high throughout the course of the experiment. In three cattle that became infected, no increase in postexposure titer was observed until the time of parturition. There was no apparent correlation between maximum postvaccinal titers and maximum postexposure titers. The animal in which a postvaccinal titer was not detected later proved to be Brucella resistant.

There was little difference among the three vaccinal age groups in the number of cattle that became infected and in the number of abortions. Similarly, when the Index of Infection (6) criteria as suggested by the Brucellosis Research Conference was applied to the results, no major group differences were demonstrable. Furthermore, statistical analysis of these results failed to show any significant differences in the degree of immunity established in each of the three vaccinal age groups. In contrast to the similarity of the results of the three vaccinated groups was the postexposure response of the nonvaccinated control cattle, where 90 percent became infected and 68 percent aborted.

**DISCUSSION**

Although differences existed in the extent and persistence of local reactions after vaccination in Experiment 1, they were unrelated to the degree of vaccinal immunity established. Similarly, the severity of postvaccinal systemic reactions, such as anorexia and temperature rise, apparently are no indication of the degree of immunity established.

Although demonstration of postvaccinal bacteremia was shown in only a few animals, there were no significant differences between the postexposure immunologic or serologic responses of these animals and those in which no postvaccinal bacteremia was demonstrated. However, it is possible that had hemocultures been made more frequently, bacteremia may have been
demonstrated in more of the animals. Kerr (9) and Manthei (16) vaccinated calves with *Brucella abortus* at 30 days of age or younger and failed to get any serologic response. It would be interesting to know if such calves would later be resistant to *Brucella abortus*, since it has been shown in our study that production of a detectable postvaccinal titer was not essential for the establishment of immunity to *Brucella abortus*. Apparently the age at which active immunity to brucellosis can be first produced in calves is still a matter for further study.

Since postvaccinal titers are lower and recede sooner in calves vaccinated at four months of age and the degree of immunity established compares favorably with that produced in calves vaccinated at older ages, it appears that the problem of persistent postvaccinal titers would be safely minimized or eliminated by vaccinating calves at the younger age.

**SUMMARY**

Twenty-four calves were vaccinated with *Brucella abortus* strain 19 vaccine at four months of age, 22 others were vaccinated at six months of age, and a third group of 23 calves was vaccinated at eight months of age.

Midway through their first gestations these vaccinated cattle and a group of 22 nonvaccinated controls were exposed to approximately $7 \times 10^5$ cells of virulent *Brucella abortus* strain 2,308 by the conjunctival route. Postvaccinal and postexposure serologic studies were correlated with the postexposure immunologic responses of the individuals and of the groups.

Postvaccinal titers were lower and receded to a negative status (50 or lower) sooner in the four-month group than in the six- or eight-month groups.

Increases in postexposure titers occurred first in the vaccinated cattle followed in about two weeks by an initial rise in titers of the nonvaccinated cattle.

Proof of infection was based upon isolation of *Brucella abortus* from one or more of the following sources: milk, blood, uterine contents, fetus, or tissues obtained at necropsy.

Eight (33 percent) of the 24 calves vaccinated at four months of age became infected, five of which aborted. Eight (36 percent) of the 22 calves vaccinated at six months of age became infected, five of which aborted. Seven (30 percent) of the 23 calves vaccinated at 8 months of age became infected, six of which aborted. Twenty (90 percent) of the 22 nonvaccinated controls became infected, 15 of which aborted.

Although no significant differences in the degree of vaccinal immunity could be shown among the three groups of animals vaccinated at different ages, vaccination at four months of age materially reduced the problem of persistent postvaccinal titers.
REFERENCES


SWINE BRUCELLOSIS AS A PUBLIC HEALTH PROBLEM

STANLEY L. HENDRICKS,* D.V.M., M.P.H.

The concerted nationwide program to eliminate brucellosis in cattle which has been in progress since World War II is reflected in the marked decrease in the number of human cases of brucellosis reported during the same period. The number of human cases reported has dropped from more than 6,000 in 1947 to less than 1,000 in each of the last four years. (Table 1.) In view of

TABLE 1

Reported Human Brucellosis

United States 1946-1960*

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1946</td>
<td>5,887</td>
</tr>
<tr>
<td>1947</td>
<td>6,321</td>
</tr>
<tr>
<td>1948</td>
<td>4,991</td>
</tr>
<tr>
<td>1949</td>
<td>4,235</td>
</tr>
<tr>
<td>1950</td>
<td>3,510</td>
</tr>
<tr>
<td>1951</td>
<td>3,139</td>
</tr>
<tr>
<td>1952</td>
<td>2,537</td>
</tr>
<tr>
<td>1953</td>
<td>2,032</td>
</tr>
<tr>
<td>1954</td>
<td>1,823</td>
</tr>
<tr>
<td>1955</td>
<td>1,444</td>
</tr>
<tr>
<td>1956</td>
<td>1,300</td>
</tr>
<tr>
<td>1957</td>
<td>983</td>
</tr>
<tr>
<td>1958</td>
<td>924</td>
</tr>
<tr>
<td>1959</td>
<td>892</td>
</tr>
<tr>
<td>1960</td>
<td>741†</td>
</tr>
</tbody>
</table>

† Provisional Data for 1960.

this progress, an examination of the probable sources of infection of recent human brucellosis seems indicated.

The United States Public Health Service has summarized epidemiologic case histories collected by the various State health departments (1). The part of these data pertaining to probable sources of infection for cases which occurred during the four-year period, 1957-1960, is shown in Table 2.

* Assistant Director, Preventable Disease Division, Iowa State Department of Health.

100
TABLE 2
Probable Sources of Human Brucellosis Cases in U. S. 1957-1960

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent*</td>
<td>No.</td>
<td>Percent*</td>
</tr>
<tr>
<td>Cattle</td>
<td>170</td>
<td>36.8</td>
<td>98</td>
<td>35.8</td>
</tr>
<tr>
<td>Swine</td>
<td>64</td>
<td>13.8</td>
<td>40</td>
<td>14.6</td>
</tr>
<tr>
<td>Cattle and Swine</td>
<td>92</td>
<td>20.0</td>
<td>58</td>
<td>21.2</td>
</tr>
<tr>
<td>Raw Milk—Family Cow</td>
<td>34</td>
<td>7.3</td>
<td>9</td>
<td>3.3</td>
</tr>
<tr>
<td>Raw Milk</td>
<td>40</td>
<td>8.6</td>
<td>15</td>
<td>5.5</td>
</tr>
<tr>
<td>Packing House</td>
<td>39</td>
<td>8.4</td>
<td>43</td>
<td>15.7</td>
</tr>
<tr>
<td>Rendering Plant</td>
<td>4</td>
<td>.8</td>
<td>1</td>
<td>.4</td>
</tr>
<tr>
<td>Vaccine Accidents</td>
<td>19</td>
<td>2.0</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>Sheep and Goats</td>
<td>1</td>
<td>.2</td>
<td>1</td>
<td>.4</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>2.1</td>
<td>6</td>
<td>2.1</td>
</tr>
<tr>
<td>Total—Sources Stated</td>
<td>463</td>
<td>274</td>
<td>454</td>
<td>273</td>
</tr>
<tr>
<td>Sources Not Stated</td>
<td>174</td>
<td>95</td>
<td>204</td>
<td>95</td>
</tr>
<tr>
<td>Total</td>
<td>637</td>
<td>369</td>
<td>658</td>
<td>368</td>
</tr>
</tbody>
</table>

Data Source Ref. 1.
* Percent of stated sources.
† Includes laboratory accidents.
‡ Provisional data.

While the information is not available for all cases, it would appear to be sufficiently complete to be of significance. The percentage of cases probably due to cattle decreased from 36.8 in 1957 to 15 in 1960. During the same period cases traced to swine as a source increased from 13.9 to 49 percent. The probable sources of other cases are shown in Table 2. An examination of the geographic distribution of human brucellosis cases reported in 1960 (Figure A) reveals much similarity with the distribution of the swine population in the United States. (Figure B). In 1960, 21 percent of the hogs produced in the United States were raised in Iowa, 18 percent were slaughtered in Iowa and 43 percent of all human brucellosis cases were reported from Iowa. (Table 3.) Since the public health aspects of swine brucellosis appear

TABLE 3

Numbers of Hogs Raised and Slaughtered and Human Brucellosis Cases
United States and Iowa 1960

<table>
<thead>
<tr>
<th></th>
<th>U. S.</th>
<th>Iowa—Percent*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hogs Raised</td>
<td>88,492,000</td>
<td>18,714,000</td>
</tr>
<tr>
<td>Hogs Commercially Slaughtered</td>
<td>78,955,000</td>
<td>14,455,000</td>
</tr>
<tr>
<td>Human Brucellosis Cases</td>
<td>741</td>
<td>307</td>
</tr>
</tbody>
</table>

Data Source
U.S.D.A.
Ref. No. 1

* Percent of United States Total.
FIGURE B
SWINE RAISED IN UNITED STATES 1960

Data Source: U.S.D.A., A.M.S.
to center in Iowa, the remainder of this paper pertains to the problem as it occurs in this State.

The public health significance of brucellosis in swine in Iowa was recognized as early as 1930 when epidemiologic and bacteriologic studies by Hardy, Borts, Jordan and Hardy indicated that swine were implicated as a source of human brucellosis (2). These investigations also demonstrated the importance of direct contact as a means of transmission and of the skin as a portal of entry. Subsequent reports (3-5) emphasized the role of swine as a source of human cases. Direct contact with infected animals or their fresh tissues appeared to be the most common method of transmission. However, in a paper presented at the Forty-sixth Annual Meeting of this Association in Chicago in 1942, Doctor Jordan described two milk-borne outbreaks due to \textit{brucella suis}, one involving 77 human cases. In these epidemics persons became infected by drinking raw milk containing \textit{B. suis}. The cows shedding the organism had been maintained in a lot with brucella infected sows (6). \textit{B. melitensis} associated with bacteriologically proven human cases was first recovered from hogs in Iowa in 1946 by Borts, McNutt and Jordan (7).

During the period 1930-1961 brucella organisms have been recovered or identified from 555 human cases by Doctor Borts and associates at the State Hygienic Laboratory (8). Of these 334 (60 percent) were \textit{B. suis}, 135 (24.4 percent) were \textit{B. abortus}, 82 (14.8 percent) were \textit{B. melitensis}, and four (0.7 percent) were unclassified. During the two-year period ending July 31, 1961, 12 isolates of \textit{B. suis}, 14 of \textit{B. melitensis} and two of \textit{B. abortus} were recovered. Of the two \textit{B. abortus} isolates, one was recovered from a granuloma of the lung of a 61-year-old retired farmer. The only history the patient could recall that seems to be significant was that he had been ill about 1929 at a time during which his heifers were aborting. In retrospect the signs and symptoms the patient reported having had at that time are compatible with brucella infection. The other strain of \textit{B. abortus} was recovered from a worker in the specialty meats department of a packing plant that slaughtered cattle and hogs.

Most human cases occur among certain occupational groups because their work brings them in direct or indirect contact with infected animals or their fresh tissues (5). However, women and children sometimes become infected. A rural housewife bottle-fed an orphan pig which was the only one remaining from a litter of pigs that were born dead or weak. Four weeks later the woman developed signs and symptoms of brucellosis. \textit{Brucella suis} was recovered from her blood. Another swine breeder had a group of six sows that produced only one live pig. It was bottle-fed by the owner's children for two days before it died. According to the breeder, 60 percent of 30 gilts in an adjoining lot failed to conceive. Blood agglutination tests confirmed the attending veterinarian's clinical diagnosis of brucellosis. During the next six weeks, the owner, his four-year-old son, his 12-year-old son who helped with the hog chores, and the hired man became ill with brucellosis. While this is an example of children becoming infected, also it illustrates multiple cases in a household. Most human brucellosis occurs as single cases in a family.
Human infection associated with baby pigs emphasizes the seasonal nature of human brucellosis in Iowa farm workers. Previous studies have shown an increase of cases following the spring and fall farrowing seasons (5).

The probable sources of a number of human cases that occurred in Iowa during the four and one-half year period, January 1, 1957-June 30, 1961 are shown in Table 4. This summary was prepared from routine case reports submitted by practicing physicians and from questionnaires answered by patients. It does not include all cases for the period since the information was not available in some instances. While incomplete, the probable sources of 619 of 875 cases are indicated.

In ascertaining the probable sources, all available pertinent information was considered. This included species of animals contacted by the patient, type of contact, presence or absence of clinical manifestations of brucellosis in the animals, results of serologic tests of animals if done and pasteurization status of milk consumed. Some farmer patients reported contact with both cattle and hogs but gave no information on the health of these animals. The probable sources of such cases are listed as cattle and swine. The sources of packing house cases are shown as cattle or swine depending upon the species butchered by the patient. If the patient worked on more than one species or if the species was not stated the source is classified as “packing house.” The rather large number shown as “not stated” represents several occupational groups including some farmers on which information regarding probable source was not available.

The data in Table 4 indicate there has been a decrease in the proportion of cases due to cattle during this period. Since January 1, 1960 the percentage of cases due to swine has increased markedly. The figures for the State

### Table 4

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>21</td>
<td>25.0</td>
<td>26</td>
<td>31.0</td>
<td>19</td>
<td>16.9</td>
<td>24</td>
<td>9.5</td>
<td>12</td>
<td>14.1</td>
</tr>
<tr>
<td>Swine</td>
<td>22</td>
<td>26.2</td>
<td>10</td>
<td>11.9</td>
<td>26</td>
<td>23.4</td>
<td>151</td>
<td>59.5</td>
<td>45</td>
<td>53.0</td>
</tr>
<tr>
<td>Cattle and Swine</td>
<td>24</td>
<td>28.8</td>
<td>10</td>
<td>11.9</td>
<td>26</td>
<td>23.4</td>
<td>151</td>
<td>59.5</td>
<td>45</td>
<td>53.0</td>
</tr>
<tr>
<td>Raw Milk</td>
<td>1</td>
<td>1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Packing House</td>
<td>14</td>
<td>16.6</td>
<td>25</td>
<td>29.7</td>
<td>22</td>
<td>19.6</td>
<td>44</td>
<td>17.3</td>
<td>19</td>
<td>22.4</td>
</tr>
<tr>
<td>Rendering Plant</td>
<td>1</td>
<td>1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccine Accident</td>
<td>1</td>
<td>1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total—Sources</strong></td>
<td><strong>84</strong></td>
<td><strong>84</strong></td>
<td><strong>112</strong></td>
<td><strong>80</strong></td>
<td><strong>254</strong></td>
<td><strong>192</strong></td>
<td><strong>308</strong></td>
<td><strong>22</strong></td>
<td><strong>99</strong></td>
<td><strong>11.2</strong></td>
</tr>
<tr>
<td>Source Not Stated</td>
<td>61</td>
<td>72.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>145</strong></td>
<td><strong>131</strong></td>
<td><strong>192</strong></td>
<td><strong>80</strong></td>
<td><strong>254</strong></td>
<td><strong>192</strong></td>
<td><strong>308</strong></td>
<td><strong>22</strong></td>
<td><strong>99</strong></td>
<td><strong>11.2</strong></td>
</tr>
</tbody>
</table>

* Percent of Stated Sources.
† First 6 months.
beginning in 1960 have been influenced to a large extent by a great increase
in cases in two packing plants, one of which slaughters only swine. A detailed
study (9) of 128 cases from the swine slaughtering plant that were reported
during a nine-month period revealed attack rates of 33 percent among
workers on the killing floor, 24 percent in the casings department and 12
percent on the cutting floor. Isolated cases occurred in the head trim, freezer,
inedible, maintenance, fresh pork pack, curing and boning departments and
among the night clean-up crew. There were no cases in the smoked meats,
sliced bacon, sausage, canning, lard, livestock, luncheon meats, warehouse
and shipping departments. There were two cases among 11 government
inspectors working on the killing floor, but no cases among seven inspectors
in other parts of the plant. Eleven strains of \textit{B. melitensis} and seven strains of
\textit{B. suis} were recovered from 18 of 69 blood cultures of these patients.

\textit{Reservoir of Infection in Swine}

The occurrence of an outbreak of this nature with swine as a source raises
questions about the reservoir of infection among the hogs. Information about
the actual incidence of brucellosis in Iowa swine is incomplete. Despite the
limitations of the agglutination test as applied to an individual hog (10),
agglutination surveys would seem to provide the best available indicator of
the extent of brucella infection in swine. A survey in February 1960 of
hogs slaughtered in the plant mentioned above revealed that 0.9 percent of
1,156 butcher hogs and 2.2 percent of 405 sows reacted positively in dilutions
of 1:160 or higher. This compares with one percent of 25,304 swine specimens
tested in 1960 at the State-Federal Brucellosis Laboratory at Iowa State
University, which had titers of 1:100 or higher (11). The 25,304 specimens
represented 2,198 different herds of swine. Ninety of the herds contained one
or more reactor animals. (Table 5.)

\begin{table}[h]
\centering
\begin{tabular}{lrrr}
\hline
 & \textbf{No.} & \textbf{Percent} & \textbf{No.} & \textbf{Percent} & \textbf{Total Tested} \\
\hline
\textbf{Herds} & 2,108 & 90 & 4.1 & * & 2,198 \\
\textbf{Animals} & 24,625 & 258 & 1.0 & 421 & 1.6 & 25,304 \\
\hline
\end{tabular}
\caption{Results of Swine Blood Specimens Tested at State-Federal Brucellosis Laboratory 1960}
\end{table}

* Herds with suspects but no reactors were classed as negative.

It must be remembered that the specimens tested at the State-Federal Laboratory represent herds and animals that are selected and are not chosen on a random sample basis. The specimens are submitted by practicing veterinarians for specific reasons including confirmation of clinical impression, differential diagnosis, herd certification, exhibit and sale. The results of tests on such specimens may or may not be similar to results of tests of a random sample. Some factors that influence the selection of herds and animals to be
tested would tend to result in a higher rate of positive animals and herds than tests on a random sample. Other factors would tend to give a lower than actual rate. The net result of these factors is unknown.

Assuming that the incidence of positive agglutination test reactors is an indicator of the actual extent of infection it would appear that only about one percent of the hogs in Iowa are infected. The percentage of infected herds also appears low when compared with bovine infection rates at the beginning of bovine brucellosis eradication efforts. Despite the apparently low infection rate among herds and individual animals, a significant public health hazard exists. Swine raisers, their families and swine handlers are exposed to infected animals. In a large packing house a single infectious hog passing through the slaughtering line may expose dozens or even hundreds of employees. Even with only one percent of the hogs infected, as many as 50 or more infected hogs may be slaughtered during a single day in one of the larger packing houses. Thus it is likely that each employee on the killing floor and in some other departments would be intimately exposed by direct contact to fresh warm infectious tissues repeatedly during each day of normal operations. Other employees may be subjected to indirect exposure through contaminated environment.

Summary

A review of probable sources of human brucellosis, epidemiologic investigations and a study of brucella agglutination tests of swine indicate that hogs constitute a major reservoir and source of brucella infection in man. Eradication of the disease from swine as well as cattle is necessary in order to prevent human brucella infection.

REFERENCES

11. BLAKE, GRANT E.: Personal Communication.
COOPERATIVE STATE-FEDERAL BRUCELLOSIS ERADICATION

C. K. MINGLE, D.V.M., M.S.C.*
Washington, D. C.

A great deal of knowledge and experience has been gained over the period in which the cooperative State-Federal brucellosis eradication program has been operating. In the beginning progress seemed to be satisfactory but the disease continued to break out in herds previously freed of the disease. The explosive nature of brucellosis required more than an accurate, simple diagnostic test to bring it under control. Strain 19 vaccine filled this gap, and has enjoyed increasing acceptance since it became available in 1941. However, the big break-through came in 1952 when the brucellosis ring test was officially adopted. With more and more of the cattle population becoming resistant to the disease through vaccination and with the brucellosis ring test being available to quickly detect infected dairy herds, brucellosis eradication became a realistic and attainable goal. This fact was recognized in 1954 when the cooperative program was greatly intensified. Mass approach was necessary in order to deal quickly with infected herds. The recommended Uniform Methods and Rules, formulated and adopted by this Association, have proved adequate in dealing with brucellosis in most areas where they have been utilized.

Target Date for Completing the Brucellosis Program Established

For a number of years certain groups and individuals have expressed doubts and misgivings concerning the eventual outcome of the brucellosis eradication program. These uncertainties are due largely to the fact that some people are not yet fully aware of the evolving nature of this program. With this in mind, we believe the time has come to spell out clearly the timetable and goals for the program that now appear realistic.

A tentative date of 1975 has been established for completing the eradication of brucellosis in the United States. By complete eradication is meant not only eradication of bovine brucellosis but, also elimination of the disease in all species of domestic livestock.

In order to complete the eradication effort in 1975, it will be necessary for all states to move logically and systematically through appropriate phases of the program. Initial modified certified area status should be completed on a nationwide basis in 1965. In the meantime, brucellosis-free area programs must be adopted by all modified certified states as soon as possible after completing initial certification. The Brucellosis Committee of this Association has been alert to the changing requirements of the program and whenever

*Chief Staff Officer, Brucellosis Eradication, Agricultural Research Service, United States Department of Agriculture.
justified the Uniform Methods and Rules have been revised accordingly. By this continued review of program needs it has been possible to operate along lines that are progressively more effective and practical.

In order for the program to advance as rapidly and efficiently as possible it is essential that surveillance programs be utilized extensively in all areas. The effectiveness of brucellosis milk ring testing increases proportionately to the frequency of application. It is important, therefore, that every effort be made to establish the brucellosis ring test program on a four times per year schedule. Market cattle testing provides another valuable tool for achieving and maintaining maximum progress in the eradication program. Full use of these two screening procedures, together with follow-up investigations of presumptively infected herds, will provide an economical and effective means of locating the last vestiges of infection.

In those areas approaching the final stages of eradication, a limited number of herds are being disclosed in which brucellosis has persisted in spite of the application of routine procedures. These so-called “problem herds” are requiring special handling by personnel qualified in this field. In order to meet this need, men are being trained along these lines and assigned to help those States where the “problem herd” situation is acute. The brucellosis “problem herd” program should be adopted promptly in all certified States.

The role vaccination continues to play in the eradication effort is outstanding. It has provided a bulwark against rapid extension of the disease in many areas that otherwise could never have been controlled. Vaccination must be continued at high levels until the goal of eradication is assured. At that time, it should be possible to design a gradually declining program for vaccination. Such action would, of course, be supported by the application of suitable precautionary measures relative to the movements of animals into and within the country and the maintenance of effective surveillance procedures. Under these conditions, vaccination can eventually be discontinued entirely without jeopardizing the brucellosis-free status of the country.

The experience of Sweden with respect to vaccination is interesting and significant. That Country withdrew Strain 19 vaccine from the official program six years before the final outbreak of brucellosis was reported. The last infected animal was removed in 1957 and since that time no further reactors have been found. There is every reason to believe that brucellosis has been eradicated from Sweden.

INCREASED PROGRAM ACTIVITIES

The additional funds available for the Cooperative State-Federal Brucellosis Eradication Program during fiscal year 1961 provided for increased field activities over this period.

Table I gives a comparison of operations conducted in fiscal years 1960 and 1961.
Herds and Cattle Blood Tested:

The number of lots blood tested during fiscal year 1961 increased by 147,000 over 1960 for a total of 1,332,651. Of these, 58,068 or 4.4 percent contained reactors. This compares with 5.1 percent infected lots disclosed in 1960.

Cattle blood tested during 1961 increased by 1,000,000 over 1960 for a total of 13,418,657. Of these, 139,894 or 1.04 percent were reactors. In 1960, 1.19 percent of the cattle tested were classed as reactors.

Blood test data are becoming less significant as such screening devices as ring testing and market cattle testing are more extensively employed. These procedures are channeling more and more blood testing into presumptively infected herds.

Brucellosis Ring Testing:

The ring testing program for brucellosis continues to be one of the brightest spots in the brucellosis eradication effort. In comparison with fiscal year 1960, the number of tests reported was increased by 150,000, and the number of suspicious herds dropped more than 16,000. In 1960, 3.7 percent of the ring tests were suspicious, while in 1961 only 2.4 percent...

<table>
<thead>
<tr>
<th>Activities</th>
<th>Fiscal Year</th>
<th>Percent Change for 1961</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herds—Lots</td>
<td>1,185,562</td>
<td>1,332,651 (+12.4)</td>
</tr>
<tr>
<td>Reactor Herds—Lots</td>
<td>60,835</td>
<td>58,068 (—4.5)</td>
</tr>
<tr>
<td>Percent</td>
<td>5.1</td>
<td>4.4 (—0.7)</td>
</tr>
<tr>
<td>Cattle Tested</td>
<td>12,468,476</td>
<td>13,418,657 (+7.6)</td>
</tr>
<tr>
<td>Reactor Cattle</td>
<td>147,805</td>
<td>139,894 (—5.3)</td>
</tr>
<tr>
<td>Percent</td>
<td>1.19</td>
<td>1.04 (—0.15)</td>
</tr>
<tr>
<td>Ring Tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herd Tests</td>
<td>1,593,642</td>
<td>1,744,526 (+9.5)</td>
</tr>
<tr>
<td>Suspicious Herd Tests</td>
<td>58,457</td>
<td>42,049 (—28.1)</td>
</tr>
<tr>
<td>Percent</td>
<td>3.7</td>
<td>2.4 (—1.3)</td>
</tr>
<tr>
<td>Vaccinations (Calfhood)</td>
<td>6,438,497</td>
<td>6,721,296 (+4.4)</td>
</tr>
<tr>
<td>Certification of Counties</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified Certified</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New and Reinstated</td>
<td>311</td>
<td>216</td>
</tr>
<tr>
<td>Removed</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>1,946</td>
<td>2,154</td>
</tr>
<tr>
<td>Certified Brucellosis-Free</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New</td>
<td>22</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>62</td>
</tr>
<tr>
<td>Total Certified Counties</td>
<td>1,968</td>
<td>2,216 (+12.6)</td>
</tr>
</tbody>
</table>

( ) Percent difference.
suspicious herds were disclosed. This downward trend in ring test suspicious herds is continuing during the first two months of fiscal year 1962, with only 1.8 percent of the tests conducted during that period being suspicious. The effectiveness of this procedure in helping establish and maintain certified areas is reflected in the continued reduction in infection rates throughout the Nation.

**Vaccination:**

Except for fiscal year 1960, when funds were low, there has been a consistent yearly increase in the volume of official calf vaccinations. There is no question but that this program has contributed much toward the progress being made in eradicating bovine brucellosis. At this stage of the brucellosis eradication program there is still a need to further improve the vaccination coverage. For the year ending June 30, 1961, a total of 6.7 million official vaccinations were recorded. This represents an increase of about 300,000 or 4.4 percent over the previous year. From the standpoint of eligible calves, about 52 percent were vaccinated in 1961.

**Area Certifications Continue to Advance**

If all remaining counties request a complete area program within the near future, Nationwide Modified Certification could be attained by 1965. At the end of fiscal year 1961, 2,216 counties or 70.3 percent of all counties were qualified either as Modified Certified or Certified Free Areas. In addition to the counties certified at that time there were 321 other counties engaged in programs leading directly to Modified Certification. With 80.4 percent of our counties now operating a brucellosis eradication program on an area basis, prospects of achieving the 1965 goal for the entire country are encouraging. As of October 1, 1961, this percentage had risen to 82.0.

While there was an upsurge in all program activities over 1960, this was not reflected in an increased number of counties certified during the year due to the fact that it takes more than 12 months on the average to complete the qualification of counties. This resulted in a net gain of only 248 certified counties as compared with 315 during 1960. Moreover, the number of new counties that can be qualified each year with available manpower and funds becomes less as the maintenance of certified areas requires increased attention. For example, during fiscal year 1961, a total of 762 counties were recertified. For the current year a total of 642 counties will require the same attention. Thus, it is understandable why the number of new counties qualified during 1961 is under the number qualified in the preceding year.

Two States—Idaho and Indiana achieved State-wide Modified Certified Brucellosis status during fiscal year 1961. As of June 30, 1961, there were 26 States, Puerto Rico and the Virgin Islands listed as Certified States and Territories. Unfortunately, since then the State-wide certification of one State was lost because one county failed to qualify for recertification. It is hoped that the county involved will requalify soon and permit this State to be listed again. In this connection, it should be pointed out again that everything possible is being done to insure the validity of all certified areas.
The Certified Brucellosis-Free Area program has shown encouraging progress since recommended procedures for establishing and maintaining such areas were adopted by this Association in 1959. By the end of fiscal year 1961, a total of 62 counties had achieved this distinction. Since then additional counties have qualified so that as of October 1, 1961, there were 84 Certified Brucellosis-Free Areas in 11 States. This number includes the entire State of New Hampshire. It appears that Maine will be the second State to achieve State-wide brucellosis-free status, as only three of its 16 counties remain to qualify.

Based on experience gained during the past year and a half, no difficulty is anticipated in maintaining the status of brucellosis-free areas after they are properly qualified. As expected, the brucellosis ring test is proving to be a valuable aid in this regard.

Figures 1 and 2 depict area certification progress on a nationwide basis.

Figure 1

ADEQUACY OF CERTIFICATION PROCEDURES CONFIRMED

Final data have been assembled and tabulated for the project designed to evaluate the brucellosis status of cattle moving interstate from Modified Certified Areas. This investigation was initiated in July, 1957, and carried through September, 1960. A total of 76,725 cattle from Modified Certified Areas were tested at destination in representative areas of the country. Of these, only 31 reactors were found, giving an incidence of infection of 0.04 percent. This represents one reactor for each 2,475 animals tested.
During the same survey, 30,561 cattle were tested which originated in noncertified areas. Of these, 201 or 0.65 percent were reactors. This represents one reactor for each 152 animals tested.

It is important that incentives be provided for the establishment of certified areas. The acceptance of cattle from such areas without further test is an important factor in this regard. If such recognition will help encourage wider participation in area type programs, then the risk of one reactor out of about 2,500 animals becomes insignificant.

At the present time, 24 States accept beef cattle from Modified Certified Areas in accordance with the Uniform Methods and Rules. This number compares with six in 1956 and 21 in 1959 which granted the same privileges. It is hoped that other States will take similar action, thereby contributing to their own protection by furthering the eradication effort in all sections of the country.

Figure 3 shows the States that have been added to the list of those accepting beef cattle from Modified Certified Areas during the past five years.

**Figure 3**

**STATES ACCEPTING BEEF CATTLE**

*From Certified Areas In Accordance With Uniform Methods and Rules*

- **October 1956-Total (6)**
- **October 1959-Total (21)**
- **June 1961-Total (24)**

MARKET CATTLE TESTING EXPANDED

The market cattle testing program continues to expand in many parts of the country as its practical advantages and usefulness are more clearly understood. It is encouraging to note the interest that has developed in semi-range and non-range areas of the country for screen-testing cattle at
auction markets. The purpose of market cattle testing is, of course, served equally well by tests conducted on identified animals of this type at any point, including ranches, concentration centers, such as auction markets, or at packing plants. Previous indications that this procedure will reduce by about 97 percent the ranch level testing otherwise required for recertification of areas, still holds true. Thus, the market cattle testing program can provide effective means for eradicating brucellosis at a minimum of inconvenience to owners.

At present, 41 States are actively participating in market cattle testing. This represents an increase of 15 over the 26 States that were conducting such a program at the time of our report last year. Moreover, 104 counties in 10 States have incorporated market cattle testing results for purposes of recertification. It is also significant to note that blood samples are now being collected at packing plants in 44 States.

Figure 4 presents a comparison of States conducting market cattle testing as of June, 1960 and October, 1961.

**Figure 4**

**STATES CONDUCTING A MARKET CATTLE TESTING PROGRAM**

With rapid progress being made in eradicating bovine brucellosis, the problem of brucellosis in other species of domestic animals becomes increasingly important. It will never be possible to attain a brucellosis-free country until all reservoirs of infection are eliminated. The most important reservoir of infection, outside of cattle, has always been and continues to be swine. It is estimated that about six percent of the Nation's 1.8 million swine herds are affected with brucellosis.
During the past several months a number of National organizations have expressed, by resolutions, the need for intensifying activities in connection with swine brucellosis eradication. These groups include the National Brucellosis Committee of Livestock Conservation, Inc., The Chief Livestock Sanitary Officials of the Northcentral, Northeastern, and Southern States, a special meeting of representatives of this Association and the National Brucellosis Committee, and the National Association of Swine Records.

There are three main reasons for this interest in eradicating brucellosis from swine. First of all, the economic losses cannot be ignored. It has been conservatively estimated that brucellosis costs the swine industry about 10 million dollars each year. Then, there is the danger of interspecies transmission of the disease between swine and cattle. Such spread has been known to occur and presents a potential hazard to completing the eradication of brucellosis from cattle. Finally, swine brucellosis constitutes a public health threat to farmers, livestock handlers, veterinarians, meat processors and butchers through contact with infected animals. United States Public Health Service reports show that in 1957, 40 percent of the human brucellosis cases was believed to have originated in swine. Three years later, in 1960, this figure increased to 60 percent. The obvious indications are that fewer reservoirs of infection now exist in cattle and as a consequence the brucellosis situation in swine is becoming more prominent. Regardless of the interpretation placed on these findings, it is high time that an intensified effort be made to eradicate swine brucellosis.

While the eventual eradication of brucellosis from all swine herds must be the goal of the intensified cooperative program, emphasis in the beginning should be on the "Validation" of individual herds as brucellosis-free. The term "Validated" is proposed to replace the old terminology of Certified Brucellosis-Free. The need for such a change lies in the fact that purebred swine producers and others in the swine industry have come to associate the term "Certified" with production standards—such as, "Certified Litter" and "Certified Sire".

The three plans adopted by this Association and approved by the Department in 1958 have proved effective in eradicating brucellosis from individual herds under varying field conditions. There is no apparent reason, therefore, why these basic procedures cannot be used in all States to establish and maintain brucellosis-free swine herds.

In spite of the gradually increasing interest in controlling swine brucellosis, the qualification of brucellosis-free swine herds has never reached a significant level. As of January 1961, 31 States were prepared to designate herds as brucellosis-free. However, at that time there were only 451 such herds listed in all of these States.

No doubt some of this delay has resulted from the swine industry not being fully aware of the needs for eradicating this disease. However, of more importance in this regard is the failure of some States to recognize the brucellosis-free status of animals originating in herds declared free of the disease. This has applied to both intrastate and interstate movements. It
appears that certain States are uncertain as to the effectiveness of the procedures employed by other States in qualifying brucellosis-free swine herds. We believe the solution to this problem lies in establishing and applying uniform procedures in all States. The Department hopes that cooperative arrangements can be made with all States so the validity of all swine herds declared brucellosis-free throughout the country will be insured. This problem is readily apparent in the fact that only 13 of the 31 States with brucellosis-free herd programs will accept swine from qualified brucellosis-free swine herds in other States without additional tests. This situation discourages many producers from undertaking a swine brucellosis eradication program.

The tools and knowledge are available for the eradication of swine brucellosis. With full cooperation of everyone concerned, real progress can be made in this direction. The Department recognizes the urgency of swine brucellosis eradication and has initiated a wide-scale information program dealing with the disease.

A new exhibit dealing with various aspects of swine brucellosis has been prepared recently by the United States Public Health Service. This display should be useful in helping encourage participation in a swine brucellosis eradication program.

ANTICIPATED PROGRESS IN FISCAL YEAR 1962

With only 18 percent of the counties left to participate in the area program, it is expected that approximately one-third of these will be enrolled by next June 30. This will bring participation to 88 percent of all counties in the United States.

From the standpoint of initial modified certifications, an increase of 275 counties should be attained during fiscal year 1962. If this is accomplished, 75 percent of the Nation will be certified. During the first three months of this year 57 counties were initially qualified.

State-wide certifications should increase by seven to a total of 32 during the current year. The seven additional States are California, Illinois, Virginia, Arkansas, Kansas, South Carolina and Missouri.

By the end of this fiscal year 100 new counties should achieve Certified Brucellosis-Free status. This will bring the total number of such areas to almost 200. Maine is the only State that is expected to qualify as brucellosis-free on a State-wide basis during the current fiscal year.

COMMENTS

A realistic estimate of annual losses now sustained by the cattle industry as a result of brucellosis approximates 25 million dollars. As further progress is made in the eradication program these losses will continue to decline. With the changing economics involved, we could be faced with efforts to prematurely reduce State and Federal financial support of the program. Such action could jeopardize the cooperative project to the extent final eradication
would never be realized. It is hoped that everyone concerned will encourage the support necessary to carry the brucellosis eradication program on to a successful conclusion.

Field studies conducted over the past year in brucellosis "problem herds" have been quite productive and encouraging. These investigations have shown that most of these herds respond rather quickly when available supplemental tests are applied under competent supervision. It has been found also, that some of the so-called "false positive" ring test results may be due to occasional failures of the blood serum agglutination test to identify infected animals. In a significant number of these cases virulent Brucellae have been recovered from animals which are only suspects or in some cases even negative to the blood test. These findings emphasize the danger of disregarding milk ring test reactions that cannot be associated immediately with diagnostically positive blood reactions. It is beginning to look as if the ring test may be more specific for Brucella infection than expected.

As States complete the Modified Certification program and proceed toward final eradication it becomes increasingly urgent that all remaining infected herds be freed of the disease as quickly as possible. The establishment of the "problem herd" program, in which all available supplemental procedures are used, has proven to be an effective solution to the problem of persisting infections.

For purposes of routine testing, leading to Modified Certification of areas, the rapid plate test is both effective and practical. However, in the final stages of the eradication program sufficient evidence is being found to justify substitution of the tube agglutination test for the plate test. In the case of many so-called "problem herds" residual infections can be detected and quickly eliminated through testing by the tube method.

During fiscal year 1961, 6,251 infected herds were disclosed in the 26 Modified Certified States, Puerto Rico and the Virgin Islands. This is 0.46 percent of the 1.3 million herds under supervision in these areas. Of these infected herds, 3,029 had no history of past infection and can be considered newly infected. This new infection should alert us to the continued menace of brucellosis and dispel any tendency toward complacency following the attainment of Modified Certification.

The continued expansion of market cattle testing and ring testing is providing innumerable benefits to the brucellosis program. When eradication is achieved, great credit must be given to these screening devices. Without these, it is doubtful if we could ever have halted the extension of brucellosis in cattle to the extent necessary for eventual eradication. Although ring testing reached a level of 1.7 million herd tests in 1961, there is still need for an increased volume. The efficiency of this procedure is directly related to the frequency of application. During this same period, about 500,000 market cattle test results were recorded. In order to be fully effective it is believed that this number should be increased to at least two million tests yearly. So far this year, market cattle testing is being conducted at the rate of about one million tests per year. The importance of further expanding this phase of the program cannot be overemphasized.
With the advantages of market cattle testing for recertification fully established, a statistical evaluation of this procedure has been carried out to determine its possible effectiveness for initial certification of areas. Results of this analysis show that, with adequate safeguards, market cattle testing can provide a highly efficient and practical method for initially qualifying Modified Certified Areas. It is believed, therefore, that this procedure should be adopted as an alternate method of attaining Modified Certified Area status under the Recommended Uniform Methods and Rules for Bovine Brucellosis Eradication.

Swine brucellosis becomes increasingly important as the incidence of brucellosis in cattle is reduced. It is believed that the proposed new term "Validated" should be adopted for the designation of brucellosis-free swine herds and it is hoped that an intensified herd validation program will be undertaken in all swine producing areas of the country. The economic and public health hazards of this disease can no longer be ignored. The Department is now cooperating with the State of Georgia on a county-wide swine brucellosis eradication program. The work in Dooly County, Georgia is designed to evaluate, on an area basis, the recommended eradication plans adopted by this Association in 1958. It is hoped that results of this field study will provide guide lines for swine brucellosis eradication beyond the validation of individual herds.

We now have within our grasp an outstanding accomplishment in the field of animal disease eradication. Therefore, it behooves us all to keep our sights on the goal of brucellosis eradication until the program is completed. It would be most unfortunate, at this late stage, to relax our efforts and lose the momentum generated over the past few years.
The serious nature of the swine brucellosis problem in the Corn Belt States is recognized by producers, veterinarians and public health officials. The degree of loss suffered in an infected swine herd varies from sterility which may be unsuspected to the more spectacular “storm” of abortions in which an entire pig crop may be destroyed before birth (1). The most serious loss, however, occurs when a member of the farm family contracts brucellosis. This disease is especially serious in man, since in spite of the most modern therapy, recovery may be delayed for months or years. Current records show that over 75 percent of the human cases in Iowa are contracted from swine (2). Also over 40 percent of all the human cases of brucellosis in the United States occurred in Iowa during 1960. People contract brucellosis through contact with infected animals or meat or milk—if there was no brucellosis in lower animals there would be no brucellosis in man.

The Veterinary Medical Research Institute of Iowa State University has had this disease under active study for more than 25 years. By 1947 considerable information had been gathered on the basic aspects of swine brucellosis, including its pathogenesis, and the disease had been eradicated from certain special study herds. On the basis of the information obtained from these studies, it was decided to undertake comprehensive field trials, supported by laboratory work, to determine the practicability of establishing and maintaining brucellosis-free herds—to test whether or not eradication of swine brucellosis was practical under the usual farm conditions.

It was expected that the information obtained in such field trials would aid in the development of a still larger, overall eradication plan. The results of these field trial studies did, indeed, lead to such a plan, and the “Iowa Certified** Brucellosis-Controlled Swine Herd Program” was formulated and officially accepted in October 1949.† This report deals with some of the information obtained and certain of the conclusions reached as a result of the laboratory studies, the field trials and the eradication measures applied in infected herds.

Before a new herd was admitted to the eradication trials, the field collaborator obtained a complete herd history concerning swine brucellosis and

* Field Collaborator, Veterinary Medical Research Institute, Iowa State University, Ames, Iowa.

† Professor of Veterinary Science, University of Wisconsin, Madison, Wisconsin.

** It has been proposed that the term “validated” be employed instead of “certified” in reference to brucellosis-free swine herds.

‡ Because of the effectiveness of the program, it has not been altered in the past 12 years. The procedures are essentially the same as those adopted by the United States Livestock Sanitary Association in 1958.
other diseases, the breeding practices, number of animals, value of blood lines, equipment including housing and pasture, and other factors involved in swine production in the particular herd. On the basis of these findings, the most practicable plan for eradication was adopted. Frequent visits were made to each farm and the animals in the herds and the records on such animals were examined. Fetuses, placentas, baby pigs, testicles and post-mortem tissues, were collected and transported immediately to the laboratory for examination. As expected, the mass of information obtained in all of the above studies and examinations made it possible to evaluate the overall programs and to clarify individual herd situations.

**ESTABLISHING CERTIFIED BRUCELLOSIS-FREE HERDS**

Herds were certified for a one-year period on the basis of two consecutive negative blood tests conducted within a period of 30 to 90 days provided all accessory regulations were faithfully met.

Certification was renewable on an annual basis by the completion of a single negative blood test on all boars, sows and gilts over six months of age. The plans employed for the handling of brucella-infected herds were those commonly accepted as follows:

**PLAN I—MARKET THE ENTIRE HERD FOR SLAUGHTER**

This plan is most often employed for the usual commercial herd. After the entire infected herd was sold for slaughter, the premises were cleaned as much as possible and the houses and equipment employed for hogs were cleaned and disinfected. Because infected lots and pastures are especially dangerous for swine, time for self-disinfection of these areas was allowed. Naturally, repopulation was from breeding stock coming from herds known to be free of brucellosis.

**PLAN II—TEST AND SEGREGATION OF WEANLING PIGS**

This plan has been used mostly in herds where blood lines were to be saved. It is based largely on two facts: (1) the main source of infection is the older animals in the herd and (2) pigs weaned at 42 days of age are rarely infected, even though born of infected dams. A minimum amount of testing and the maximum amount of isolation are required. Naturally, it fails if the weanling pigs are not completely separated from the old infected herd and from contaminated premises.

The young pigs were separated from their dams at 42 days of age and moved to an entirely clean area where there was no chance of them coming into contact with animals in the old herd or with other hogs. The old herd was disposed of as soon as possible. (This plan was more easily applied if there was but a single farrowing during a 12-month period.) The young isolated gilts were tested before breeding. Only those negative to the test were kept. These gilts were farrowed in individual pens and held until again tested after farrowing. The gilts negative to the agglutination test and their
pigs were handled separately, in isolation from the rest of the herd. Pigs from the negative sows, weaned at 42 days of age, became the basis of the new clean herd. This cycle was repeated when necessary to achieve final eradication.

**PLAN III—TEST AND ELIMINATION OF REACTORS**

It seemed that this plan had limited application in small herds where only a few reactors were found or where the swine were kept in separate groups and one or more of the groups were apparently free of the infection. It was so recommended. The animals that reacted to the agglutination test were sold for slaughter and the herd retested in 30 to 90 days. When no reactors were found on two consecutive tests, the herd was considered free of the disease. It appears that if a herd is not freed of infection in a reasonable time by this plan, it should be discarded in favor of one of the other plans.

The eradication program in Iowa was more effective than anticipated. During the calendar year 1954, the Division of Animal Industry of the Iowa Department of Agriculture certified 76 herds as brucellosis-free. Sixty-six or 86.9 percent of these were recertified at least once. Three herds or 3.9 percent of the 76 herds were dispersed. Six herds did not apply for recertification. Only one herd or 1.3 percent was reinfected. Thus, of the 67 that attempted to recertify, 66 or 98.7 percent were recertified on a single test—only one herd failed to recertify because of new infection.

In the light of the experience in Iowa as well as elsewhere there is every reason to believe that brucellosis in swine can be eradicated in a practical manner with the knowledge and tools now available. For successful eradication, it is essential that none of the basic principles concerning the disease be compromised (3). The development of the Certified Brucellosis-Free Herds is an important initial step in the direction of total eradication. This appears to be especially true because reinfection of clean swine herds has been found to be rare. The brucellosis-free area seems to be less important in swine than in cattle. Moreover, the certified brucellosis-free herds provided a safe source of clean stock for replacement purposes. Breeding stock from certified herds sells at a premium and the program is increasing in popularity. A great deal less testing is required in certified herds than in noncertified herds. Without certification it is necessary that individual animals be tested prior to sale. Certified herd testing can be done at a time of year when the inventory of mature animals is lowest.

The place of certified brucellosis-free herds in providing a safe source of clean breeding stock looms large in swine production because essentially all swine brucellosis outbreaks can be traced to the introduction of infected breeding stock and especially to infected boars.

It has been said that the blood serum agglutination test is more reliable in cattle than in swine. The above trials and studies did not prove this to be true. When properly interpreted and its limitations recognized the agglutination test applied to swine blood was just as reliable as the same test employed with cattle (4). With the recognized economic and public health
hazards associated with swine brucellosis, it is the hope that the industry will institute efforts to free swine herds of this disease.

REFERENCES


2. Personal communication. Dr. I. H. Borts, Director, State Hygienic Laboratory, University of Iowa, Iowa City, Iowa.


REPORT OF THE COMMITTEE ON BRUCELLOSIS


When God made the oyster, He guaranteed him absolute economic and social security. He built the oyster a house, a shell to protect him from other animals and other oysters. When hungry, all the oyster has to do is to open up his shell and food rushes in.

But when God made the eagle, what did He do? He said, "The blue sky is the limit. Get out, and build your own house." And the eagle goes out and builds his house on the highest mountain crag, where danger and disaster threaten him every day. For food, he flies through a thousand miles of rain and snow and wind.

The eagle and not the oyster is the emblem of America.

The Agricultural Statistics for 1959 tell us that farm assets and claims amounted to approximately 200 billion dollars, or three-fifths of the entire assets of all the industries in the United States.

We have all read and heard the statement that "Livestock is the Cornerstone of America's Agricultural wealth and that the Veterinary profession is its greatest safeguard." This statement can have only one meaning and that is unless livestock diseases are controlled and when possible, eradicated, there can be no successful agriculture. Down through the years, regulatory officials (both Federal and State), with the cooperation of the practicing veterinarians, the livestock owners and allied interests, have waged a constant and unrelenting fight against all contagious and infectious diseases of our domestic animals.

When God made the eagle and told him to get out and fight for his existence He must have had in mind the part that agriculture would play in the existence of mankind and the continued fight that man would have to make to establish and maintain a healthy agriculture.

The men and women of this country from the pioneer days down through the years have met the challenge of agriculture in all of its complicated phases.

The history of Animal Disease Control and Eradication goes back to the very earliest pioneer days. In the report of the Fifteenth Annual Meeting of the United States Livestock Sanitary Association in 1911, E. S. Wood, of the Kentucky Agricultural Experimental Station stated in his address entitled
"Infectious Abortion in Cows and Mares," "Tuberculosis and infectious abortion are two diseases causing a tremendous loss annually to the breeders of beef and dairy animals, not only in this country but in foreign countries as well. In fact, I believe that abortion is becoming as much a nightmare to our dairymen as is tuberculosis. The loss in the United States yearly amounts to many millions of dollars. There is no doubt that there is such a thing as infectious abortion among our domestic animals for it has been recognized for many years not only by stockmen themselves, but by veterinarians in many parts of the world."

From 1911 and maybe earlier than this date, Contagious Infectious Abortion, later known as Bang's Disease, and now as Brucellosis, has had a prominent place on the programs of this Association. That there was much to be learned on the subject is evident from the early reports.

Records show that definite and satisfactory progress has been made in recent years in the eradication program and all indications are that all States will qualify as a Modified-Certified Area during the next few years providing sufficient funds are made available.

While the prime object might be to test all animals within a given area and reduce the infection to the percentage required to qualify as a Modified-Certified Brucellosis Area, we must not neglect or lose sight of the fact that continued testing and eternal vigilance must be exercised in all of the States and areas which have already qualified if we are to hold the line and advance our fronts in new areas.

Last June at a special meeting of The National Brucellosis Committee the following additional alternative method of certification was proposed for discussion and ultimate acceptance by appropriate disease control authorities:

THE PROPOSED ALTERNATE PLAN

Using approved back tagging and market testing procedures, when five percent of all cattle 30 months of age and over in all herds in a control area have been screened annually for three years and level of infection is found to be below minimum official levels and an official vaccination program of an intensive level has been carried out concurrently, such procedures should be acceptable for official initial certification.

SWINE BRUCELLOSIS

Also at the June meeting of The National Brucellosis Committee, swine brucellosis eradication was discussed at length. The need for substituting the term "validated" for "certified" in identifying officially recognized brucellosis-free swine herds was unanimously accepted by those present.

Under date of August 18, 1961, your chairman received the following resolution from The National Association of Swine Records, signed by R. E. Judd, President:
WHEREAS, swine brucellosis is a serious problem to swine producers, both from the economic losses it causes and from the standpoint of the hazards to human health; and

WHEREAS, concerted action at the National, State and local levels is necessary to eradicate this disease;

NOW THEREFORE BE IT RESOLVED by the National Association of Swine Records meeting in Champaign, Illinois on July 12,

1. That an effective swine brucellosis eradication program be initiated, such a program to be based upon procedures recommended by the United States Livestock Sanitary Association, and approved by the United States Department of Agriculture.

2. That all States adopt these same procedures for the purpose of establishing brucellosis-free swine herds;

3. That those swine herds which qualify under these procedures be designated as 'Validated Brucellosis-Free Herds'; and

4. That all States take action to allow individual animals from such validated brucellosis-free herds to move interstate without further test.

BE IT FURTHER RESOLVED, that the United States Department of Agriculture, through its Animal Disease Eradication Division, provide the membership of the associations comprising the National Association of Swine Records with information on swine brucellosis, its prevention and eradication.

Both of these recommendations were presented to and discussed at length at the open hearing on brucellosis last Tuesday.

Each year as we meet in convention, new plans backed up by research and controlled experiments are presented for consideration of your Committee. Many resolutions and suggestions for improvement of the program have been received during the past 12 months from different sections of our country. All of these have been considered in public hearings which started Tuesday morning in this hotel. We believe that everyone has had an opportunity to present his case and every proposal has been given careful consideration.

This coveted goal of attaining a Modified-Certified Brucellosis-Free status is the desire of every State. Upon achieving it, however, a State may be in its most dangerous period. There is the real prospect of a let-down in the vigorous effort to attain final and complete eradication. While State and Federal expenditures can be reduced considerably following attainment of the Modified-Certified Brucellosis-Free status, industry and professional pressure must be continued, not only to attain final eradication but to prevent losing hard-fought ground.

Therefore, your Committee wishes to emphasize that there is no short road to success in disease eradication, and that increasing warfare against all contagious and dangerous economic livestock diseases demands of us constant watchfulness and service.
Your Committee on Brucellosis is made up of men from all parts of our country and Canada. All are educated and well versed in the business of Animal Industry. It has been said that an educated man is one who keeps his mind open on every question until the evidence is all in. He always listens to the man or men who know. He never laughs at new ideas. He cross-examines his day-dreams. He knows his strong points and plays them. He knows when to think and when to call in the experts to think with him. You can't sell him magic. I am sure the members of your Brucellosis Committee will all qualify according to the above formula.

Last Tuesday from early morning until late evening all phases of Brucellosis eradication were discussed. All interested parties were given an opportunity to be heard. All resolutions were given serious consideration and study.

With these introductory remarks, we are now ready to report to you, what, in our best judgment, should be incorporated into the uniform methods and rules governing the program of brucellosis eradication in the United States.

The following Resolution of the American National Cattlemen's Association was presented by Robert Laramore, Cheyenne, Wyoming:

RESOLUTION

Using approved back tagging and market testing procedures, when five percent of all female cattle 30 months of age and over in all herds in a control area have been screened annually for three years, or herds not so complying shall test in compliance with progressive chart, of the uniform methods and rules, and the level of infection is found to be below minimum official levels and an official vaccination program. (Percent of vaccinated heifer calves retained or added annually to be determined by local area option.) Such procedures shall be acceptable for official initial certification.

The percentage of replacement heifer calves to be vaccinated to comply with the section may be varied by local conditions and agreed upon by Federal and State regulatory officials and the affected cattle owners in the area.

This Resolution was adopted in principle by your Committee. Its operation will be provided for in the uniform rules and regulations.

In view of the excellent progress being made in the cooperative brucellosis eradication program, your Committee strongly recommends that adequate State and Federal funds be provided to assure completion of the Program as quickly as possible.

Your Committee further recommends the adoption by all States of the word Validated in lieu of “Certified” for designating brucellosis-free swine herds.

BOVINE BRUCELLOSIS ERADICATION

PROPOSED CHANGES IN THE UNIFORM METHODS AND RULES—1961

Part V, Certified Brucellosis-Free Areas.

The provisions of the individual certified herd plan that relate to quarantining, cleaning and disinfecting shall apply to Certified Brucellosis-Free
Areas. The official tube agglutination test shall be utilized in all blood serum agglutination tests conducted in relation to establishing and maintaining Certified Brucellosis-Free Areas. Animals classed reactor must be immediately marketed for slaughter in accordance with Section II, Paragraph 7, of this Part.

Part V, Section I, Paragraph 2.

2. All herds in the area except those covered in Paragraph 11 of this Section have been included in one of the following within 18 months immediately preceding the request for Certified Brucellosis-Free Area status:

Paragraphs 4, 5, 6, 7 and 8.

4. Not more than one percent of the herds, or one herd, whichever is greater, shall have been found to be infected during the 18 months immediately preceding the request for Certified Brucellosis-Free Area status. For purposes of this calculation, herds maintained in accordance with Paragraph 11 of this Section are not to be included.

5. Not more than 0.2 percent of the cattle shall have been found to be reactors during the 18 months immediately preceding the request for Certified Brucellosis-Free Area status. For purposes of this calculation, cattle maintained in accordance with Paragraph 11 of this Section are not to be included.

6. All suspects to the blood serum test shall:
   (c) be a part of a herd included under 2 (a) or (b), or Paragraph 11 of this Section, or

7. All herds (except those which are being maintained in accordance with Paragraph 11 of this Section) in which brucellosis has been known to exist have been legally released from quarantine.

8. All herds in which brucellosis has been found during the latest certification period shall have been retested not less than three months following removal of the last reactors, or be a herd included under 2 (a) or (b), or Paragraph 11 of this Section.

Part V, Section I, Paragraph 11.

11. Beef-type cattle may be maintained for purposes of dry lot feeding in Certified Brucellosis-Free Areas, or in areas seeking such status, provided they are inspected on the premises where held under quarantine, to be moved to slaughter under permit, at the end of the feeding period.

Part V, Section II, Paragraphs B, 1, (d), and 4, 5, and 6.

(d) a complete herd blood test conducted within 18 months prior to the termination of the certification period.

Part V, Section II, B.

1. All herds in the area, with the exception of those maintained in accordance with Paragraph 11, Section I, are represented in one of the following:
4. The number of herds found infected during the entire certification period does not exceed one percent of the area herd population, or one herd, whichever is greater. For purposes of this calculation, herds maintained in accordance with Paragraph 11 of Section I are not to be included.

5. Herds, other than those maintained in accordance with Paragraph 11 of Section I, in which brucellosis has been found have been retested and legally released from quarantine, and, in addition, have been retested not less than three months following removal of the last reactors or be a herd included under 1 (a) or (b) of Paragraph B, this Section.

6. All suspects to the blood serum test shall:

   (c) be a part of a herd included under 1 (a) or (b) of Paragraph B, this Section, or Paragraph 11 of Section I, or

Part V, Section III, Paragraph A.

A. To enter a Certified Brucellosis-Free Area, cattle (except those maintained under Paragraph 11 of Section I, and calves under eight months of age) must be from one of the following:

BRUCELLOSIS ERADICATION

PROPOSED CHANGES IN THE UNIFORM METHODS AND RULES—1961

Part IV, Section I, Area Certification.

Proposed New Paragraph D.

(If proposed new paragraph D is adopted, present paragraphs D, E, and F will be redesignated E, F, and G, respectively.)

D. An Area may be declared a Modified Certified Brucellosis Area under combined market cattle testing and milk ring testing programs provided each herd within the area qualifies according to one of the following four methods listed below and provided vaccination of replacement heifer calves is practiced at a level determined by State and Federal regulating officials in consultation with cattle owners in the area involved. Herds in which reactors are found shall be quarantined until they have passed one negative complete herd blood test at least 30 days following removal of the cattle classed positive, except cattle consigned for immediate slaughter under permit. In the last test of all herds blood tested, the number of reactors shall not exceed one percent of the area cattle population and the number of infected herds shall not exceed five percent.

Methods of Herd Qualification:

1. Brucellosis milk ring tests shall be conducted on dairy herds in the area over a three-year period, with follow-up blood tests of suspicious herds. Each negative herd qualifying under this paragraph must be included in at least two consecutive semi-annual milk ring tests during the third year.

2. Blood tests of breeding cattle over three years of age, and those less than three years of age which are parturient or post-parturient, which are
consigned to market or sale for any purpose. Each negative herd qualifying under this paragraph shall have been enrolled in this program for at least 18 months and the number of animals tested shall be at least 15 percent of the animals over three years of age. Tests may be accomplished at farm or ranch premises, concentration points, sales yards, packing plants or elsewhere. Herds of origin of market cattle reactors disclosed within 15 days after leaving the premises of origin shall have had a complete herd blood test prior to the date certification of the area is requested, or qualify by the following subparagraph.

3. A blood test of a representative sample group of the breeding cows over three years of age in each herd, the sample to contain enough animals to provide a 95 percent probability of finding brucellosis, if present, at the two percent level of disease incidence among the animals tested. (See Graph CA 4-4 to determine sample size for each herd.) The blood test is to be conducted within 18 months prior to the date area certification is requested.

4. A complete herd blood test conducted within 18 months prior to the date area certification is requested.

Part IV, Section I, Paragraph E.

E. If the test of an area as outlined under Part IV, Section I, Paragraphs A, B, or C results in more than two percent positives, or if an initial retest of infected herds as under Section I, Paragraph E, does not qualify the area for certification, it shall be necessary to make a complete area retest in accordance with Paragraphs A, B, C, or D of this Section.

SWINE BRUCELLOSIS

1. The important first step in eradication of swine brucellosis is to require by State and Federal legislation or regulations the movement of only brucellosis-free breeding swine for breeding purposes. Without such a requirement, eradication is not a practical reality.

2. Second only to No. 1 above, is an effective system of identification of slaughter boars, stags and sows that they can be tested at slaughtering establishments and reactor animals traced back to herds of origin.

3. The recommendations of the United States Livestock Sanitary Association on Swine Brucellosis at its 1958 Convention are reaffirmed. These regulations were designed for herd validation. They may be used for Area Validation at the discretion of State and Federal Cooperative officials.

4. Your Committee further recommends that all States enter into cooperative agreements with Animal Disease Eradication officials for the purpose of establishing Pilot Programs to develop Validated Brucellosis-Free Areas.

UNIFORM PLANS FOR ERADICATING SWINE BRUCELLOSIS

A. Validation of Swine Herds as Brucellosis-Free

Validation is made on the basis of two consecutive negative tests on the entire breeding herd 90 days apart. This includes all breeding animals six
BRUCELLOSIS

months of age and over. This validation holds for 12 months. Revalidation is made annually by the passing of a single negative test on the entire herd.

B. Plans for Eradication in Infected Herds

Plan 1. This plan is recommended for commercial herds.

1. Market the entire herd of swine for slaughter as soon as practicable.
2. Clean and disinfect houses and equipment.
3. Replace with stock from validated brucellosis-free herds, placing them on clean ground.
4. Following two consecutive negative tests 90 days apart, the herd is eligible for validation.

Plan 2. This plan is recommended for use in purebred herds where it is desirable to retain valuable blood lines.

1. Separate pigs from sows at 42 days of age or younger and isolate.
2. Market infected herd for slaughter as soon as practicable. If sows are held for later litters, complete isolation is essential.
3. Test the gilts to be used for the following breeding season about 30 days before breeding. Save only those gilts which are negative. Breed only to negative boars.
4. Retest the gilts after farrowing and before removing them from individual farrowing pens. Should reactors be found, they should be segregated from the remainder of the herd. Select only pigs from negative sows for breeding gilts.
5. If herd is not negative at this time, the process is repeated. When the entire herd passes two consecutive negative tests 90 days apart, it becomes eligible for validation.

Plan 3. This plan is not recommended in general but has been found useful in herds where only a few reactors are found and where no clinical symptoms of brucellosis have been noted.

1. Remove reactors from farm.
2. Retest herd at 30-day intervals, removing reactors, until entire herd is negative.
3. Two negative tests, 90 days apart, qualify the herd for validation.
4. If the herd is not readily freed of infection, abandon this plan in favor of Plan 1 or Plan 2.

C. Accessory Regulations

1. Blood samples are to be taken by approved accredited veterinarian.
2. Reactors must be sold for immediate slaughter.
3. Replacement swine may be added without test if procured directly from a validated brucellosis-free herd.

4. All other replacement breeding animals shall have passed a negative agglutination test and be held in isolation until passing a second negative agglutination test. The second test shall be at least 30 days after the first, in the case of boars and open gilts, or after farrowing in the case of breed sows and gilts.

5. All swine on the farm kept for feeding purposes shall be segregated from the breeding herd until moved for slaughter.

6. (A) Negative animal from a herd of unknown or infected status means an animal that disclosed no agglutination in test dilutions of 1/25 or higher.

   (B) Negative animal from a validated herd is one showing a reaction no greater than incomplete in 1/100 dilution.

   (C) Negative herd means a herd that discloses no animal reacting more than incomplete in dilutions of 1/100.

   (D) Reactor animal means an animal that discloses a reaction of complete in the dilution of 1/100 or higher.

   (E) Infected herd means a herd that discloses one or more animals reacting complete in the dilution of 1/100 or higher, than any animal in the herd showing a reaction of complete in dilution of 1/25 or higher shall be considered a reactor.
LEPTOSPIROSIS IN SWINE: REVIEW AND COMMENTS

E. H. Bohl, D.V.M., Ph.D.*

Leptospirosis in swine has been recognized in the United States for only about 11 years. Yet, during this relatively short period there has been considerable interest in this condition. Aside from the increased attention which any newly recognized disease may receive, interest has stemmed from the observation that an appreciable number of swine become infected; that the disease is rather widespread in the United States; that an economically important clinical manifestation of the infection may occur, namely abortion; and that swine may be a source of infection to other animals including man. In addition, rather simple and inexpensive laboratory methods are available which permit the extensive study of various aspects of this disease, and thus the research worker finds it amenable to the collection of considerable data.

In a discussion of leptospirosis in swine it should be kept in mind that there are several species of leptospiras that are known to infect swine under natural conditions; for example, *L. pomona*, *L. canicola*, *L. icterohaemorrhagiae*, and *L. hyos*. At least in the United States, infection of swine with *L. pomona* appears to be the most prevalent and the most important, and will be the principal object of this report. The results of various surveys have shown that from two to 22 percent of the swine tested in the United States have been serologically positive with *L. pomona* (1, 2, 3, 4). However, *L. hyos* has also been incriminated as the cause of abortion in swine (5) and in some countries an appreciable number of swine are serologically positive (5, 6, 7). Although this species has not been isolated from swine in the United States, further attention should be given to its probable presence.

Pathogenesis

When swine are infected with leptospiras the sojourn of the organisms within the infected animal would probably be very similar regardless of the species involved. Briefly, the usual course of events with *L. pomona* would be as follows (3, 8, 9): After entrance of the organism, either through the mucous membrane or broken skin, a leptospiremia of about four to six days’ duration occurs. A febrile reaction, as well as inappetence, for one or two days may be observed during this period. The organisms then become localized in the lumen of the uriniferous tubules of the kidneys. In this location they find proper nutrients for growth and escape the action of antibodies which are then found in high titer in the blood. Leptospiras will then be eliminated in the urine of these apparently healthy swine for a variable period of time, usually for three to five months but in the rare animal possibly for a year (8, 9).

* Department of Microbiology, the Ohio State University, Columbus, Ohio.

133
Most of these infections, regardless of the leptospiral species, occur as a benign and inapparent infection. However, a few possible exceptions will be enumerated.

(1) If infection with *L. pomona* (10, 11) or *L. hyos* (5) occurs in pregnant swine abortion or birth of weak pigs may result. Kemenes (12) reports that abortion is most apt to occur if infection with *L. pomona* takes place in swine that are at least 60 days in the gestation period. Abortion usually occurs within the last three weeks of gestation. In some litters, especially those born near term, the condition of the individual pigs may vary considerably, from dead or weak pigs to those that appear normal.

The course of events leading up to the abortion appears to be as follows: A pregnant sow becomes infected and during the leptospiremic phase the organisms cross the placenta and infect some, if not all, of the fetuses. The fetus provides a suitable location for the propagation of the leptospiroses, especially since antibody from the sow does not reach the fetus nor is the fetus capable of producing antibody. Thus, infection of the fetus often leads to death with subsequent abortion, which frequently occurs about three or four weeks following the initial infection of the sow. The sow usually shows no illness. Aborting sows, thereafter, usually have no impairment in breeding efficiency, and appear to be solidly immune to subsequent infection with the same leptospiral serotype (13).

(2) On rare occasions, illness, including anemia, icterus and hemoglobinuria have been reported in swine infected with *L. pomona* (8, 2, 14). Concurrent infections may aggravate what would normally be a benign infection (15). In contrast to cattle which often show some evidence of anemia, icterus or hemoglobinuria when infected with *L. pomona*, pigs rarely do so. This might be explained on the basis that porcine erythrocytes are much more resistant to the lytic effect of the *L. pomona* hemolysin than are bovine erythrocytes (16). Jaundice and death have been reported in pigs infected with *L. icterohaemorrhagiae* (17, 18).

(3) *L. pomona* readily localize in the kidneys producing varying degrees of inflammation which can be observed microscopically and which may persist for several months (20). However, it is doubtful if this renal damage is severe enough to produce any clinical manifestation (19). Pertinent to this is the report of Morse *et al.* (9) that "the difference between the average daily gain of *L. pomona*-infected swine and that of their uninfected littermates was not statistically significant."

**Transmission**

Swine become infected through the medium of the urine from infected animals. Since it has been shown that many different wild and domesticated
LEPTOSPIROSIS IN SWINE: REVIEW AND COMMENTS

animals (21) may be urinary shedders of \textit{L. pomona}, and since interspecies transmission is known to occur, it may be difficult to know from what animal source an infection originated. However, among the domesticated animals, swine are considered the primary reservoir of \textit{L. pomona} since they usually shed the organism in greatest numbers and for the longest period of time. In many herd outbreaks, the source of infection can be traced to the introduction of infected swine, often a boar (22).

A knowledge of the ability of the leptospirobes to survive outside of the body is of help in understanding its mode of transmission. Dessication and freezing, as occurring under natural conditions, and a low pH will kill them (23). However, under suitable conditions of temperature (10° to 34° C.), pH (6-8.4) and moisture (surface water or supersaturated soil) \textit{L. pomona} may survive for days or weeks (23, 24). There is one report of this organism surviving for 183 days in soil supersaturated with water (23). Surface water and especially small streams, containing urine from infected animals, are probably the best avenues for the spread of leptospiral infections.

Increased use of artificial insemination in swine warrants the further examination of this procedure as a possible mode of transmission. Semen from infected boars could readily contain leptospiral organisms from passage through the urethra. Whether the method of handling, processing and storing the porcine semen would kill the organism I do not know. In this respect, it would be advisable to use serologically negative boars and to vaccinate them with an \textit{L. pomona} bacterin once a year. If it is necessary to use a serologically positive boar, then treatment with an effective chemotherapeutic agent would be indicated.

\textbf{Diagnosis}

A suspicion of leptospirosis in swine would normally occur in those herds where abortion or the birth of weak pigs is a problem. In such cases a laboratory confirmation of a clinical diagnosis can be made by detecting antibodies in high titer (usually above 1:3200 by the agglutination-lysis test) in the serum of several of the involved sows. Also, it is frequently possible to detect numerous viable leptospirobes from the tissues (especially the pericardial fluid and kidney) of those fetuses that are still alive or those that have been dead for just a few hours. Such tissues may be examined for leptospirobes by direct darkfield examination, by inoculation of special culture media, or by animal inoculation.

The determination of a renal carrier state by the use of serological tests is rather uncertain since animals will retain antibodies for a longer period than they remain carriers. However, by determining the antibody titer some idea as to the state of the infection can be obtained. As the agglutination-lysis test is used in our laboratory, we interpret an antibody titer of 1:1600 or above as indicative of a rather recent infection and highly suggestive of a renal carrier state.
Public Health Aspects

Man is susceptible to *L. pomona*, with infected animals, principally swine and cattle, serving as the source of the infections. As the physicians increase their index of suspicion for this disease, more cases are being diagnosed. Most cases are confined to those individuals—farmers, packing house workers, veterinarians—having close contact with infected cattle or swine, or to those who have been exposed by swimming in water contaminated with the urine of infected animals (21, 25, 26, 27). Many of the infections have been diagnosed as a meningitis or influenza. The more common clinical signs are: high fever, severe headache, and pains in the neck, back, joints or calves of the legs (25). Fatal cases must be extremely rare, as no deaths due to uncomplicated causes could be found in the literature.

Infection of swine with *L. canicola* does occur and on several occasions swine have been incriminated as a source of canicola fever in man (28, 29, 30). Coghlan et al. (30) state that "the pig may be as effective a carrier of *L. canicola* as the dog."

When a diagnosis of leptospirosis is made in either swine or cattle it would seem advisable for the veterinarian to call to the farmer's attention the possibility of human infection, without unduly alarming him. Furthermore, certain precautions should be pointed out so as to minimize exposure.

Prevention and Control

Fortunately, there are several basic procedures which can be effectively used in preventing or controlling leptospirosis. How these procedures can be most economically and effectively used will depend upon the local situation or the problem at hand, and thus it may be unwise to make sweeping or general suggestions.

1. Immunization—An *L. pomona* bacterin can be effectively used to prevent or at least diminish the severity of an infection following an exposure (14, 31, 32). Immunization of susceptible gilts or sows prior to breeding should receive serious consideration if there is any likelihood of exposure. Whether immunization will entirely prevent a leptospiruria is somewhat doubtful (32). The antibody titer following immunization will be low and, thus, it is not confused with the high titer that results from a recent infection.

   The voluntary immunization of swine and cattle prior to exhibition at fairs is recommended, which would seem to be a more positive and practical approach than demanding that such animals be serologically negative.

   As soon as the diagnosis of leptospirosis is made in a breeding herd, immunization of all swine on the farm is usually suggested. Obviously, most value will be obtained if the diagnosis and immunization is accomplished in the early stage of the herd infection; that is, before the infection has spread to the majority of the animals. Also, in an actively infected herd, abortions may continue for three to four weeks following immunization.
(2) Serological tests may be used to determine if animals have been infected. Thus, such tests may be effectively used in preventing the entrance of previously infected animals, especially boars, into clean herds, such as is done in the case of brucellosis. However, it should be again emphasized that serologically positive animals are not necessarily carriers.

(3) Treatment of the renal carrier state by the use of chemotherapeutic agents may be of value in special cases. The following antibiotics and dosages have been reported effective in swine: Chlortetracycline, at a level of 200 mg. per pound of feed, free-choice for 14 days (33); tetracycline (polyotic) given intramuscularly (3 mg./lb.), daily for five days (33); dihydrostreptomycin given as one injection of 10 mg. per pound (34); oxytetracycline fed for seven days at a level of 500 to 1,000 gm. per ton of complete feed (35).

In an infected herd of pregnant animals, treatment with a chemotherapeutic agent may be of value in reducing the incidence of abortions or birth of dead or sick animals. Chlortetracycline, fed for 10 days at a level of 400 gm. per ton of feed (14), and oxytetracycline fed for 14 days at 500 gm. per ton of total ration (36), have been reported useful in this respect.

The inauguration of legislative measures to control leptospirosis in swine is viewed, at present by the author, with hesitancy for the following reasons:

1. A simple, standardized serological test, amenable to a proper interpretation of the carrier state of the animal, is not available. However, research with this object in mind is being conducted so that this objection may not be valid in the future.

2. With the number of different species of wild and domesticated animals known to be renal shedders, the problem of control may obviously be magnified. However, the true role which these infected wild animals play in transmitting the infection to domesticated animals is not known.

3. It is questionable if infected swine pose a public health problem of sufficient significance to warrant a control program of doubtful success.

4. The control procedures now available would seem to be more workable and efficient when used on a herd level than on a State or national level. Furthermore, the control of this disease on a herd basis seems to be within the capability, and possibly the responsibility, of the herdsman and his veterinarian.
BIBLIOGRAPHY

LEPTOSPIROSIS IN PENNSYLVANIA—A PROGRESS REPORT

L. G. Clark

Kennett Square, Pennsylvania

The presence of leptospiral infections in Pennsylvania is well established (1, 2, 3). An epizootiologic investigation of the role of wildlife in bovine leptospirosis has been under way in Southeastern Pennsylvania since August, 1959. Our findings to date are summarized in this report.

MATERIALS AND METHODS

Herds with leptospirosis are located by serologic testing* of cattle sera submitted by cooperating veterinarians. Urine specimens from animals demonstrating high leptospiral antibody titers are then cultured. Detailed information concerning cultural and serologic procedures has been published elsewhere (2).

Water samples from ponds and streams on infected premises are tested for pH and inoculated into guinea pigs. Kidneys from guinea pigs are removed 30 days post-inoculation and cultured for leptospires.

After completion of serologic tests and urine cultures, an intensive wildlife trapping program is begun on the immediate premises of the infected herd. Approximately 100 wildlife specimens are collected in each trapping area; an attempt is made to include all species normally present in any given area. Live-trapped specimens are transported to the Leptospirosis Laboratory at New Bolton Center where each animal is assigned a tag number. Larger animals are killed by asphyxiation in a carbon monoxide chamber; chloroform is used for small animals.

All wildlife specimens, except small rodents, are exsanguinated, fixed to a board, cleaned by hosing, and disinfected with Benzylkonium chloride.\textsuperscript{Rx} One kidney is removed aseptically from each animal and cultured.

\textsuperscript{Rx} — Rocal, Winthrop Laboratories, New York, N. Y.

* — Microscopic agglutination test (11).

All cultures are examined by darkfield microscopy at 10-14 day intervals. When growth is detected, subcultures are made and sent to the Leptospirosis Unit, National Animal Disease Laboratory, Ames, Iowa, for identification.

RESULTS

From August, 1959, through July, 1961, we have studied 397 cattle in 34 herds. In each herd the presence of infection was verified by serological and/or cultural means. Cattle with high antibody titers were always present; in a number of herds it was possible to isolate the infecting serotype.

Trapping activities have been completed on 16 infected premises; we are currently trapping two additional farms. Results of completed studies are
LEPTOSPIROSIS IN PA.—PROGRESS REPORT

summarized in Table I. The infecting herd serotype as well as wildlife isolates are indicated. *L. pomona* was the most commonly occurring herd serotype (11/16 herds) in this area. *L. hardjo* was next (4/16), and *L. ballum* (1/16) was third. In 18 additional herds, not listed in Table I, *L. pomona* was the predominant serotype in 14 herds, *L. hardjo* in two herds, and *L. icterohemorrhagiae* in two herds.

Wildlife studies are summarized in Table II. The data includes species identification, numbers trapped, percent of each species yielding leptospiral isolates, and infecting serotypes.

Streams on two premises yielded isolates (Table III).

**DISCUSSION**

The results of this limited study indicate that many wildlife species in Southeastern Pennsylvania are natural hosts for leptospires. However, wildlife species do not appear to constitute important reservoirs of infection for cattle. In fact, as cattle are the most important carriers of *L. pomona* in this area, it is entirely possible that they constitute a serious source of infection for wildlife.

It is noteworthy that certain serotypes are isolated more commonly from some species than from others: *L. ballum* and *L. pomona* from the opossum (*Didelphis virginiana*); *L. icterohemorrhagiae* and *L. pomona* from the raccoon (*Procyon lotor*); *L. ballum* from the common mouse (*Mus musculus*); and *L. pomona* from the red fox (*Vulpes fulva*) and the skunk (*Mephitis mephitis*), (Table II).

Nine new leptospira-host relationships have been established in this study: *L. pomona* in the woodchuck (*Marmota monax*) (5); *L. ballum* in the vole (*Microtus pennsylvanicus*) (8); *L. icterohemorrhagiae* in the raccoon (*Procyon lotor*) (4); *L. icterohemorrhagiae* in the common mouse (*Mus musculus*) (2); *L. pomona, L. ballum* and *L. icterohemorrhagiae* in the red fox (*Vulpes fulva*) (6); and *L. icterohemorrhagiae* in the muskrat (*Ondatra zibethica*) (7) and gray fox (*Urocyon cinereoargenteus*) (4).

Owing to the many difficulties involved in recovering leptospires from cattle, field isolations alone are probably not truly representative of the serotype spectrum in this area. Identification of infecting serotypes required the application of serologic as well as cultural techniques.

Wildlife did not appear to play a role in herd infections, with the possible exception of herd H-1-60. *L. icterohemorrhagiae*, isolated from eight wildlife specimens on this premise, appeared to be present in the herd as well.

Leptospires were isolated from wildlife on all but two of the 16 premises trapped. However, the epidemiological significance of many isolates is probably negligible. Of 140 common mice (*Mus musculus*) trapped, 16 yielded *L. ballum*. Though all 16 infected mice lived in close proximity to herds under investigation, only herd C-3-60 presented serologic evidence of exposure to *L. ballum*. Similarly, eight of 145 raccoons (*Procyon lotor*) were carriers of *L. icterohemorrhagiae*, but only herd H-1-60 appeared to have experienced infection with *L. icterohemorrhagiae*. 
A total of 1766 wildlife specimens have been cultured. Of these, only 34 (1.9 percent) yielded *L. pomona* isolates, yet this serotype was present in 11 of 16 herds investigated. This level of infection in the wildlife does not appear to be significant.

Only six of the 20 species encountered in this area appear to be true carriers of leptospirosis (Table II): skunks (*Mephitis mephitis*), gray foxes (*Urocyon cinereoargenteus*), red foxes (*Vulpes fulva*), common mice (*Mus musculus*), Norwegian rats (*Rattus norvegicus*) and opossums (*Didelphis virginiana*). Owing to the small numbers trapped, the importance of the gray fox as a leptospirospiral carrier is not yet clear.

Cultures of 37 streams and ponds on 34 premises yielded one isolation each of *L. pomona* and of *L. ballum* (Table III). For survival in surface waters, leptospires require a pH near neutrality (9, 10).

The two water isolations obtained were from streams with a neutral or nearly neutral pH. The pH of a majority of streams in this area falls within required limits. However, most streams or ponds cannot be adequately tested, as too large a volume of water and too many guinea pigs are required.

**SUMMARY**

Cultural and serologic studies of 397 cattle in 34 herds have been completed. A total of 1,766 wildlife specimens comprising 20 genera, trapped on 16 infected premises, have also been tested. *L. pomona* was the predominant herd serotype in 11 herds, *L. hardjo* in four herds, and *L. ballum* in one herd. In 18 additional herds the predominant serotypes were: *L. pomona* (14), *L. hardjo* (2) and *L. icterohemorrhagiae* (2). Nine new leptospira-host relationships were established: *L. pomona* in the woodchuck (*Marmota monax*) and red fox (*Vulpes fulva*); *L. icterohemorrhagiae* in the raccoon (*Procyon lotor*), mouse (*Mus musculus*), red fox, gray fox (*Urocyon cinereoargenteus*) and muskrat (*Ondatra zibethica*); *L. ballum* in the meadow vole (*Microtus pennsylvanicus*) and red fox *L. pomona* and *L. ballum* were isolated from surface waters.

**AUTHORS**

Lawrence G. Clark, D.V.M., and Joseph I. Kresse, B.S.

Robert R. Marshak, D.V.M.

Charles J. Hollister, D.V.M.

Edward A. Carbrey, V.M.D.

United States Department of Agriculture, Agricultural Research Service, A.D.E.D., Laboratory Services assigned to the University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania.


In Charge, Leptospirosis Unit, National Animal Disease Laboratory, Ames, Iowa.
### TABLE I

<table>
<thead>
<tr>
<th>Herd Code</th>
<th>Predominant Serotype in Herd</th>
<th>Wildlife Host</th>
<th>Wildlife Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1-59</td>
<td>L. pomona (I)</td>
<td></td>
<td>No isolations</td>
</tr>
<tr>
<td>J-1-59</td>
<td>L. pomona (S)</td>
<td>skunk</td>
<td>L. icterohemorrhagiae</td>
</tr>
<tr>
<td>J-1-59</td>
<td></td>
<td>raccoon</td>
<td>L. pomona</td>
</tr>
<tr>
<td>J-1-59</td>
<td></td>
<td>2 raccoons</td>
<td>L. icterohemorrhagiae</td>
</tr>
<tr>
<td>J-1-59</td>
<td></td>
<td>opossum</td>
<td>L. ballum</td>
</tr>
<tr>
<td>J-1-59</td>
<td></td>
<td>common mouse</td>
<td>L. ballum</td>
</tr>
<tr>
<td>J-3-59</td>
<td>L. pomona (S)</td>
<td>opossum</td>
<td>L. ballum</td>
</tr>
<tr>
<td>J-3-59</td>
<td></td>
<td>2 raccoons</td>
<td>L. pomona</td>
</tr>
<tr>
<td>J-3-59</td>
<td></td>
<td>3 skunks</td>
<td>L. pomona</td>
</tr>
<tr>
<td>J-3-59</td>
<td></td>
<td>common mouse</td>
<td>L. ballum</td>
</tr>
<tr>
<td>J-4-59</td>
<td>L. pomona (S)</td>
<td>opossum</td>
<td>L. pomona</td>
</tr>
<tr>
<td>J-4-59</td>
<td></td>
<td>skunk</td>
<td>L. ballum</td>
</tr>
<tr>
<td>J-6-59</td>
<td>L. pomona (I)</td>
<td>skunk</td>
<td>L. pomona</td>
</tr>
<tr>
<td>J-6-59</td>
<td></td>
<td>opossum</td>
<td>L. pomona</td>
</tr>
<tr>
<td>J-6-59</td>
<td></td>
<td>opossum</td>
<td>L. ballum</td>
</tr>
<tr>
<td>J-6-59</td>
<td></td>
<td>2 common mice</td>
<td>L. ballum</td>
</tr>
<tr>
<td>J-7-59</td>
<td>L. pomona (I)</td>
<td>woodchuck</td>
<td>L. pomona</td>
</tr>
<tr>
<td>J-7-59</td>
<td></td>
<td>opossum</td>
<td>L. pomona</td>
</tr>
<tr>
<td>J-7-59</td>
<td></td>
<td>4 opossums</td>
<td>L. ballum</td>
</tr>
<tr>
<td>J-7-59</td>
<td></td>
<td>3 common mice</td>
<td>L. ballum</td>
</tr>
<tr>
<td>K-14-59</td>
<td>L. pomona (I)</td>
<td>red fox</td>
<td>L. pomona</td>
</tr>
<tr>
<td>K-14-59</td>
<td></td>
<td>3 common mice</td>
<td>L. ballum</td>
</tr>
<tr>
<td>L-1-59</td>
<td>L. pomona (S)</td>
<td>red fox</td>
<td>L. pomona</td>
</tr>
<tr>
<td>A-3-60</td>
<td>L. pomona (I)</td>
<td>opossum</td>
<td>L. pomona</td>
</tr>
<tr>
<td>C-4-60</td>
<td>opossum</td>
<td>L. ballum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Adjacent farms trapped at</td>
<td>red fox</td>
<td>L. pomona</td>
</tr>
<tr>
<td></td>
<td>same time)</td>
<td>2 raccoons</td>
<td>L. pomona</td>
</tr>
<tr>
<td></td>
<td></td>
<td>raccoon</td>
<td>L. icterohemorrhagiae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 skunks</td>
<td>L. pomona</td>
</tr>
<tr>
<td>C-1-60</td>
<td>L. pomona (S)</td>
<td></td>
<td>No isolations</td>
</tr>
<tr>
<td>C-3-60</td>
<td>L. ballum (S)</td>
<td>skunk</td>
<td>unidentified</td>
</tr>
<tr>
<td>C-3-60</td>
<td></td>
<td>red fox</td>
<td>L. pomona</td>
</tr>
<tr>
<td>C-3-60</td>
<td></td>
<td>opossum</td>
<td>unidentified</td>
</tr>
<tr>
<td>H-1-60</td>
<td>L. hardjo (I)</td>
<td>5 raccoons</td>
<td>L. icterohemorrhagiae</td>
</tr>
<tr>
<td>H-1-60</td>
<td>L. icterohemorrhagiae (S)</td>
<td>muskrat</td>
<td>L. icterohemorrhagiae</td>
</tr>
<tr>
<td>H-1-60</td>
<td></td>
<td>grey fox</td>
<td>L. icterohemorrhagiae</td>
</tr>
<tr>
<td>H-1-60</td>
<td></td>
<td>common mouse</td>
<td>L. icterohemorrhagiae</td>
</tr>
<tr>
<td>H-1-60</td>
<td></td>
<td>skunk</td>
<td>L. pomona</td>
</tr>
<tr>
<td>H-1-60</td>
<td></td>
<td>opossum</td>
<td>L. ballum</td>
</tr>
<tr>
<td>Herd Code</td>
<td>Predominant Serotype in Herd</td>
<td>Wildlife Host</td>
<td>Wildlife Isolates</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------</td>
<td>---------------</td>
<td>------------------</td>
</tr>
<tr>
<td>J-3-60</td>
<td>L. hardjo (I)</td>
<td>4 common mice</td>
<td>L. ballum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>meadow vole</td>
<td>L. ballum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red fox</td>
<td>L. ballum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>skunk</td>
<td>L. ballum</td>
</tr>
<tr>
<td>K-5-60</td>
<td>L. hardjo (S)</td>
<td>2 skunks</td>
<td>L. pomona</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red fox</td>
<td>L. pomona</td>
</tr>
<tr>
<td></td>
<td></td>
<td>common mouse</td>
<td>L. ballum</td>
</tr>
<tr>
<td>A-3-61</td>
<td>L. pomona (S)</td>
<td>2 raccoons</td>
<td>L. pomona</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 skunks</td>
<td>L. pomona</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 rats</td>
<td>L. icterohemorrhagiae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>red fox</td>
<td>L. icterohemorrhagiae</td>
</tr>
<tr>
<td>F-1-61</td>
<td>L. hardjo (S)</td>
<td>woodchuck</td>
<td>L. pomona</td>
</tr>
</tbody>
</table>

(I) = serotype determined by isolation.
(S) = serotype determined by serologic testing.
<table>
<thead>
<tr>
<th>Host</th>
<th>No. of Trapped</th>
<th>No. of Isolations</th>
<th>Percent Infected</th>
<th>Serotype Serotypes Isolated</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>woodchuck (Marmota monax)</td>
<td>324</td>
<td>3</td>
<td>0.9</td>
<td>L. pomona* (5)</td>
<td>3</td>
</tr>
<tr>
<td>vole (Microtus pennsylvanicus)</td>
<td>249</td>
<td>1</td>
<td>0.4</td>
<td>L. ballum* (8)</td>
<td>1</td>
</tr>
<tr>
<td>opossum (Didelphis virginiana)</td>
<td>186</td>
<td>16</td>
<td>8.6</td>
<td>L. ballum</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. pomona</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>unidentified</td>
<td>2</td>
</tr>
<tr>
<td>squirrel (Sciurus carolinensis)</td>
<td>165</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>raccoon (Procyon lotor)</td>
<td>145</td>
<td>15</td>
<td>1.0</td>
<td>L. icterohemorrhagiae* (4)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. pomona</td>
<td>7</td>
</tr>
<tr>
<td>common mouse (Mus musculus)</td>
<td>140</td>
<td>18</td>
<td>12.9</td>
<td>L. icterohemorrhagiae* (2)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. ballum</td>
<td>16</td>
</tr>
<tr>
<td>deer mouse (Peromyscus supp.)</td>
<td>79</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rat (Rattus norvegicus)</td>
<td>74</td>
<td>7</td>
<td>9.5</td>
<td>L. icterohemorrhagiae</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. ballum</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>unidentified</td>
<td>4</td>
</tr>
<tr>
<td>red fox (Vulpes fulva)</td>
<td>69</td>
<td>7</td>
<td>9.86</td>
<td>L. pomona* (6)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. ballum*</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. icterohemorrhagiae*</td>
<td>1</td>
</tr>
<tr>
<td>rabbit (Sylvilagus floridanus)</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>feral cat (Felis domesticus)</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>skunk (Mephitis mephitis)</td>
<td>54</td>
<td>18</td>
<td>33.3</td>
<td>L. pomona</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. ballum</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>unidentified</td>
<td>1</td>
</tr>
<tr>
<td>shrew (Blarina spp.)</td>
<td>53</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chipmunk (Tamias striatus)</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>deer (Odocoileus virginianus)</td>
<td>28</td>
<td>1</td>
<td>3.6</td>
<td>L. pomona</td>
<td>1</td>
</tr>
<tr>
<td>muskrat (Ondatra zibethica)</td>
<td>25</td>
<td>1</td>
<td>4.0</td>
<td>L. icterohemorrhagiae* (7)</td>
<td>1</td>
</tr>
<tr>
<td>gray fox (Urocyon cinereoargenteus)</td>
<td>11</td>
<td>2</td>
<td>18.1</td>
<td>L. icterohemorrhagiae* (4)</td>
<td>2</td>
</tr>
<tr>
<td>feral dog (Canis familiaris)</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mole (Scalopus aquaticus)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>weasel (Mustela frenata)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = establishes new host for serotype indicated. (5) = reference.
TABLE III

Stream Isolations

<table>
<thead>
<tr>
<th>Herd Code</th>
<th>Herd Serotype</th>
<th>Stream pH</th>
<th>Water Volume Inoculated Per Guinea Pig</th>
<th>Serotype Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-3-60</td>
<td>L. pomona</td>
<td>6.9</td>
<td>0.1 ml.</td>
<td>L. pomona</td>
</tr>
<tr>
<td>J-3-60</td>
<td>L. hardjo</td>
<td>7.0</td>
<td>5.0 ml.</td>
<td>L. ballum</td>
</tr>
</tbody>
</table>

REFERENCES

REPORT OF COMMITTEE ON LEPTOSPIROSIS

E. E. ROTH, Chairman, Baton Rouge, Louisiana; E. H. BOHL, Columbus, Ohio; R. J. BYRNE, Silver Spring, Maryland; E. A. CARBREY, Ames, Iowa; J. A. HOWARTH, Davis, California; D. E. HUGHES, Beltsville, Maryland; S. G. KENZY, Pullman, Washington; A. W. MONLUX, Stillwater, Oklahoma; C. S. ROBERTS, Auburn, Alabama; W. L. SIPPEL, Kissimmee, Florida; L. W. TURNER, Nashville, Tennessee; M. J. TWIEHAUS, Manhattan, Kansas.

In past years this Committee has reviewed the available knowledge of leptospirosis and has considered its possible application for eradication of the disease. It is the consensus of this Committee that leptospirosis is not amenable to eradication. This view is due to the widespread occurrence of several serotypes in many species of wild and domestic animals. It is further complicated by the difficulty in detecting carrier animals. We believe that economic losses attributable to leptospirosis can be reduced by the judicious application of control measures. Most of these details have been presented in previous reports and will not be repeated here.

Field use of the macroscopic plate test by inexperienced persons has resulted in misunderstanding and confusion because of false positive reactions. Leptospirosis tests should be performed in diagnostic laboratories by qualified personnel. Furthermore, we recommend that the diagnostic laboratories adopt the agglutination-lysis test as outlined in last year's report.

Continued isolations of *Leptospira hardjo* from cattle in Louisiana and isolations from cattle in Nebraska and Pennsylvania suggests that this serotype may be a factor in bovine leptospirosis. An effort should be made by the Agricultural Research Service to investigate the incidence of *L. hardjo* infection on a national scale. Serological studies and cultural isolations are necessary to establish whether or not there is a need for bacterins prepared from serotypes other than *L. pomona* as well as to provide information on the possible occurrence of other leptospiral serotypes among domestic animals. Many of these serotypes have already been isolated from wild animals in the United States.

The promulgation of regulations which discriminate against animals having low antibody titers is not desirable. The employment of such practices creates in the minds of livestock owners the misconception that serological tests will identify the animals spreading the disease. Serological findings should be used only as evidence to aid in the diagnosis of leptospirosis.

*Leptospira pomona* is still recognized as the predominant cause of leptospirosis in cattle and swine. Evidence of infection with other serotypes (*L. hardjo* and *L. canicola* in cattle) continues to accumulate. Until more information about these infections is available, vaccination with serotypes other than *L. pomona* is not justified.
As is evident from the preceding remarks, additional research and field studies are necessary to define the leptospirosis more clearly. A new tool that will accurately detect the carrier state is urgently needed. New information should be evaluated regularly and used, when indicated, for periodic revision of past recommendations. We respectfully suggest that this Committee remain active and submit this report to the Executive Committee for approval.
AN EVALUATION OF EXISTING AND PROPOSED MASTITIS
CONTROL PROGRAMS AND PROPOSALS OF THE
NATIONAL MASTITIS COUNCIL

R. W. METZGER

New York

This discussion of bovine mastitis control programs is presented as a
function of the Committee on Control Programs and Procedures of the
National Mastitis Council.

Organization

In the spring of 1960, the executive board of the International Association
of Milk and Food Sanitarians appointed a special committee to organize an
invitational meeting for the purpose of presenting a review of the problem
of bovine mastitis to interested leaders in the dairy industry, to health and
agriculture agencies of Federal, State, and local government, and to certain
professional groups in the hope of initiating a uniform national effort for
the control of this disease.

This special committee, known as the Mastitis Action Committee, was
composed of representatives of the Farm Methods Committee of the I.A.M.F.S.,
the American Veterinary Medical Association, the American Farm Bureau
Federation, the National Milk Producers Federation, the United States Depart-
ment of Agriculture, the United States Public Health Service, dairy trade
publications, and dairy trade associations. This Committee developed a
conference which was held on October 29, 1960, in Chicago with over 200
people present, representing producers, processors, public health and regu-
larity agencies, and educational institutions. The conference program con-
sidered four areas:

1. The Public Health Problem  3. The Status of Research
2. The Economic Problem  4. The Regulatory Problem

From this October 29 meeting came a directive to the I.A.M.F.S. Farm
Methods Committee to develop a continuing organization to serve as a
national force in furthering mastitis research and control. The conference
recommended that a national committee for mastitis action should be estab-
lished composed of one to three representatives of appropriate organizations
interested in the program.

Following the conference, the Farm Methods Committee of I.A.M.F.S. took
steps as directed to create a continuing national organization. A document
of Incorporation was established. Over a dozen organizations indicated a
desire for membership. Officers were elected and standing committees
established to begin the work of the new organization. The National Mastitis
Council is now incorporated in Illinois as a nonprofit corporation. Its work is being done by representatives of its member organizations. Representatives of the United States Department of Agriculture and the United States Department of Health, Education and Welfare, serve as consultants without vote rather than as a part of the policy making body. The National Mastitis Council now has a president, executive secretary, and treasurer. Standing committees have been appointed on finance, research, education, and programs and procedures.

The Problem

The problem caused by mastitis is one of many facets involving public health and economic considerations and unique requirements for the development of control programs.

Consider the public health problem. Human infection may result from the consumption of raw milk containing the organisms which are often involved in bovine mastitis. This group of organisms includes Escherichia coli, Corynebacterium pyogenes, Pasteurella multocida, and Salmonella species, and certain streptococci. Food poisoning may result from staphylococcus enterotoxins which may be present as a result of the growth of Staphylococcus aureus. Such food poisoning outbreaks have been reported in people who have consumed foods made with spray-dried skim milk powder. These instances have occurred in England and Puerto Rico. Staphylococcus enterotoxin food poisoning has been reported in several midwestern states due to eating cheddar cheese containing the enterotoxin. Still another public health problem (although now under apparent control) is the presence of antibiotic residues in milk. Persons who develop sensitivities to certain antibiotics may experience severe allergic reactions after consuming milk or milk products containing antibiotic residues.

Consider the economic problem. Mastitis is the most costly disease affecting the dairy industry. Yet it may not be very dramatic or even particularly apparent to the individual dairyman. Although it may be constantly leaching away a dairyman's profits, it does not alert him in a dramatic way to the serious losses which he is constantly suffering as would the loss of a total herd from a positive tuberculin test or a positive blood agglutination test for Brucellosis. The United States Department of Agriculture publication, *Losses in Agriculture, 1954,* reported an estimated loss due to mastitis of $225,805,000 per year. This figure represents only the loss of animals and milk. When you consider the cost of therapy, milk withheld following mastitis treatment, and the value of unsalable milk discarded because of untreated mastitis, the total annual loss will approach a half billion dollars per year. This figure would be considerably higher if the time spent by the dairy farmer in feeding, raising feed, and other activities were given a dollar value and included in the total losses. The cost of therapy has been increasing. Bulk antibiotics increased 1.3 million dollars from 1958 to 1959. This may indicate an increased usage of antibiotics as well as an increase in the incidence of mastitis. It may also indicate that with greater availability, antibiotics have
been used more freely and probably without the benefit of an accurate diagnosis.

Consider the control problem. Bovine tuberculosis and bovine brucellosis are diseases caused by specific organisms and lend themselves quite readily to specific test and elimination procedures. They can both be controlled primarily by the action of State and Federal government agencies. Control of these diseases is dependent upon a single test and upon elimination of the animals found to be infected on any given test. Mastitis is a disease caused by a variety of microorganisms and aggravated by a multitude of stress factors. The control of mastitis will depend primarily upon the dairyman and his management practices and, secondarily, upon the professional assistance he may be able to obtain.

Programs

The Committee on Programs and Procedures of the National Mastitis Council has attempted to make a study of what is currently being done in the field of mastitis control. In making this study, the Committee sent questionnaires to several potential sources of information in each State. These sources included the State health department, the State department of agriculture, agriculture experiment station or agriculture extension service, college of agriculture, and college of veterinary medicine (17 states). The return from these questionnaires was very gratifying and shows a significant effort being made and widespread planning for further control activities. One must remember that this report is a strict evaluation of the information given in the replies to the questionnaire.

Most states have some type of educational program. These programs consist mainly of distributing educational literature containing information on suggested mastitis control measures. Many states conduct courses for county agriculture agents or agriculture leaders instructing them in control methods. States having schools of veterinary medicine periodically hold refresher courses for veterinarians.

Seven states have proposed programs. In these states committees have been formed, procedures outlined, and in some cases studies have been made of active programs in other states.

Ten states indicate that they have an active control program. However, none of these is capable of providing complete service to even the Grade A milk producers. These programs vary from a state-wide effort to research and control projects limited to small areas of the State. Just two states have what could be called a full-fledged program offering service to the entire State.

Eleven states reported funds available for mastitis control activities. Two of these did not indicate the amount of money. The remaining nine had funds ranging from $1,500 to $235,000 per year.

Thirty-five states reported a total of 86 laboratories equipped to conduct mastitis diagnostic work. Sixty-three of these are State laboratories, seven are operated by the dairy industry, and 16 are privately owned laboratories. Several of the states reporting active programs did not indicate that laboratory facilities were available. The existence of more facilities is possible.
Judging from the reports, two states have what the National Mastitis Council considers to be a State mastitis control program. Connecticut was the first State to develop a program having had State funds appropriated each year since 1940. One laboratory at the University of Connecticut conducts the laboratory examinations on the quarter samples collected by trained field technicians. *Streptococcus agalactiae* is eliminated first and other organisms dealt with as they appear. The participating dairymen must work with a veterinarian to assure effective treatment and elimination of the disease. Educational activities are conducted through leaflets sent to dairymen.

New York's program organized in 1956 is quite similar to that of Connecticut, except that New York State may give more consideration to abnormal secretions or abnormal milk than does the Connecticut program. Both states have developed an impressive number of *Streptococcus agalactiae* free herds. Indication of the value of elimination of *S. agalactiae* is found in reports from Connecticut as well as New York State. Reports from Connecticut, a few years ago, revealed that a group of *S. agalactiae* free herds produced an average of 10 percent more milk than they did when infected. The 1960 Annual Report of the New York State Veterinary College shows that in herds where 35 percent of the cows were infected with *S. agalactiae*, there was an incidence of 13 percent abnormal secretion; whereas, in the herds free of *S. agalactiae* the incidence of abnormal secretions was six percent. This is a very good example of the effect *S. agalactiae* elimination can have on milk quality.

Another indication of milk quality in relation to mastitis control also appears in the 1960 New York State report. In 111 herds not under an organized mastitis control program 70 percent delivered milk with one million or more leucocytes; whereas, 94 herds under a mastitis control program revealed 23 percent with a count of one million or more cells.

Some other states have programs that are not as extensive as those in Connecticut and New York. California has a mastitis control project involving 35 counties under the direction of the School of Veterinary Medicine and the Extension Service. This project provides services to 540 herds containing about 85,000 cattle. In this project the California Mastitis Test is used for screening purposes. Seventeen laboratories furnish the laboratory support for the project. Much attention is directed toward the milking equipment as the major source of stress predisposing the cow to mastitis. Funds for the project are obtained from fees charged for the California Mastitis Test. Training courses are available to veterinarians, extension personnel, and dairymen.

Florida has a mastitis prevention and control program stressing good herd management, milking techniques, and proper milking machine installation. This program is operated on an annual budget appropriation. One laboratory services the project.

Maine conducts surveys on milk as received at the dairy plant. Dairymen having positive samples are notified and advised to contact their local veterinarians. Dairymen with unusual problems may seek assistance from the State.
Michigan is beginning a State program consisting primarily of educational activity.

New Hampshire operates a service program, making a small charge for tests. Dairymen are currently demanding more service.

Vermont has a prevention and control program operated by the State extension service. Training sessions and demonstrations are provided which explain the California Mastitis Test, proper milking equipment operation, and good herd management practices. (Since this survey, Vermont has organized a State Mastitis Council and is represented in the National Council.)

Wisconsin has a program which is an extension of the experimental project originating at the University of Wisconsin. Consideration is currently being given to extending this project to a state-wide program which will include checking milking equipment and milking procedures, along with the laboratory examination of quarter samples and the provision of advice to dairymen. This is a cooperative effort of the University of Wisconsin, the State dairy industry, and practicing veterinarians.

Puerto Rico does not have a program but it is interesting to note that in recent field surveys using the California Mastitis Test as many as 72 percent of the cattle in one area were positive.

In addition to State programs, several local studies have been made and in one case, a regulatory program is being developed. The New York City Department of Health, in cooperation with the New York State Mastitis Control Program and the State Veterinary Medical Society, inaugurated a “screening” test for detection of inferior quality of milk due to mastitis or abnormal udder secretions. Modified Whiteside Tests were conducted on milk collected from bulk or weigh tanks delivered to six plants supplied by 640 farms. It was found that about eight percent of the milk contained markedly abnormal secretions. Farm visits were made by plant sanitarians where abnormal or high cell counts were indicated. The dairyman was given 72 hours to correct the condition. If it was not cleared up in that time, he was then required to employ a veterinarian to make a mastitis examination of his herd and introduce such therapeutic and corrective measures as indicated. Repeat offenders or those with a very serious mastitis condition were required to utilize the New York State mastitis control program. The number of grossly abnormal samples was substantially reduced during the four-month test period. This program will continue on a broader scale this coming year, substituting the screening test and follow-up for three of the quarterly physical examinations which are now required by that department on farm bulk milk. It was thought that pursuing the program in this manner will give added data in regard to its applicability on a widespread scale.

It appears that a workable test which would identify mastitis through poor quality milk due to mastitis, followed by farm visits by the milk sanitarian, together with the dairyman’s knowing that such a test is being used, creates a strong educational psychological incentive that will lead to improved milk quality.
Saginaw, Michigan, also has what appears to be an excellent screening program having high potential. Milk samples are taken monthly from each producer's delivery. A microscopic examination of the milk is made for presence of leucocytes. Dairymen producing milk with a count in excess of 1,000,000 cells are notified that there is evidence of mastitis in their milk. They hope to reduce this eventually to a standard of 500,000 per ml. Farmers exceeding the one million count receive a written notice and, if repetition occurs during the following month, the producer receives a second notice together with a form for his veterinarian to use for reporting the results and action taken when he checks the herd. There is also provision for a third notice, but this is apparently seldom necessary. In addition to examining deliveries of milk at Saginaw, they also use the California Mastitis Test for examination of cows in herds showing an indication of mastitis. Following is a quotation from the report to the National Mastitis Action Committee sent by Mr. E. D. Shreve, Milk Sanitarian, Saginaw, Michigan.

"Good mastitis control results in better quality milk. Those same factors which lessen the danger of udder irritation, injury, or infection, also lessen bacterial contamination of the raw milk supply. In July 1960, 21 percent of our herds showed evidence of mastitis as determined by a direct microscopic examination of a stained smear of raw milk taken from each producer's supply. During this same period, 59 percent of the producers received a Blue Ribbon card for having produced milk having a bacteria count of less than 25,000 per ml. In January 1961, four percent of our herds showed evidence of mastitis, and 83.2 percent of our producers received a Blue Ribbon card."

As a comparison of work in the United States with other countries, Dr. H. G. Hodges, on a recent visit to Sweden, Denmark, and England, found that mastitis workers in those countries also utilize a screening test for detecting abnormal milk.

During the past several years there has been an increased interest by all segments of the dairy industry in mastitis control. The lack of similarity in existing programs and varying degrees of effectiveness indicates a very great need of serious consideration being given to development of uniform and practical approaches to the problem. It is recognized that certain areas of dairying have their own peculiarities, but basically the cow is the same and the disease mastitis is quite similar wherever we find it in the United States. No great success would have been achieved with tuberculosis or brucellosis had we not had a uniform and standard procedure of control and elimination. Bovine mastitis as a disease is, to be sure, a much different condition but without a well designed and properly organized approach, we will not get far in combating this disease.

Since the Mastitis Action Committee held its first meeting in Chicago in October 1960, there seems to have been a marked increase in the interest in mastitis programs in several states. This is an excellent and healthy development. However, there are certain points that we believe to be imperative when
organizing a mastitis control program in any State. If we restrict a program to any one phase of the problem without giving attention to the over-all picture, definite limitations will occur.

A well balanced program should be based on several different factors or phases.

1. A well developed education program.
2. Screening tests through examination of milk supplies as an indication of the existence of mastitis in individual herds.
3. Diagnosis of mastitis either physically or bacteriologically in the herds indicated to have problems.
4. Installation of an over-all good management program to include regular inspection of the milking equipment by qualified technicians.

An approach which ignores any one of the above mentioned phases will have little success. If we should follow the diagnostic approach without any recognition given to management and prevention, our success would be nullified, because it takes something more than diagnosis and treatment to control bovine mastitis. On the other hand, if we approach it from management alone without giving recognition to the fact that bovine mastitis is a disease of the cow's udder and associated with many different types of bacteria, again our success will be limited. These organisms within the udder will continue to cause trouble unless they are recognized and dealt with in the manner indicated.

Furthermore, should we introduce an educational program without any attention given to recommendations for control programs, no great success can be expected.

The most practical approach to the problem is one of the well organized program no matter how small. At a meeting of the National Mastitis Council, the following recommendation was made:

"The states should establish committees with representatives of milk producers, milk processors, public health and agricultural agencies, veterinary medical associations, education, and other allied groups to evaluate the extent of the mastitis problem; determine the resources for mastitis control and research; actively encourage and support research in mastitis control; and perform such other functions as are recommended by the National Mastitis Council."

Here is a very definite need for an organization with aggressive leadership. One key participant is, of course, the dairyman. Without his cooperation, participation and assistance, no program can be a success. There are great potentialities with proper leadership, proper organization, and a good relationship among these various groups, combined with a well organized approach to mastitis control.

No group is in a better position to assist with a mastitis control program than are milk sanitation agencies. Excellent examples, as reported earlier, are found in Saginaw, Michigan, and New York City, N. Y. Location of
the herds with serious trouble with mastitis can best be accomplished through screening tests, such as the California Mastitis Test, or modified Whiteside Test, on bulk or weigh tank milk samples. A mastitis program which combines a physical examination, diagnosis and treatment has value; but bacteriological examination of carefully collected milk samples provides information on infection that gives the veterinarian a good indication of the most satisfactory control procedures. This approach must be preceded by a good educational program to teach dairy farmers better sanitation, management, and attention to the care of the cow's udder. Here is a wonderful opportunity for sanitarians to work in close cooperation with the Extension Service in teaching dairy farmers these important methods.

We know that we do not have all the answers to the problems of mastitis, and there is a great need of more research activities than now exists. We not only need new research projects, but coordination of existing research. In this area, the Committee on Research of the National Mastitis Council will prove to be an invaluable tool to researchers in the field of mastitis.

The National Mastitis Council is a young organization struggling to give people in the United States a better concept of practical, workable mastitis control programs and such assistance as may be needed by states wishing to organize such programs. One pertinent activity is the preparation of information of successful programs and procedures for the information of states, organizations and individuals who wish to do something about the problem of bovine mastitis.

In conclusion I would like to recommend that the United States Livestock Sanitary Association consider membership in the National Mastitis Council, Inc. and the appointment of a representative to take an active part in the activities of the council and the formulation of its future policies.

By Dr. R. W. Metzger, Director of Quality Control, Dairymen's League Co-op. Ass'n. Inc., President, National Mastitis Council, Inc.
PATHOGEN-FREE SEMEN FOR LIVESTOCK BREEDING:
A RESPONSIBILITY OF ALL SEMEN PRODUCERS


Ames, Iowa

The use of artificial insemination in cattle has had a phenomenal growth since 1938 when the first cooperative was organized in New Jersey (13). Initially, emphasis was placed on using a bull that had a desirable genetic potential and the ability to produce fertile semen. Little attention was paid to the disease status of the bull; by this, we mean that only occasional tests were made to assure that the bull was free from tuberculosis and brucellosis. Frequent testing for these diseases was required only in milkshed areas. Some states required that insemination be done by veterinarians, because they understood the need for sanitary techniques. After World War II started in 1941, a shortage of veterinarians developed, and lay technicians were then trained to do the inseminating. Some studs encouraged professional veterinary treatment of repeat breeders through a plan of reduced insemination fees for treated animals.

During these early years of artificial insemination, research was directed toward improving diluents for semen. Egg yolk added to buffer solutions improved the keeping quality of semen, improved the fertility rate, and permitted increases of the dilution rate (9). The addition of sulfanilamide in 1946 further improved the keeping qualities of semen (8). The incorporation of the antibiotics, penicillin and streptomycin (1), in 1949 increased both the storage time and the fertilization rate of certain semen. These improvements made it possible to dilute semen further and to reduce the bacterial count.

The use of frozen semen began in 1952 (14) and has expanded rapidly. Fifty-nine of 69 recognized bull studs in the United States are reported in 1960 to be using frozen semen in part or entirely in their operations. Frozen semen is also being used by private owners. Thirty-seven percent of dairy cattle inseminated in 1960 are reported to have been inseminated with frozen semen (6). Some semen has been kept longer than seven years without harm to its fertilizing qualities (6). A problem, which is of concern, is that freezing also preserves infectious organisms. We can visualize the possible spread of venereal (2) (4) (12) as well as other infections with the expanded use of frozen semen unless control of disease through planned sire health regulations becomes a reality throughout the whole industry.

From the National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.

157
Artificial insemination is the means of choice to control and eliminate venereal diseases providing it employs essential sanitary techniques and uses disease-free bulls. There is no better means of protecting the 37 percent of the dairy industry that is enrolled in the artificial insemination program.

In 1954, at the request of the National Association of Artificial Breeders (NAAB), the American Veterinary Medical Association (AVMA) Committee on Artificial Insemination and Animal Reproduction developed a guide of essential sanitary operating procedures for the control of the several diseases transmissible through artificial insemination, especially vibriosis and trichomoniasis (5). The need for a guide or code was recognized when instances of Vibrio fetus (11) (15) and Trichomonas foetus (2) (4) infections were attributed to artificial insemination. State-Federal programs were inadequate. Some required only annual tests for tuberculosis and brucellosis for all bulls and pre-entry tests for new bulls entering studs. Venereal diseases were completely ignored. The A.V.M.A. Committee outlined a code of minimum standards (5) which the N.A.A.B. members accepted and chose to call Sire Health Regulations (3). There was no official agency enforcing compliance with the “Regulations” as the word might suggest. Rather, the N.A.A.B. volunteered to do the policing.

A review was made in January, 1959, by the N.A.A.B. Committee on sire health management with Dr. E. R. Carlson as chairman, to determine the effectiveness of these Sire Health Regulations (3). The Committee summarized data from 43 respondents of the 65 studs, which had been solicited. This summary revealed that failures to comply were significantly high.

The A.V.M.A. sponsored a consulting panel to revise and update the A.V.M.A.-N.A.A.B. Code of Minimum Standards for health of bulls used in artificial insemination (7). The panel made specific revisions for clarity. The principle of the several tests and procedures was not changed. The panel recommended that the A.V.M.A. issue a document signifying approval of those organizations complying with the A.V.M.A.-N.A.A.B. Code. However, at its annual meeting in August, 1961, the A.V.M.A. did not approve this recommendation since such endorsements are contrary to A.V.M.A. policy.

Most N.A.A.B. bull studs are doing a good job, and we commend their personnel for the progress that has been made. The N.A.A.B. has developed a gigantic program, but there are many bulls that are outside the operation of the association and do not come under a health program. This situation is extremely important when one recognizes that a single bull has the theoretical potential of transmitting disease to as many as 50,000 females in thousands of different herds. If infectious diseases that interfere with reproduction are to be controlled, a minimum health code must be practiced, not only in the artificial insemination industry but also in the use of frozen semen by private owners. Today, many bull studs freeze, store, and distribute semen for private owners (10). It is not unusual to find independent technicians who travel from farm to farm to collect, freeze, and store semen
PATHOGEN-FREE SEMEN FOR LIVESTOCK BREEDING

for private owners, often on the owner's own farm. In this way, large collections of frozen semen are accumulated. Some owners may use this semen in their own herds, sell it for use in other herds, or exchange it for semen from other breeding herds. This traffic is especially hazardous since these bulls are outside the N.A.A.B. sphere, and State-Federal regulations simply do not exist. Some owners do not appreciate the importance of disease control. Although many understand the need, nothing is done because of inconvenience and cost. Many privately owned bulls are not limited to artificial insemination and are constantly exposed to infection through natural service.

In many areas of the country, it is customary for one farmer to keep a bull and sell semen or service to other farmers in the community. This community bull may either be kept in one location or be transported from farm to farm. A community bull is a constant potential spreader of venereal diseases, which might be controlled through enforcement of a health code for vendors of semen.

The organized segments of the artificial insemination industry have done a reasonable job of disease control within their bull population. It is obvious that the independent operators cannot be reached through a voluntary system. Therefore, adequate control of disease potentials inherent in the collection, storage, and use of semen must be vested in a central authority.

Initially, control should be established over interstate shipment of semen. Later, intrastate control, based upon the interstate regulations, could achieve a high degree of uniformity throughout the country. This would assist in international trade agreements as well. Here is a unique opportunity for interested parties to formulate practical, effective, and acceptable controls to protect a large segment of our entire animal industry from disease.

REFERENCES

SALMONELLOSIS IN FLORIDA CATTLE

EDWIN M. ELLIS, D.V.M., PH.D.*

Kissimmee, Florida

You will notice in your programs that the title of this report is Salmonellosis of Cattle. The material to be presented is part of the report of your Committee on Diseases of Cattle and concerns salmonellosis of cattle in Florida. The purpose of this presentation is to emphasize the importance of salmonellosis as a disease of cattle.

The discussion will include remarks concerning the disease as seen in adult cattle, the disease in calves, and some observations as to the epidemiology.

A review of the literature will be omitted. For those interested, an excellent review on salmonellosis is that by A. Buxton, published by the Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.

Salmonellosis is causing renewed concern to livestock and public health officials. A crash program now completed has once again emphasized the finding of Salmonella spp. in commercial feeds. The most likely source of these organisms is from meat by-products. Due to the lack of quality control by feed companies, some Florida feeds have become contaminated with Salmonella organisms.

Salmonellosis is more prevalent among dairy and beef animals than reports would indicate. Nearly 100 outbreaks of salmonellosis have been diagnosed at the Florida Department of Agriculture’s Animal Disease Diagnostic Laboratory at Kissimmee, Florida, over a period of two years. Forty of these cases have represented cattle and 18 of the 40, calves.

Veterinarians alerted to the possibility of this disease have been diagnosing it with greater frequency and submitting organs, feces, and live animals for necropsy and culture.

Both acute and chronic forms of salmonellosis are recognized. Acute outbreaks have been seen among younger stock, the clinical signs of which are severe with heavy losses. Older animals may have a diarrhea, become chronic shedders, and carry the organisms indefinitely. In Florida, salmonellosis is a serious disease of cattle and studies need to be done to firmly establish the sources of infection. Cattle feeds have been condemned without exact knowledge as to their importance as carriers of Salmonella.

There is a recognized need for careful epidemiologic studies, especially where calves are infected in large numbers. Little is known as to where these infections originate. It is only with such information that the veterinarians can effectively combat the disease.

A current text book on cattle diseases devotes less than one page to the discussion of calf salmonellosis and only mentions the condition in adult

* Present address: National Animal Disease Laboratory, Ames, Iowa.
cattle. This lack of emphasis would seem to indicate that proper methods of investigation have been lacking in our laboratories and that proper research studies have not been undertaken. No one, for example, has adequately determined what happens when Salmonella-containing feeds are fed to normal cattle. It would seem that this needs to be done.

Observations in Florida would indicate that adult cattle are readily infected with Salmonella organisms. One ranch visited by the author, possessed many Brahman bulls of fine breeding. Three were clinically ill and *Salmonella anatum* was isolated from the feces of these and of seven asymptomatic animals. Treatment was begun and a search made for other diseases that might produce symptoms of intoxication and diarrhea. Results were negative. Of the three clinically ill animals, only one recovered. The other two died after about two weeks' illness.

Of 40 laboratory diagnosed Salmonella outbreaks, 21 have involved adult cattle only. In each case either feces or fresh organs taken at necropsy were cultured. Numerous adult beef cows as well as dairy cows have died wherein salmonellosis was the only laboratory diagnosis made. In many instances it appeared that conditions present at the probable time of infection greatly influenced the effect of the Salmonella intoxication. Carrier animals exposed to physiological insults such as dipping, undue exposure while being shipped, branding, etc., have developed clinical signs and have died from salmonellosis. Two cases illustrate this. In each instance, the animal had been put through a dipping vat only to develop a bloody diarrhea three days later. *Salmonella* organisms were isolated from the liver and spleen. Necropsy examination of these animals indicated a severe enteritis with toxemia. Examinations for insecticide were negative. On many occasions adult cattle have been observed to sicken and die with Salmonella infection as the only finding. Microscopic examination of the tissues usually revealed enteritis and toxic hepatitis of unknown etiology. In these cases it was impossible to rule out all diseases. Anaplasmosis, brucellosis, leptospirosis, virus diarrhea and Johne's disease were, in most cases, ruled out. In herds where adult cattle have died, feces from asymptomatic animals have been positive for Salmonella organisms. Serologic types isolated from adult cattle have included *S. miami*, *S. litchfield*, *S. newport*, *S. typhimurium*, *S. anatum*, *S. habana*, *S. java*, and *S. montivideo*. Typing has been kindly carried out by the Communicable Disease Center at Atlanta. Attempts to associate these serologic types with the feed have not been very successful due mainly to lack of cooperation of the owner. In one outbreak, where feed samples were collected, the same serologic type was isolated from both the feed and the cow organs.

Salmonellosis has been of greater importance in calves by virtue of the numbers lost. Eighteen calf outbreaks have been laboratory diagnosed. These outbreaks have not been limited to dairy breeds as several devastating outbreaks among beef calves have been discovered. In the latter, the rancher was alerted to the problem by finding dead calves on range. Others staggered and in some a bloody diarrhea was noticed. Diarrhea was not always a sign in acute and rapidly fatal disease. Diagnosis was made only by laboratory examination. In few cases was the condition diagnosed in the field.
Perhaps the most serious outbreaks have been observed in dairy calves. In three of these, dairymen were raising calves for heifer replacement purposes. Individual pens were used on two premises. Fatalities in one outbreak resulted in the loss of 75 calves and more may have died following contact with the laboratory. On two other premises, 35 to 45 calves died. In two cases where enteritis was the most prominent sign and Salmonella organisms were not isolated, calves were examined for pathogenic serologic types of E. coli. Two such outbreaks were found wherein serologic types 0119 and 045 were considered to be the responsible agents. These, among other E. coli types, have been cited as causative agents of calf enteritis.

In the total calf Salmonella cases, an estimated 200 animals have died. When it is realized that the findings reported here probably represent a small sampling of the entire State, some realization of the size of the problem can be gained.

The question of epidemiology of this disease arises. A Florida survey carried out by the United States Public Health Service during the past year has shown at least five rendering establishments to be infected with Salmonella species. Salmonella were found in the finished product. Meat scraps from such plants are included in various cattle feeds sold in Florida and elsewhere. A survey of Salmonella in feeds has been carried out for a period of a year by the Animal Disease Diagnostic Laboratory, Florida Department of Agriculture. Fourteen feeds have been found containing Salmonella spp. A well known mineral mix containing meat scraps sold widely in Florida has recently been shown to contain Salmonella organisms. While no definite studies have been initiated to show that these feeds produce salmonellosis in normal cattle and calves, it can be assumed that the spread of these bacteria over the country certainly presents an optimal opportunity for infection. It would seem that our first obligation to the livestock interests would be the removal of such known threats to the health of our animals.
<table>
<thead>
<tr>
<th>Case</th>
<th>Date</th>
<th>Adults</th>
<th>Calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>1832</td>
<td>10/1/59</td>
<td></td>
<td>S. hartford</td>
</tr>
<tr>
<td>1874</td>
<td>10/9/59</td>
<td></td>
<td>S. newport</td>
</tr>
<tr>
<td>1952</td>
<td>10/21/59</td>
<td>S. miami</td>
<td></td>
</tr>
<tr>
<td>2056</td>
<td>11/4/59</td>
<td></td>
<td>S. typhi-murium</td>
</tr>
<tr>
<td>2086</td>
<td>11/8/59</td>
<td></td>
<td>S. typhi-murium</td>
</tr>
<tr>
<td>2251</td>
<td>12/2/59</td>
<td></td>
<td>S. typhi-murium</td>
</tr>
<tr>
<td>2353</td>
<td>12/22/59</td>
<td></td>
<td>S. newport</td>
</tr>
<tr>
<td>2452</td>
<td>1/13/60</td>
<td>S. litchfield</td>
<td></td>
</tr>
<tr>
<td>2634</td>
<td>2/15/60</td>
<td>S. newport</td>
<td></td>
</tr>
<tr>
<td>2827</td>
<td>3/12/60</td>
<td>S. typhi-murium</td>
<td></td>
</tr>
<tr>
<td>2860</td>
<td>3/16/60</td>
<td></td>
<td>S. typhi-murium</td>
</tr>
<tr>
<td>4752</td>
<td>11/29/60</td>
<td></td>
<td>S. typhi-murium and S. anatum</td>
</tr>
<tr>
<td>5016</td>
<td>1/3/61</td>
<td>S. anatum</td>
<td></td>
</tr>
<tr>
<td>5050</td>
<td>1/6/61</td>
<td>S. anatum</td>
<td></td>
</tr>
<tr>
<td>5280</td>
<td>2/1/61</td>
<td>S. habana</td>
<td></td>
</tr>
<tr>
<td>5304</td>
<td>2/3/61</td>
<td></td>
<td>S. anatum</td>
</tr>
<tr>
<td>5313</td>
<td>2/3/61</td>
<td>S. anatum</td>
<td></td>
</tr>
<tr>
<td>5339</td>
<td>2/8/61</td>
<td>S. anatum</td>
<td></td>
</tr>
<tr>
<td>5376</td>
<td>2/10/61</td>
<td>S. anatum</td>
<td></td>
</tr>
<tr>
<td>5377</td>
<td>2/10/61</td>
<td></td>
<td>S. anatum</td>
</tr>
<tr>
<td>5466</td>
<td>2/24/61</td>
<td>S. typhi-murium</td>
<td></td>
</tr>
<tr>
<td>5586</td>
<td>3/6/61</td>
<td>S. anatum</td>
<td></td>
</tr>
<tr>
<td>6420</td>
<td>6/2/61</td>
<td>S. anatum</td>
<td></td>
</tr>
<tr>
<td>6475</td>
<td>6/8/61</td>
<td>S. montivideo</td>
<td></td>
</tr>
<tr>
<td>6495</td>
<td>6/9/61</td>
<td>S. tennessee</td>
<td></td>
</tr>
<tr>
<td>6632</td>
<td>6/25/61</td>
<td>C²</td>
<td></td>
</tr>
<tr>
<td>6807</td>
<td>7/18/61</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>6856</td>
<td>7/25/61</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>6980</td>
<td>8/7/61</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>7004</td>
<td>8/9/61</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>7034</td>
<td>8/14/61</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>7144</td>
<td>8/25/61</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>7245</td>
<td>9/7/61</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>7296</td>
<td>9/7/61</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>7349</td>
<td>9/14/61</td>
<td>S. Kentucky</td>
<td>S. bovis-morbiicans</td>
</tr>
<tr>
<td>7374</td>
<td>9/18/61</td>
<td></td>
<td>S. typhi-murium</td>
</tr>
<tr>
<td>7394</td>
<td>9/20/61</td>
<td></td>
<td>S. typhi-murium</td>
</tr>
<tr>
<td>7412</td>
<td>9/20/61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mr. Chairman, Members of the Association and Guests: With the exception of bovine mastitis your Committee is not including further reference to diseases covered last year namely: Bovine Brucellosis, Bovine Mastitis, Bovine Mucosal Disease Complex, Bovine Viral Diarrhea Diseases, Bovine Tuberculosis, Current Status of Cattle Insect Control, Infectious Bovine Rhinotracheitis, Johnes Disease, Leptospirosis, Shipping Fever, Foreign Diseases, Rinderpest, Contagious Bovine Pleuropneumonia, Lumpy Skin Disease, East Coast Fever, Malignant Catarrhal Fever, and Trypanosomiasis. We feel that these diseases were ably covered at that time.

For our report we have endeavored to review mastitis, salmonellosis, and artificial insemination through the three discussions just presented. These three entities involve a high percentage of our cattle population.

We have gone further in the field of artificial insemination. The Committee therefore recommends that the “Code of Minimum Standards for Health of Bulls and Hygiene of Bull Studs Producing Semen for Artificial Insemination” be studied for one year and a report of recommendations regarding this code be made by this Committee at the United States Livestock Sanitary Association meeting in Washington, D.C., next year (1962).

It is further recommended that the Committee on infectious diseases of cattle make recommendations regarding feeds and feed by-products containing salmonella organisms, and problems pertaining to mastitis.
TUBERCULOSIS IN NEW YORK STATE

G. S. KALEY, D.V.M.

Albany, New York

As you are probably aware, New York has the dubious distinction of having contributed about 25 percent of the four million odd reactors removed from the country's herds in the course of the national eradication effort.

We started in 1917 with half of our cattle diseased, and we eliminated more than 99 1/2 percent of this infection in 20 years. In the next 20 years we eliminated 90% of the remainder. At the start of the campaign, then, we condemned about 5,000 of each 10,000 animals tested. In 1956, we condemned only seven cows of each 10,000 tested. That was the low point. Each year since has shown an increase.

Today we condemn 18 of each 10,000 tested. This is two and a half times the 1956 rate and puts us in about the same position we were in 10 years ago. The statistical indications are that we have in New York at this very moment, 2,800 sensitized cows among which are 476 animals with gross lesions of tuberculosis.

Ordinary prudence suggested the need for a hard look at the situation to determine what factors had a bearing on this increase. Were we losing control of things? If so, what could be done about it?

Those of us concerned with the problem in New York have done a little soul-searching. We do not claim to have all the answers but we have arrived at a few conclusions. Since the problem itself is apparently not unique to New York, some of these things may be of interest to you.

Those among us who worked on tuberculosis eradication back in the early 30's or before, of necessity and by force of circumstances, developed a good working acquaintance with tuberculosis. In several ways the job was easier then than it is now. The owners were mentally conditioned to the idea that when their turn came reactors would be found. They accepted the fact that they might even lose the entire herd. Also, most of the condemned animals had gross lesions in one location or another.

Things have changed since those days.

First, we now have in practice a full generation of young veterinarians who do not know bovine tuberculosis from first hand experience. To these must be added the experienced veterinarians who have become apathetic and complacent.

Second, the fact that deviates have been found in his herd now comes as a shock to most owners. Instead of being glad to get the animals out of the herd, his first thought is that somebody has made a mistake.

Third, eighty percent of our reactors fail to show gross post-mortem lesions. If the owner suspects that his cows were condemned in error, he
becomes thoroughly convinced of it when he learns that the post-mortem find-
ingings are negative.

The more we examined the situation the more evident it became that these three factors, or perhaps more accurately, our reaction to them—were responsible for the state of our eradication effort.

Now it is an easy matter to decry carelessness on the part of some partic-

ipants or disinterestedness on the part of others. The thing becomes a bit embarrassing when it appears that much of this carelessness and disinterest-

edness merely reflects the inertia of those of us in the administrative end of the regulatory business. What were we doing to correct the situation and insure the provision of uniformly good service? In our own case it was apparent that many phases of the eradication program were suffering because of inattention to detail by our own administrative organization.

With our attention focused on other and newer disease problems, the handling of tuberculosis had become routine and perfunctory. The work of eradication was affected with a sort of creeping paralysis. In one case, we discovered, more or less by accident, that a practitioner assigned to two towns in a good dairy area had not got around to testing a single cow in two years. Neither, for that matter, had he vaccinated a calf. From another veterinarian came a report of a herd tuberculin test. The report was liberally sprinkled with X's denoting reactors. There was no evidence that he had condemned anything or even quarantined the herd. We called him on the phone. He had been testing cows for 12 years and had never seen a deviate before and didn't quite know what to do about them.

It was a series of these little things that finally woke us up to the facts of regulatory life. How much of this sort of thing was going on? We didn't know and we don't know yet but we have developed the means of finding out.

The fact that these things can happen is a good example of administrative complacency at its worst.

This word complacency has been used rather freely in connection with tuberculosis but I can't recall anyone taking the trouble to define what is meant by it. The dictionary says that it means amiable or affable or oblig-
ing. In my personal book, complacency, as applied to our attitude toward tuberculosis eradication, is merely an euphemistic term for sloth, procras-
tination, lack of attention to detail and careless work in general. Perhaps we would be better off if we used these more descriptive adjectives.

Just when complacency entered the picture in New York is uncertain. Perhaps it goes way back to November 4, 1937. That was a day to be remembered in Albany. Officially known as Achievement Day, it celebrated New York's addition to the role of Modified Accredited States. Reduction of our infection in 20 years from 50 percent to less than one-half of one percent was indeed a laudable accomplishment. It was certainly fitting that the achievement be recognized—and recognized it was in no uncertain manner.

Now a sense of achievement is a fine and necessary and rewarding thing but ours, I suspect, got out of hand. I am afraid that a lot of people went
home from that affair with the idea that the job was about done. The seeds of complacency had been sown in a welter of self-congratulation.

Certainly, a few more years like the last five will put New York right back where it was in 1937, and complacency will have played its part. If this happens I doubt if we will consider it cause for another celebration but if we do you will all be invited.

At any rate, complacency and inexperience among the participating veterinarians have been a problem, and it must be remembered that we depend on these men for all of our diagnostic procedures. From them we must have prompt service and precision of technique and interpretation if we, in turn, are to fulfill our obligation to the livestock industry.

The error-prone individual must change his ways or he must relinquish his right to participate and there can be no compromise on this point. We have learned from sad experience that as long as human nature remains what it is, you cannot turn 500 men loose in the field and expect uniformity, or if you prefer the term, quality control, unless you everlastingly work for it. I might add that this is not an area of activity designed to make friends for regulatory personnel. Regardless of this fact, it is still an area in which more work will have to be done.

To coin a cliche, our pressing and immediate need is not for a superior tuberculin but for superior use of the one we already have.

So as I have mentioned, we looked our situation over. What we saw was neither flattering nor reassuring. Some changes were long overdue.

One obvious need was for a change in attitude, a renewal of faith in what we were doing. For too long we had been content merely to defend our methods against those critics who saw only that, after years of effort, we still found reactors.

Now it is true that some $330 million has been expended on tuberculosis eradication in the U. S. and the job is not yet completed. On the other hand, it is estimated that the annual saving arising from the progress to date approximates $150 million. Viewed from a coldly analytical, dollars-and-cents approach, it would seem that any program which returns substantially its entire cost every two years is a bargain even if in the future we should no more than maintain the status quo. To this $150 million can also be added an incalculable saving in human life and human misery.

A program of which such things can be said need offer no apologies.

A second thought for the future concerns undergraduate training in regulatory veterinary medical procedures. We are fortunate in that most of our veterinarians are educated in the State. It is our hope that early discussions with the faculty of the New York State Veterinary College will lead to additional undergraduate instruction in regulatory methods.

In this general connection we will also propose to the U.S.D.A. that the privileges of accreditation be withheld from the graduating veterinarian until he has passed a comprehensive accrediting examination with a satisfactory grade. Today, accreditation is more or less automatically bestowed.
It is also essential that the young accredited veterinarian be made aware of his relation to the entire eradication effort. He must understand that the things he does and the way he does them on any farm can affect the welfare of a lot of people in addition to the owner of that particular farm.

To help on such things, we depend a great deal on our force of 24 supervising veterinarians. This will be increased to 33 at the start of the new year although the additional professional man hours available will be largely absorbed by new assignments in the meat inspection field. Among a score of lesser duties these men, with some help from four Agricultural Research Service veterinarians, conduct all retests of suspects and quarantined herds.

In addition they have immediate supervision over the accredited veterinarians in their area. Before a young veterinarian is authorized to participate in official programs he must work with the supervisor who is required to answer some 70 questions related to the attitude, interest and technical competence of his new charge.

The final question reads as follows: "Are you satisfied that this veterinarian can be depended upon to properly carry out his official disease control assignments? Yes or No?"

The questionnaire, which we have just started to use, has the added advantage of serving as a deterrent to the supervisor who might be inclined to hurry this training duty because of an impending fishing trip.

Each young veterinarian is also required to spend a day in the Albany office where all phases of each disease eradication program are discussed in detail with particular reference to the role the accredited veterinarian must play. He also meets and talks with the people who take care of the paper end of the eradication effort. This includes the heads of the units which handle such things as files, herd classification, interstate movement, quarantines, disinfection, compensation, or data processing. He gets a first hand picture, through specific examples, of the chain of trouble that can be started by a carelessly spelled name, an illegible report, a duplicated tag number or an incomplete interstate certificate.

Only then does he get his authorization.

I cannot recommend this on-the-job training method too highly. The men that complete it are incomparably better cooperators as a result. It is unfortunate that these procedures were not adopted 30 years ago.

I mentioned earlier that we had worked out a method for keeping track of the type of service provided in his area by each participating veterinarian. This will take the form of a card index file with provision for a record of the herds and cattle assigned, cattle tested, reactors condemned, lesions found, suspects reported, the results of the retests on these suspects and the number of trace-backs from infected herds or straight kill lesion cases that lead back into the area.

Now this sounds like a formidable undertaking but virtually all of it can be picked up with little effort from monthly I.B.M. reports already being prepared for other purposes. The information compiled in this way will tell us a great deal about the quality of the work as well as the quantity. The
regulatory agency that does not know these things may well be dealing with more tuberculosis than it suspects.

We also plan to have our supervisors ride with the accredited veterinarians periodically. This was a well established custom in New York at one time. It fell into disuse because of the pressure of other work. Something new will be added this time in the form of a supervisor's report covering his observations on some 20 specific points.

In view of the foregoing, and as a guide for the future, we have reduced our philosophy on professional service down to four fundamental precepts:

First, let us recognize the fact that tuberculosis has not been eradicated.

Second, let us exhibit, both publicly and privately, a little more faith in the methods and procedures that have carried us so far along the road to eradication.

Third, let us undertake every injection of tuberculin with the thought in mind that the animal under test may be one of the 2,800 undetected reactors.

Fourth, and last, let those among us who cannot do these things, conscientiously and in good faith, be separated from the eradication effort.

This pretty well covers the method by which we hope to insure uniformity of technique and interpretation. Granted this, we in return must stand behind every decision of the accredited veterinarian. This support has been forthcoming and there is no better evidence of it than our position on the no gross lesion reactor.

The no lesion problem is not a new thing. Let me quote from a letter in the Department files: "A second question that is disturbing me a great deal, and I am receiving a great many letters from breeders as well as veterinarians concerning it, is the explanation for the large number of no lesion cases following the tuberculin test of accredited herds. I am told by both veterinarians and breeders that of the animals condemned in accredited herds, ten to sixty percent fail to show the presence of tuberculous lesions on post-mortem."

That letter was written by Dr. Veranus A. Moore, Dean of the New York State Veterinary College to Dr. John R. Mohler, Chief of the Bureau of Animal Industry, U.S.D.A. on May 24, 1920.

The official Division position on the no lesion case has taken some amazing twists and turns over the years, some of which are frankly beyond my comprehension. For example the following rule was in effect for a rather protracted period.

"An accredited herd in which reactors are reported, all of which reactors on post-mortem examination show no visible lesions or skin lesions only, shall be immediately reinstated to the accredited status."

Nothing could have been better designed to impede tuberculosis eradication and it could well be that some of our current problems stem from it. Let us consider, as an example, what might have been the end result if that rule had been applied in a recent Long Island Case.
One animal in a herd of 122 reacted. The post-mortem report—no gross lesions. Two more reactors were found 60 days later. Both had marked thoracic lesions. On the next subsequent retest the fourth and fifth reactors were removed, in one of which mediastinal lesions were found. The herd is not yet due for retest.

A few years ago that herd would have been automatically reaccredited following receipt of the first post-mortem report. It is this sort of experience that comes to mind when an irate owner takes pen in hand to give us his frank opinion of a program which, in his words, "turns a careless, irresponsible, incompetent veterinarian loose with a syringe to condemn his perfectly healthy cow." The answer we give to such correspondents adds up to unqualified support of the testing veterinarian. If, on a rare occasion, we have some mental reservation about a particular case, we take the matter up directly with the veterinarian involved.

I am glad to report that the rule quoted above is no longer in effect in New York State although the regulation which replaced it is still weighted heavily in favor of the no gross lesion reactor.

In fact, the idea that a gross lesion reactor represents tuberculosis infection while a no gross lesion reactor may mean something else is well on the way to becoming a state of mind.

The Uniform Methods themselves appear to lend support to the theory that the no gross lesion case is in some way less reprehensible than the lesion case. You will find the distinction in the very first paragraph (I-l-a).

"Herds in which reactors occur shall be quarantined and must pass a negative tuberculin test after a period of at least 60 days before the herd may be released from quarantine. If there is an indication of advanced tuberculosis in one or more reactors, a second herd test at least 60 days from the prior negative test, and such additional tests as the cooperating State and Federal officials deem necessary shall be applied before releasing the herd from quarantine."

Now there are some unpleasant possibilities inherent in making this distinction between the lesion and the no lesion reactor: Witness the following case history:

<table>
<thead>
<tr>
<th>Date</th>
<th>Number</th>
<th>Lesion Type</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>April, 1958</td>
<td>9 reactors</td>
<td>9 No gross Lesions</td>
<td></td>
</tr>
<tr>
<td>July, 1958</td>
<td>Negative herd retest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>October, 1959</td>
<td>Straight Kill Generalized Lesions</td>
<td>1 cow</td>
<td></td>
</tr>
<tr>
<td>November, 1959</td>
<td>22 reactors (entire herd)</td>
<td>4 Generalized</td>
<td>12 Localized</td>
</tr>
</tbody>
</table>

We certainly had warning enough on that one. A nine reactor warning, and we let the entire herd get away from us, lulled into complacency by a negative post-mortem report. Another retest in the fall of 1958 would in all probability have kept another 15 or 20 reactors out of the record books.

Today, about 17 percent of our New York reactors show gross lesions on routine meat inspection post-mortem. Another two percent are reported to
have skin lesions. We are inclined to the view that had we been able to subject the tissues of the remaining 81 percent to more searching scrutiny, perhaps even to culture, some logical explanation of the deviation observed by testing veterinarian would have been forthcoming in a high percentage of the cases.

Perhaps this thought could be better expressed in another way. We have not yet lost faith in the tuberculin test as a diagnostic weapon.

We are also trying to stimulate our eradication program in a number of other ways. Time will not permit the details but a brief mention of some of them might be in order.

Fee Schedules: It is axiomatic in personnel circles that, other things being equal, you get just about the kind of service you are willing to pay for. An increase last year has made our fees, if not good, at least reasonably adequate. The result has been increased interest and activity all along the line.

Records: There are only two things you can do with records. They can be stuffed into a file drawer for possible future reference or they can be put to work to get essential information to those who need it when they need it. We now send out monthly lists of suspects, infected herds and trace back contacts to supervising veterinarians. Once on the list, a name reappears each month until a report of the retest is in our hands. Master lists with all essential information on every herd assigned to each of our 500 accredited veterinarians are now coming from our data processing machines. A new system for keeping track of cattle moving through dealers and commission sales is in effect. Full use of good live records has done much to get our eradication effort in high gear again.

Suspects: When a veterinarian is in doubt we encourage him to report the deviate as a suspect. There are those who disagree with such encouragement of the suspect status. We are satisfied that our policy opens the door to infected herds that we might not otherwise get into. I have one word of caution in connection with the handling of suspects. Never focus your attention on the suspicious individual to the point where you forget that tuberculosis is invariably a herd problem.

We are also attempting to improve our disinfecting procedures and our quarantine enforcement. Both now leave much to be desired. Avian tuberculosis needs attention it is not getting. We look to an expanded meat inspection program to provide us with improved straight kill post-mortem report service. Our first State-wide tuberculosis eradication conference was held in September. Small scale versions of this conference will be held in local areas throughout the State to carry the message to all practitioners.

None of the things we are doing are particularly new or different or spectacular. In fact, they represent our reaction to the belief that new and spectacular innovations in the field of bovine tuberculosis control are not likely to be forthcoming. They are the result of our belief that the remaining
infection will have to be dug out of our herds with the tools now in our hands.

They are, finally, the result of our belief that the job can be done but can be done only by uncompromising insistence on precision of technique and interpretation, rigid adherence to the established principles of epidemiology and constant attention to detail. We can be amiable and affable and obliging on these points only at the risk of living with bovine tuberculosis forever.
A PROGRESS REPORT OF TUBERCULOSIS RESEARCH
AT MICHIGAN STATE UNIVERSITY

W. L. MALLMANN*

An increase of bovine tuberculosis has occurred during the past few years, particularly in the areas of high dairy cattle population. Although many tuberculin positive animals show gross lesions at slaughter, approximately 85 percent are listed as no gross lesion (NGL) reactors. Some herds upon repeated tuberculin testing yield only N.G.L. reactors. The finding of N.G.L. reactor animals is not new and has been of concern to research workers and veterinary enforcement officials from the early phases of the eradication program.

Hastings et al. (1) in 1924 reported the following figures:

<table>
<thead>
<tr>
<th>No. of Reactors in Herd</th>
<th>No. of Herds Tested</th>
<th>No. of Animals Slaughtered</th>
<th>No. of Animals N.G.L.</th>
<th>Percentage N.G.L.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,063</td>
<td>1,063</td>
<td>458</td>
<td>44.0</td>
</tr>
<tr>
<td>2</td>
<td>346</td>
<td>692</td>
<td>248</td>
<td>35.8</td>
</tr>
<tr>
<td>3 or 4</td>
<td>110</td>
<td>699</td>
<td>188</td>
<td>26.9</td>
</tr>
<tr>
<td>5 to 9</td>
<td>207</td>
<td>1,167</td>
<td>135</td>
<td>13.1</td>
</tr>
<tr>
<td>10+</td>
<td>120</td>
<td>2,105</td>
<td>145</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Although the maximum percentage of N.G.L. reactors was only 44, the data reveal that the N.G.L. problem existed and that the herds with low incidence of tuberculin-positive cattle gave the largest percentages of N.G.L. reactors. An examination of current data would be similar except that the percentage of N.G.L. reactors is higher.

The high percentage of N.G.L. reactors in lesion herds and the occurrence of only N.G.L. reactors in some herds over a period of repeated examination raises many questions. Are N.G.L. herds infected by Mycobacterium bovis? Has the animal husbandry practices upset sensitivity to tuberculin? Is the tuberculin test too sensitive? Is the tuberculin test specific? If the tuberculin test is specific, why the increase in bovine tuberculosis? Is tuberculosis caused by other acid-fast bacilli? Has the use of antibiotics caused the development of new types of organisms? Answers to these questions and others along the same line are needed so that control measures can be formulated and practiced. Research on bovine tuberculosis is necessary.

A research project is underway at the College of Veterinary Medicine Michigan State University as a research contract with Animal Disease and Parasitology Division and a cooperative agreement with Animal Disease Eradication Division, United States Department of Agriculture. The research contract provides for a thorough study of bovine tuberculosis as to isolation of organisms from the tuberculin positive cattle, identification of isolants,

* Department of Microbiology and Public Health, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan.
serologic reactions, sensitivity tests, and studies of N.G.L. reactors from gross lesion reactors and N.G.L. reactor herds. The cooperative agreement provides for laboratory service for tissues from gross lesion and N.G.L. reactors submitted by A.D.E. representatives.

The research program has been based on the following postulates:

1. Tuberculosis, in a broad sense is not limited to the three classical types, *M. tuberculosis*, *M. bovis* and *M. avium*.
2. Host-parasite relationships cause changes in mycobacteria.
3. Acid-fast organisms are susceptible to environmental changes.
4. Acid-fast organisms in N.G.L. reactors are in low populations.
5. Organisms in N.G.L. reactors may be found only at the primary site of invasion—lung, Peyer's patches, skin.
6. Atypical mycobacteria may be more susceptible to adverse conditions than are the tubercle bacilli.
7. Tuberculin positive test may result from mycobacterial infections other than the classical types.

In the early phases of the research program, laboratory methods have been devised to test the validity of each of the above postulates. New techniques have been developed, one has been published (2) and several are in manuscript form. All of the above postulates have proved valid, in part, if not fully. The results of the research have revealed information that must be carefully pursued in the laboratory before field applications can be made. Hasty interpretations are dangerous.

A report on research in progress is always difficult to present because conclusions are not ready, data may look promising, but the amount may be too meager to draw valid conclusions, and applications of basic information may have not been made. If the presentations made in this report are evasive or couched in conjectural phraseology, it is intentional. This report carries no information that could be immediately translated into field application or explanations of field observations. The results of the research are promising and an examination of the basic data obtained up to now indicate that the original approach was right and answers are in the not too distant offing.

Extensive studies have been made of tissues from N.G.L. reactors submitted by A.D.E. representatives from a Detroit packing plant. These tissues consist of lymph nodes of mesenteric thoracic, cervical, supramammary, prefemoral and prescapular, Peyer's patches, section of lung and any skin lesions. All nodes are delivered intact so that aseptic examination can be made by the pathologists for gross lesions. The examination of these specimens, in addition to the observations by the A.D.E. representatives submitting the specimens, determine whether the animal was a gross lesion or N.G.L. reactor.

The writer is aware that an examination of a carcass at the packing plant for lesions is incomplete. A label of N.G.L. on a reactor is based on presumptive evidence. However, seven reactors have been slaughtered in the post-mortem laboratory and have been minutely examined by the pathologists without evidence of a gross lesion. The absence of gross lesions in the
selected tissues carefully examined by a pathologist is good evidence that the animal can be classified as an N.G.L. reactor.

Histopathologic examinations are made of all tissues using the new fuchsin hematoxylin-eosin stain (2) which differentially stains both the acid-fast organisms and the surrounding tissues. With few exceptions microscopic lesions characteristic of tuberculosis were found in tissues from N.G.L. reactors. Acid-fast bacilli were found in some of these lesions but the numbers per granuloma seldom exceeded three. Thus the acid-fast bacilli populations in N.G.L. reactors were low as observed by microscopic examinations. The digestion of tissues and the concentration of acid-fast bacilli from the digest confirmed the microscopic observations.

These observations and laboratory findings do not mean that all N.G.L. reactors carry microscopic lesions with low acid-fast bacilli populations but the frequency was so high that new techniques for the isolation of acid-fast organisms were needed. Several new techniques have been developed.

The sodium hydroxide and acid digestion methods are objectionable because both acid and alkali destroy many of the acid-fast organisms in the tissues. In gross lesions where the acid-fast bacilli population is high, the loss of even 90 percent of the bacilli would not preclude the isolation of acid-fast bacilli. These methods have been successful and are widely used because the acid or alkali destroy the accompanying contaminating organisms. This makes possible the isolation of the slow growing acid-fast organisms from the gross contamination of the tissues. However, when these methods are applied to tissues with low acid-fast populations, acid-fast organisms may be missed entirely. When they are isolated, it is largely a chance isolation unless relatively large volumes of digested tissue concentrates are cultured.

Two methods for the isolation of acid-fast organisms from low population tissues have been devised, namely the enzyme-digestion-pentane procedure (3) and the enzyme digestion membrane filter technique (4). In the former the acid-fast organisms are concentrated by a flotation procedure using a hydrophobic agent, pentane, whereas in the latter procedure the acid-fast organisms are concentrated on membrane filters which are placed upon suitable media for incubation. No inhibitors are used to suppress contaminating organisms, so unless the tissues are relatively free of contamination, overgrowth contaminates the media.

Contamination has been eliminated largely by the handling and preparation of the tissue specimens. Lymph nodes are delivered in a refrigerated container to the laboratory uncut and imbedded in their covering of fat. The tissue specimens are deposited in a 1,000 ppm hypochlorite solution over night at 40° F. The following day the nodes are excised from the fatty envelope and immersed into 1,000 ppm hypochlorite solutions. The nodes are then aseptically sliced for evidence of gross lesions and sections are selected for cultural examination.

By the examination of lymph nodes freed of external contamination, the acid-fast isolants represent significant mycobacteria involved in the microscopic lesions present in the tissue. No acid-fast isolants, irrespective of their colonial characteristics, pigmentation or rate of growth, were discarded.
All isolants were injected intradermally into guinea pigs. If local or disseminated lesions were produced and tuberculin tests using mammalian and avian tuberculin were positive for one or both tuberculins, the culture was typed using rabbits and chickens. If no disseminated lesions were produced in guinea pigs, rabbits or chickens, the culture was classified as an atypical mycobacterium. These are further identified by growth and cytochemical characteristics (6).

Approximately 250 organisms have been isolated from bovine and swine tissues from tuberculin positive reactors. To show the frequency of isolations from N.G.L. reactors, out of the first 20 N.G.L. reactors examined this year, three yielded *M. bovis* and 11 atypical mycobacteria. The three N.G.L. reactors which yielded *M. bovis* were from herds with a history of N.G.L. reactors only. The organism was isolated from the lung in all three animals, one in a cervical lymph node, and one in a thoracic lymph node.

Skin lesions have been submitted for examination from various parts of the United States. Out of 84 skin lesion examinations that have been completed, 50 isolations have been made. Over 130 skin lesions have been received to date. All of the cultures isolated have been classified as atypical mycobacteria.

Soil samples have been obtained from barnyards of farms where tuberculin positive cattle have been found. Most of the acid-fast organisms isolated have been classified as saprophytes but some significant atypical mycobacteria have been found.

Atypical mycobacteria have been found in humans suffering from tuberculosis. These organisms have been classified by Runyon (5) as follows:

I. Photochromogens—do not produce pigment in the dark
II. Scotochromogens—do produce pigment in the dark
III. Non-pigmented
IV. Rapid growing organisms

Other tests besides pigment production and growth rate are also used such as catalase production, arylsulfatase production, nicotinic acid (Niacin test, neutral-red reaction, and incubation temperatures (37-45 C). A type III—organism designated “Battey” is responsible for pulmonary infections in the Southeastern Section of the United States (7).

It is interesting to note the isolation of atypical mycobacteria from tuberculin positive cattle and swine in our laboratories. The atypicals isolated from animal tissues do not classify well into the Runyon Classification, however organisms that fit roughly into each group have been isolated. The group III organisms show the greatest virulence, although all four groups show pathogenicity upon intradermal injection into guinea pigs. A research report with specific data is being presented at the Annual Meeting of the American Public Health Association, November 13, 1961, at Detroit, Michigan, by Dr. Virginia H. Mallmann, a member of the tuberculosis research team (6).

All of the atypical mycobacteria showing pathogenicity in guinea pigs induce a positive tuberculin test to both or either mammalian and avian tuberculin.
Studies are underway to evaluate the pathogenicity of a selected group of atypical mycobacteria in calves. These organisms present a new problem in the diagnosis of tuberculosis in animals. The answers will be forthcoming when the behavior of these organisms in cattle are fully understood.

Along with the calf infectivity studies, serological tests of various types are being studied as well as the specificity of sensitins that are being prepared from selected atypical mycobacteria.

A brief review of the research has been presented without an attempt to evaluate the results. However, a few comments can be made that have practical significance.

1. *Mycobacterium bovis* has been isolated from three N.G.L. reactors from herds with N.G.L. reactor histories.
2. Atypicals of all four groups have been isolated from N.G.L. reactors.
3. The tuberculin test detects mycobacterial experiences in cattle, as evidenced by the isolation of mycobacteria from tuberculin positive cattle.
4. Atypical mycobacteria have been isolated from skin lesions.
5. Atypical mycobacteria have been isolated from G. L. animals.
6. No observations in the research studies would indicate the need of any change in the bovine tuberculous eradication program.

Acknowledgments—The writer wishes to express his appreciation to the research team who are responsible for the laboratory work in these studies and the development of the program, especially Virginia H. Mallmann, Ph. D., James A. Ray, D.V.M., M. S., Robert Lipe, Ph. D., Don Willigan, D.V.M., Ph. D. and Jude Swalley, D.V.M. He is also appreciative of the cooperation of Asa Winter, D.V.M., R. M. Scott, D.V.M., A. B. Park, D.V.M. and Ralph Jenner, D.V.M., M.P.H. of A.D.E.

LITERATURE CITED
3. Lipe, Robert, Mallmann, W. L., and Ray, J. A.: An enzyme-pentane procedure for the concentration of mycobacteria in low population from tissues. (In manuscript.)
4. Wright, George, and Mallmann, W. L.: A membrane filter digest method for isolating mycobacteria from low population sputa and tissues. (In manuscript.)
STATUS OF STATE-FEDERAL COOPERATIVE TUBERCULOSIS ERADICATION

A. F. Ranney and O. D. Corson

Through the years, since the inception of the tuberculosis eradication program, there have been varying degrees of interest in the national effort to sustain the program. A giant step to eradicate the disease had been taken by 1940 when all counties were modified accredited as having less than one-half of one percent infection. This accredited status was meaningful, and apparently in the minds of most individuals associated with the livestock industry, it had provided the desired results. It seemed that tuberculosis in livestock had ceased to be a problem and that is precisely why we arrived at the low point of interest in the program during the years of World War II and the decade that followed. Even during the last five years, in spite of the attention given to publicizing the fact that tuberculosis had not been eradicated, there is apathy in many states about the existing hazard to the livestock industry.

There are, however, some states that have recognized the need for continuing this program to a successful conclusion, and they have promoted, and are continuing to promote, expanded State budgets and personnel to deal with the problem. Other states have not as yet seen the need for intensifying their efforts to meet the goal of tuberculosis eradication. They seem to be content with the status quo. These states need to be reminded that this disease is not a passive foe. Rapid transportation facilities and increasing movements and concentration of livestock and people preclude the theory that the hazards of spread of tuberculosis can be overlooked. As long as there remain living organisms of tuberculosis, so long will there remain an opportunity for their continued propagation. State lines and wishful thinking are not barriers to infection.

It is not necessary to remind the members of this Association that we are committed to a goal of eradication. The increased interest and attention given to the program in recent years is most encouraging; but we should take cognizance of the fact that in several states the cooperating animal disease eradication officials have stimulated increased activity only to be plagued with the problem of curtailing those activities because of a decrease in financial support. And, as has been pointed out in prior reports to this Association, there has been in recent years a moderate increase in the number of dollars from State appropriations annually available for supporting

*Dr. A. F. Ranney, Chief Staff Officer, Tuberculosis Eradication, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Washington, D. C.

Dr. O. D. Corson, Assistant Chief Staff Officer, Tuberculosis Eradication, A.D.E.-A.R.S., United States Department of Agriculture, Washington, D. C.
the program while those from Federal sources have remained practically unchanged during the past 20 years.

PROGRAM PROGRESS

During the past year we have made progress in dealing with herds categorized as "Red Flag Herds." There are herds presently under quarantine from which gross lesion reactors have been found on repeated tests. Some of these herds have been infected for many years. The number of these herds in the nation has been reduced from 239 in July 1960, to 101 in July 1961. This 58 percent reduction supports the statement frequently made that we do have the tools and the know-how to eradicate the disease when we are willing to dig in and stamp it out.

A comparative record of slaughtered reactors shows that in the Fiscal Year 1961, there were 273 whole bovine carcasses condemned as unfit for food and 43 additional carcasses passed for cooking. This is a 60 percent increase for cattle in these categories over the previous two years. We assume that a large part of this increase is the result of concentrated eradication efforts in Red Flag Herds.

For a considerable period of time, certain responses to the tuberculin test have been disregarded in some areas as being of little importance. Considerable effort is being directed toward having all responses to the tuberculin test recorded. More and more animals with questionable responses are being classified as suspects and held under quarantine for a retest. As a result, the total number of animals reported as suspects is showing a considerable increase. In fiscal year 1960 there were 14,149 reactors and 7,763 suspects. This past year (fiscal year 1961) there were 14,579 reactors and 13,600 suspects. Approximately the same number about nine and one-half million cattle were tested each year.

The amount of testing in species other than cattle was about the same in 1961 as 1960. In certain areas based on limited tests, it appears that the number of poultry and swine infected may be significant as sources of infection for cattle—causing reactions in animals in which gross lesions of tuberculosis are not found.

The high incidence of tuberculosis found in mature chickens slaughtered under Federal inspection between March and July 1961 and the geographic area having the greatest number of this class of birds, correspond significantly with the geographic area generally considered the problem states in the tuberculosis eradication program.

Over one-third of the total mature chickens slaughtered were slaughtered in the east-north central and west-north central areas. For the country as a whole, 98.72 percent of the total mature chickens condemned because of tuberculosis were slaughtered in these two areas. When one considers that most of these infected birds undoubtedly came from farm flocks, the potential hazard to the cattle industry in these areas becomes readily apparent.

In the early 1940's more attention was given to tuberculosis in poultry than is currently the case. In one rather extensive study conducted during
that period, which involved the testing of more than 1,400,000 birds, the infection rate was 2.8 percent. But, tuberculosis in mature chickens is still commonly found in some areas of the country, and considerably more attention should be directed toward eradicating it.

There are large numbers of swine raised in these same areas. The incidence of the disease in swine slaughtered under Federal inspection approaches three percent nationally. Most of these infections are generally considered to be caused by the avian type organism. Additional laboratory studies are planned to determine the relative importance of the three types of tuberculosis in swine as well as cattle.

It is essential that we incorporate into the cooperative programs all of the knowledge available to us to eradicate the disease wherever it exists. Detailed epidemiological studies must be used more generally wherever infection is disclosed in any specie. We must not overlook the need for determining the tuberculosis health status of animals that may have associated with cattle that respond to the tuberculin test. All too frequently there has been an obvious disregard of other livestock which may be the source of continuing trouble.

Testing cattle for the prime purpose of reaccrediting areas has detracted from applying basic eradication principles in some areas. The attention to details of rooting out sources of infection wherever they may be found is our intent and purpose.

During the past year, approximately 120 State and Federal veterinarians were given a short but intensive course in epidemiological orientation. Three training sessions were held: one in Atlanta, one in Kansas City, and one in Salt Lake City. The purpose of these training courses was to encourage those who attended to apply their training at the field level to the various disease eradication programs including tuberculosis.

We recognize that we are still confronted with many unanswered questions about tuberculosis. As reported previously, we continually encourage research help. Interesting leads are being developed from research projects and field studies that may resolve some of our problems. We cannot report that there has been sufficient evidence developed as yet to indicate a need for revision of the basic program as expressed in the Uniform Methods and Rules. These methods and rules are used primarily to provide means of reaccreditation, but they are also intended to guide us in locating foci of infection which must be found and eliminated in order to achieve eradication.

In addition to research and field studies, more emphasis is being placed on adequate identification through back tags, ear tags, or other means, for animals moving in commerce and particularly to slaughter, so that trace backs of infection to herds of origin can be improved.

These records are used to illustrate contrasting conditions that exist in two areas of the cattle population.

In the three eastern states, a larger percentage of the cattle are identified by ear tags. The ear tag numbers are included on test records. The states maintain records of tags issued to specific officials. Dealers are licensed and
Comparative Results of Investigations Following Reports of Tuberculosis Lesions on Regular Kill in Two Groups of States

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Three Eastern States</th>
<th>Three Midwest States</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases Investigated</td>
<td>54</td>
<td>78</td>
</tr>
<tr>
<td>Herds Tested</td>
<td>75</td>
<td>132</td>
</tr>
<tr>
<td>Reactor Herds</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>Percent Herds Tested—that had Reactors</td>
<td>37.3</td>
<td>9.0</td>
</tr>
<tr>
<td>Percent Cases Investigated With Reactor Herds</td>
<td>51.8</td>
<td>15.3</td>
</tr>
<tr>
<td>Cattle Tested</td>
<td>3,725</td>
<td>3,964</td>
</tr>
<tr>
<td>Reactors</td>
<td>244</td>
<td>72</td>
</tr>
<tr>
<td>Percent Reactors</td>
<td>6.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Cattle Reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear Tag * (%)</td>
<td>65.4</td>
<td>22.0</td>
</tr>
<tr>
<td>Sale Tag Only (%)</td>
<td>25.4</td>
<td>8.7</td>
</tr>
<tr>
<td>Ear and Sale Tag * (%)</td>
<td>45.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* Or other identification—fiscal year 1961.

required to maintain records. Sales agencies maintain records that are very effective in tracing cattle movements. Considerable effort is directed toward cooperative action with meat inspection services (Federal and State or municipal).

Many of these favorable conditions do not prevail in the other states where there is a greater mass movement of unidentified cattle, especially those of the beef breeds.

In order to obtain better results, it is necessary to acquire the coordinated efforts of all of the above mentioned agencies. The support of the livestock producers and packers is also essential.

In addition to this, an effort is being made to bring all slaughter plants (State, municipal, and county) into the plan of reporting tuberculous lesions found on regular kill.

The following table indicates the extent that gross lesions have been reported from straight kill operations in Federal meat inspection establishments.

<table>
<thead>
<tr>
<th>Year</th>
<th>Bovine Carcasses Retained for Tuberculosis</th>
<th>Bovine Carcasses Condemned and Passed for Cooking</th>
</tr>
</thead>
<tbody>
<tr>
<td>1917</td>
<td>195,488</td>
<td>49,214</td>
</tr>
<tr>
<td>1940</td>
<td>8,384</td>
<td>1,998</td>
</tr>
<tr>
<td>1961</td>
<td>382</td>
<td>87</td>
</tr>
</tbody>
</table>

However, we have reason to believe that a significant number of tuberculous animals slaughtered under straight kill operations are not detected, or if detected, are not reported to the appropriate authorities. It is probable that many lesions are not reported in small plants that have limited inspection service, and further, that a significant percentage of culled dairy cows are slaughtered without any inspection. It should be noted that many of the slaughter plants not covered by Federal inspection are located in areas that are considered most critical so far as bovine tuberculosis is concerned.
Since this class of animal is potentially most likely to have tuberculosis, we believe that we cannot overstress the importance of improving identification and inspection procedures as an aid of efficient tuberculosis eradication.

However, the importance of testing a significant number of cattle on a continuing basis must be brought to the attention of the whole livestock industry. That there is value to be derived from expanding the amount of area testing can be pointed out by the fact that 66 percent of all new gross lesion herds during the period January 1, 1959 to April 30, 1961, were found as a result of area tests. There were 898 such herds.

This means that about 2/3 of lesion units were found in the group where 62 percent of all tests were made. Eight percent of the total gross lesion herds were found as a result of testing after tracing the origin of reacting animals, the origin of animals showing lesions on regular kill, and follow-up on exposed animals removed from infected herds.

Fifty-four percent of the reactors (2,422) in gross lesion herds were detected as a result of area testing, and 17 percent of such reactors (778) were found in herds tested following tracing procedures.

From this we may conclude that as a result of area testing, many infected herds are found in the early stages of the disease as compared to those found as a result of tracing cattle that show lesions on regular kill. The number of herd owners who are saved the shock of severe herd infection as a result of removing diseased animals early can only be estimated, but we are inclined to believe that it is considerably underestimated.

While it is true that tests of exposed cattle conducted as a result of tracing are much more likely to disclose reactors with gross lesions than regularly conducted area tests, it is apparent that many cases of disease are not being detected simply because area testing is relatively limited.

In just the past few months, four counties have been removed from the list of modified accredited areas. Two of these have since been reaccredited.

Costs of routine testing versus costs of tests resulting from tracing have frequently been considered. In this reporting period, 94 percent of the total testing was area testing or associated testing, while just over one percent was the result of tracing reactors to herds of origin, lesion cases found on straight kill, and follow-up on exposed animals. Retests of quarantined herds account for the balance. The ratio of area work results compared to tracing results at first glance appears to be so strongly favorable to the latter that it may seem to be economically unsound to have such a large percent of our work effort guided toward area testing. However, area testing at present is the only means of locating infected cattle in a relatively large number of herds. It is the primary means of locating early infection in a herd. It has been the chief way of arriving at the prevalence rate of infection for each area in the country; and since the economics of any disease eradication program must be considered from the standpoint of long time gain, it is folly to consider that area testing be curtailed because of the cost involved in locating individual animals infected with tuberculosis. Until and unless better diagnostic procedures are developed, area testing as presently defined must be continued. We believe the anticipated end results justify the means.
It is the responsibility of the livestock regulatory officials to administer the program. These officials must have the livestock industry’s support in order to make the program effective. If we fail to assume this responsibility and neglect to carry out our obligation, we will merit the criticism laid at our door. Too often the statement is offered that we do not have enough time, or personnel, or funds to take proper action when tuberculosis is reported even though all evidence indicates that tuberculosis is an active antagonist. The history of past success will not indefinitely serve as a crutch to support the program. Tuberculosis has not been eradicated, and permissive disregard of the potential danger in any case of disclosed infection is permissive negligence. The price of tuberculosis eradication is constant vigilance.
REPORT OF COMMITTEE ON TUBERCULOSIS


The 1960 Report of the Committee on Tuberculosis, which was adopted by this Association in Charleston, West Virginia, contained in part the following. "It is recommended that in the future a mathematical formula based on cattle population density, cattle movement, and incidence be used as a method to find statistically the soundest approach toward locating the specific infection probability for the area to be tested. This would be an objective statistical approach toward determining the specific number of cattle to be screened in a given area to most efficiently locate infection, and would result in a wiser expenditure of monies and manpower."

At the time the 1960 report was under consideration by the Committee, suggestions were made that the Committee consider the definition of a tuberculosis-free area in its recommendations. After much discussion, the Committee finally agreed unanimously that it was not practical at that time to attempt this. The Committee did point out that the goal of eradication must be uppermost in the minds of all of us, but under existing conditions would probably be undesirable to proceed to this end until we had at hand some of the solutions needed for some of our other problems involving tuberculosis. During the year, an attempt was made by the Committee to develop information regarding the incidence of bovine tuberculosis, based on cattle densities. The figures used were the total tuberculosis testing by counties in one State only for a 6-year period, 1956-1961, involving two full accreditation periods. These figures were furnished to the office of the Federal-State statistician in the State concerned with a request that a determination be made if possible, regarding the following:

1. What, if any, relationship existed statistically between the incidence of tuberculosis and the cattle density by counties and by types of cattle operation?
2. What justification statistically could be developed for or against the established percentage rate in the Uniform Methods and Rules required for testing for accreditation?
3. Given an established minimum percentage factor for acceptable incidence, is it possible accurately to predict statistically, based on known factors (and what are those factors?), the percentage of testing required to guarantee compliance with the Uniform Rules.
4. In an attempt to achieve total eradication and to establish tuberculosis-free areas, could the same statistical approach be used in predetermining the number of reactors to be expected and what percent of the total cattle population involved should be tested?

5. As another way of putting No. 4, could a statistically sound system be developed from known factors involving tuberculosis of predetermining over a period of time the number of reactors that could be expected to exist in a given cattle population and an assumption of eradication be made when testing procedures inaugurated in this area had disclosed this number?

The statistical organization from which this information was requested spent many weeks working over the figures supplied and over additional figures that were requested from time to time and finally advised that from the figures given them, many of the answers requested could not be supplied. This Committee was advised, however, that this was due to the fact that statistics involving only one State had been furnished and as the incidence of tuberculosis was extremely low, many of the answers that emerged were borderline in their implication and could not be defended as factual. It was requested that the figures from several other States be supplied, showing varying amounts of tuberculosis from the best to the worst, so that a more accurate evaluation of the whole picture could be made. One fact did emerge, however, that appears on the surface at least to have statistical validity, and that is the fact that there is a correlation between cattle densities, type of cattle operation, and incidence of tuberculosis, both actual and predictable. Sufficient evidence developed along this line to suggest to the statisticians that the whole matter certainly was worthy of a great deal more study.

As a result of this, a request was submitted to Washington for additional statistical information from several other States. This information was supplied to the Committee and is now in the hands of the statisticians. It is your Committee's recommendation that this matter be pursued with vigor and to its logical conclusion.

The Committees on tuberculosis of this Association have for many years urged that research on tuberculosis in livestock be greatly expanded. Your Committee recognizes the excellent research being conducted on tuberculosis at Michigan State University supported by funds allocated through the Animal Disease and Parasite Division of the Agricultural Research Service. As well as the excellent effort being made at the University of Wisconsin.

It is recommended that the Secretary of this Association submit a letter to the Administrator of the Agricultural Research Service expressing the following:

1. This Association's interest in tuberculosis research and urge that funds be made available to continue the project now underway at Michigan State University.

2. Research on the tuberculosis at the new National Animal Disease Laboratory should reflect the importance of this disease in regard to the economy and health of the animal industry and the public health.
3. Studies are needed in an attempt to improve present diagnostic procedures and to develop new tools to combat the disease.
4. Para-tuberculosis in cattle and sheep and other pathological conditions caused by Mycobacteria should be included in these studies.

To facilitate animal disease eradication, it is recommended that appropriate livestock sanitary officials take immediate steps to require the individual identification of all livestock moving both intrastate and interstate by properly recorded eartags, tattoos, backtag or brand. Such identification is essential for an efficient trace-back system involving any animal found to be diseased.

During this past year members of this Committee and other regulatory officials attended the annual meeting of the National Tuberculosis Association in Cincinnati, Ohio. As there is a significant relationship between tuberculosis of man and animals, it is recommended that we all take an active interest in the activities of both the national and local tuberculosis Associations. It is further recommended that an invitation to attend our meetings be extended to the National Tuberculosis Association.

From time to time it is recognized that State and Federal officials may find, as an economical method of removing a foci of infection, a need to eliminate an entire herd badly infected with tuberculosis. It is recommended that the Director of the Animal Disease Eradication Division of the Agriculture Research Service give serious consideration to participate in making indemnity payments for animals from such herds that do not respond to the tuberculin test.

To further strengthen the tuberculosis program, it is urged that all States adopt the following program priorities:

1. Concentrate on the elimination of tuberculosis from "Red Flag" herds and other herds known to be affected with tuberculosis.
2. Thoroughly examine for tuberculosis all animal life maintained on the premises where tuberculosis is found to exist. Cooperate with public health officials in determining the health status of humans associated with the infected herd.
3. Develop procedures and provide means where necessary for identifying all market slaughter cattle in order that those found tuberculous on regular kill meat inspection may be readily identified with the herd or herds of origin.
4. Determine the origin of all reactors and test appropriate herds of origin, giving special consideration to reactors with advanced tuberculosis.
5. Follow up on all cattle believed to be exposed to tuberculosis and test the appropriate animals and herds.
6. Maintain modified accredited status as a means of locating early herd infection.
7. Develop procedures for identifying all market slaughter swine and mature chickens in order that those found tuberculous at time of slaughter
may be readily identified with the premises of origin, and take steps to eliminate the disease from the premises.

The following changes in the Uniform Methods and Rules are recommended:

1. Part I, Section 1c—change to read as follows:
   (c) When suspects to the tuberculin tests are disclosed in herds not containing reactors, such animals shall be quarantined to the premises where disclosed (accredited herd status to be suspended) and no movement of suspect animals . . . . .

2. Part I, Section 3b—delete.

3. Part II, Section 10—add the following:
   In routine area testing, all cattle twenty-four months of age and over shall be tested. All cattle in the herd may be taken into consideration in determining the percentage of infection for reaccreditation of the area. In infected herds all cattle in the herd shall be tested.

4. Part II—add Section 16:
   If an area does not qualify for reaccreditation under Section 11 through 14, the Director of the Animal Disease Eradication Division may extend the accreditation for an additional period not to exceed three years provided that all other provisions of the Uniform Methods and Rules are being complied with.
EOSINOPHILIC MYOSITIS IN CATTLE

G. MIGAKI, D.V.M. AND P. J. BRANDLY, D.V.M.*

Beltsville, Maryland

Veterinarians of the Federal Meat Inspection Division (MID) occupy a unique position which allows them to observe the numerous pathologic conditions affecting our livestock. Their experience and perception reveal many morbid and abnormal conditions which exhibit unusual characteristics and are not associated with recognizable ante-mortem signs. Many of these cases are submitted to the laboratory for specific diagnoses.

Among the most important of these conditions is a pathologic entity of unknown etiology affecting only the striated muscles which has been variously identified as sarcosporidiosis, myelogenous leukemia, myelocytoma and eosinophilic myositis (EM). The histopathologic picture is that of muscle inflammation with eosinophilic infiltration. Our experience indicates that EM occurs primarily in cattle, occasionally in sheep, and rarely in swine. The possibility of a common etiology of E.M. in dogs and cattle cannot be denied because neither disease is fully understood. However, the widespread involvement of all striated muscles, including the heart, in cattle, compared to the restriction of lesions to muscles of mastication reported in dogs, suggests a different etiology. Clinical signs in cattle have not been recognized. The paucity of American literature on this subject results from our emphasis on ante-mortem and post-mortem signs and lesions in determining the wholesomeness of meat rather than on the laboratory approach practiced in some countries. This does not imply that the veterinary histopathologists, Doctors Day, Creech, Bengston, Stiles, Davis, Seibold, Shalkop and Anderson, making diagnoses for the Federal veterinary meat inspectors (VMI), have not been fully aware of E.M. in cattle. We know of no field research program on E.M. in cattle and, because it is not of clinical significance, few studies have been made in the United States.

Our purpose in presenting this paper is to emphasize the prevalence of E.M., to review the available literature, and to describe the gross and microscopic findings. Eosinophilic myositis is the same condition identified by the late Dr. L. Enos Day in the early days of Federal Meat Inspection as sarcosporidiosis, the name by which it is still identified by many veterinary meat inspectors of long experience. Although we have studied several hundred cases, we have never been able to associate the presence of sarcocysts with eosinophilic response or muscular degeneration. The presence of sarcocysts is a common microscopic finding in otherwise normal striated muscles. The eosinophilic reaction is similar to that found in acute trichiniasis, however,

* Meat Inspection Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.
Eosinophilic Myositis in Cattle

We have never found that parasite or any other etiologic agent associated with the inflammation.

In 1958, we had hoped to study this condition microscopically and a request for sarcosporidiosis specimens was issued to the veterinary meat inspectors. In one month approximately 150 cases were received. The lesions in these cases were principally small, yellowish-green in color, and elliptically shaped, all of which probably accounted for the gross diagnosis of sarcosporidiosis. In contrast with current understanding, pathologists early in this century generally attached much pathologic significance to the order Sarcosporidia. Finding no demonstrable agent these early pathologists attributed E.M. to the readily detected sarcocysts.

Since the post-mortem inspection summary form of the M.I.D. does not designate a specific classification for sarcosporidiosis or E.M., the disposition has been included under miscellaneous inflammatory diseases, miscellaneous septic conditions, and miscellaneous parasitic conditions. In the disposal of carcasses and parts of carcasses showing evidence of E.M., the following general rules are applied: If the lesions are localized in such manner and are of such character that they can be radically removed, the nonaffected portion of the carcass may be passed for food after the removal and condemnation of the affected portions. If the carcass shows numerous lesions, or if the character of the involvement is such that complete extirpation of the lesions is difficult and uncertainly accomplished, the entire carcass shall be condemned. We conservatively estimate about one thousand carcasses are condemned annually for E.M. The importance of E.M. to the veterinary meat inspector is so great that a special category for E.M. is being included on future reporting forms.

Kennedy's (1) review of E.M. and the reference material reveals that almost all investigations have been made by European workers. Kennedy described five naturally occurring cases. In an attempt to reproduce E.M., he infected two yearling bulls and a calf with Trichinella spiralis. All developed lesions similar to, but differing in some respects from E.M. Maddy (2) discussed sarcosporidiosis and its relationship to E.M. Histologic examinations of Maddy's specimens were made by Dr. C. L. Davis who was of the opinion that E.M. was not primarily due to sarcocysts but to an undetermined parasite.

We have compiled 50 cases of E.M. chronologically received in the Biological Sciences laboratories of the M.I.D. from December, 1960, through September, 1961. This number in no way reflects the incidence of E.M. found by veterinary meat inspectors but represents only those about which new veterinary meat inspectors had questions or experienced veterinary meat inspectors desired specific microscopic findings. These specimens were received from establishments in 27 different cities in all parts of the United States during all seasons of the year. Conversations with field personnel revealed that E.M. appears in bovines of all ages. Although our data show that approximately 66 percent of the animals in this group of 50 cases were two years of age or younger, and 80 percent were in good to excellent physical condition, these factors may be related to the fact that there is greater interest in the disposition
of “choice” and “good” animals rather than to an increased incidence of E.M. in such animals. Neither the veterinary meat inspector nor our data provide evidence that sex is a factor in E.M. Thirty-nine of the 50 cases reported generalized involvement of the striated muscles including the heart, while only seven reported the involvement to be localized. The remaining four cases were found on reinspection when the carcasses were dismembered into primal cuts. When generalized, the lesions were identified as extensive and well marked in 36 cases and slight in three cases. In the localized cases, involvement of the masseter, heart, diaphragmatic or esophageal muscles was reported.

Selected representative gross descriptions given by the veterinary meat inspector on the unsolicited 50 cases are quoted:

"Lesions in the masseter muscles were extensive, cardiac-well marked, carcass slight."

"Some of the yellowish-green lesions contained a greenish exudate. The lesions were located throughout the carcass."

"Characteristic spheroid to slightly oblong yellow foci or bodies occurring numerously throughout all striated muscles."

"Muscle tissue has a grossly abnormal appearance, yellowish-green in color and widely distributed among normal appearing tissue."

"Extensive infestation in muscles of head, heart, esophagus, diaphragm, forequarters and hindquarters."

"The loin on reinspection showed a grayish-green discoloration. There was no unusual odor. The physical texture of the meat was nearly normal."

Histologic examination of sections through the lesions shows a consistent pattern, although two grossly different manifestations are found. The more common gross lesion is the multiple, small, yellowish-green, elliptical foci distributed rather uniformly throughout the muscles. The striking and less frequently found lesion is the massive involvement of the entire muscle or large muscle bundles with a uniform greenish discoloration caused by accumulation of eosinophils. The gross lesions change color from green through shades of greenish-gray to yellow to white. Eosinophilic myositis is characterized as a continuous, progressive inflammatory process advancing from peracute, acute and granulomatous to scar tissue formation and replacement with adipose tissue. The pathogenesis can be explained in this way: In response to some stimuli there is a massive invasion of eosinophils infiltrating between muscle fibers and causing separation and fragmentation of the muscle cells. Atrophy and frequent loss of striation of the degenerating muscle fibers are commonly noted. As the process continues the affected areas are composed almost entirely of eosinophils and fragments of muscle cells. The early stages of E.M. are followed by necrosis of the centers of the lesions with dead eosinophils forming a green exudate which later turns yellow. The resolution of these abscesses is accomplished by a granulomatous process characterized by necrotic centers surrounded by a zone of giant cells,
epithelioid and occasionally lymphocytes. Occasionally, calcification takes place within the lesion resulting in a persistent granulomatous process. The inflammatory process spreads in the line of least resistance between the muscle fibers; therefore, the long axes of the elliptically shaped lesions occur parallel to the muscle fibers. Resolution of the inflammatory process occurs by connective tissue proliferation, followed by replacement with fat tissue. There is no evidence of the development of immunity to E.M. because in many cases all stages of the inflammatory processes are found in the same muscle.

SUMMARY

Eosinophilic myositis of cattle is an asymptomatic pathologic entity characterized by massive eosinophil invasion in the inflammatory areas of the striated muscles. The etiology is unknown and the course is progressive with areas of necrosis, granulomatous process, fibrosis and replacement of the fibrotic tissue with fat tissue. More than 1,000 carcasses were condemned for E.M. by veterinary meat inspectors during the fiscal year 1961.

BIBLIOGRAPHY

A REVIEW OF THE TRICHINIASIS PROBLEM IN THE UNITED STATES WITH PARTICULAR EMPHASIS ON IOWA*


Veterinary Medical Research Institute
Iowa State University of Science and Technology

Ames, Iowa

Although Trichinella spiralis was first recognized microscopically in 1835 by James Paget, a British medical student, it was undoubtedly one of the primary reasons that Mosaic law declared swine unclean for human consumption. Joseph Leidy of Philadelphia in 1846 was the first to discover T. spiralis in pork and thus lay the foundation for present-day knowledge that improperly cooked pork is the primary source of trichiniasis.

Human trichiniasis is of public health interest in the United States. Gould(1), in summarizing various surveys in the U. S. during 1931-1942, found an over-all incidence of 16 percent in 11,287 autopsies. The incidence varied primarily with age, religion, nationality and occupation. Stoll (2) in 1947 estimated the United States had 20 million cases of trichiniasis, three times more than the rest of the world together.

During the 19-year period, 1942-1960, only 5,894 human cases of trichiniasis were reported to the U. S. Public Health Service (3) (4) (5) with a yearly mean of 310 cases. The yearly cases reported during this period ranged from a maximum of 487 in 1948 to 160 in 1960. The mean number of cases reported for the last four years of the above period, 1957-1960, was only 185, indicating a possible decrease in prevalence. During 1950 through 1959, 57 deaths due to trichiniasis were reported. The number of cases reported does not tend to coincide with the 16 percent incidence previously mentioned. This is due in part to the fact that trichiniasis symptomatology often resembles that of many other diseases, and is therefore not diagnosed. Also, many cases of human trichiniasis are subclinical with no symptomatology.

The cases of human trichiniasis reported annually in Iowa (6) are highly variable with 220 cases reported during the 19-year period 1942-1960. The maximum was 133 cases in 1945, with 130 cases in a single outbreak in eastern Iowa. All infected pork in this outbreak was traced to a small, local

Supported in part by research grant E-606 (C5) from the National Institutes of Health, United States Public Health Service, and Contract No. 12-14-100-5259 (93) of the United State Department of Agriculture, Meat Inspection Division.

Published as paper N.S. 619, Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University of Science and Technology.

194
packing house. Other years with relatively high reported cases included 1949 with 37, 1952 with 19 and 1948 and 1955 with 10 each. Only one death due to *T. spiralis* infection has been reported during this period, this occurring in 1958. During early 1961, an outbreak involving 18 people in eight families has been reported. The trichinae were present in home processed smoked sausage.

Studies on the trichiniasis problem in Iowa have been made during 1944-46 and 1953 to date. Emphasis for these investigations has been placed on the epidemiology and epizootiology of *T. spiralis*. This report will review these findings with those of investigators in other areas of the United States.

**SWINE**

Various studies have been made to determine the incidence of *T. spiralis* in swine. The incidence of trichinae in swine tends to vary primarily with type of feeding program. In Iowa and other midwestern states, most swine are grain-fed, garbage feeding occurring only on a limited scale. During 1960, 18,963,000 pigs were farrowed in Iowa (7) while the July 1960 report on the National Status on Control of Garbage Feeding (8) listed only 2,993 Iowa swine as being fed cooked garbage with no feeding of raw garbage reported.

### TABLE 1

**Incidence of *T. Spiralis* in Swine Diaphragms**

<table>
<thead>
<tr>
<th>Year</th>
<th>Source</th>
<th>Type</th>
<th>Number</th>
<th>+</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1953-57</td>
<td>Iowa</td>
<td>Grain-fed</td>
<td>3,597</td>
<td>6</td>
<td>0.2</td>
</tr>
<tr>
<td>1961</td>
<td>Iowa</td>
<td>Grain-fed</td>
<td>515</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>1961</td>
<td>Indiana</td>
<td>Grain-fed</td>
<td>51</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1961</td>
<td>Illinois</td>
<td>Grain-fed</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1961</td>
<td>Massachusetts</td>
<td>Cooked garbage</td>
<td>800</td>
<td>50</td>
<td>6.3</td>
</tr>
<tr>
<td>1961</td>
<td>New Jersey</td>
<td>Cooked garbage</td>
<td>505</td>
<td>26</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Zimmermann *et al.* (9) reported on studies during 1953-57 to determine the incidence of *T. spiralis* in Iowa swine. Trichinae were found in only 6 (0.2 percent) of 3,597 swine diaphragms examined (Table 1). All infected swine came from farms where no garbage leaks were reported. The maximum number of larvae recovered from a 45 g sample was 58,000. Four of the six infected samples contained less than 100 larvae.

A new series of examinations was initiated during 1961 as a cooperative project with the Meat Inspection Division, U. S. Department of Agriculture. An incidence of 0.2 percent was again found for Iowa swine; only one of 515 diaphragm samples contained trichinae. Limited sampling of grain-fed swine from Indiana and Illinois yielded negative results.

A higher incidence of *T. spiralis* exists in swine fed cooked garbage. Trichinae were isolated from 50 (6.3 percent) of 800 diaphragms from Massachusetts swine. The maximum larval count was 120,000 per 45 g sample, or 2,667 per gram. Four other samples contained more than 100
larvae per gram. Thirty of the 50 infected samples contained less than one larvae per gram.

A similar incidence was found in New Jersey swine fed cooked garbage, 26 (5.2 percent) of 505 diaphragms contained trichinae. Fourteen of the 26 infected samples contained less than one larvae per gram while only two contained more than 100 per gram. The maximum was 489 per gram of tissue.

Other studies have revealed a similar relationship between feeding programs and incidence of *T. spiralis*. Schwartz (10) found trichina larvae in only 0.63 percent of 3,031 diaphragms from midwest swine. In contrast, Schwartz (11) reported an incidence of 11 percent for 1,325 swine fed raw garbage which were examined during 1950 while the rate of infection was only 2.2 percent for 5,723 diaphragms from swine fed cooked garbage during the 1954-59 period. Kerr (12) in California found incidences of 0.5 percent in grain-fed swine, 6.4 percent in raw garbage-fed pigs, 8.3 percent in swine fed raw offal and 5.4 percent in pigs fed cooked offal. McNaught and Zapata (13) recovered trichinae from four percent of 495 garbage-fed swine in the San Francisco area. Peres (14) examined 516 southern hogs with negative results.

**PORK SAUSAGE**

Commercial pork sausage available in Iowa has been examined for the presence of *T. spiralis* larvae during the periods 1944-46 and 1953-60. A report on these studies by Zimmermann, Schwarte and Biester (15) has shown a marked change in the prevalence of trichina larvae. Studies during the 1944-46 period had shown an incidence of 12.4 percent in 460 samples of bulk pork sausage. These findings were much higher than those obtained in similar studies during the 1953-60 period when trichinae were present in only one percent of 8,402 samples of bulk pork sausage. The maximum incidence was 3.1 percent in 1953-54 with minimums of 0.3 percent in 1957-58 and 1959-60.

A similar but less marked decline was noted for the incidence of *T. spiralis* in fresh link sausage. The examination of 444 link sausage samples during 1944-46 had shown the presence of trichina larvae in 11.7 percent while during the 1953-60 period only 2.4 percent of 1,432 link sausage samples contained trichinae. The incidences varied from a maximum of four percent in 1954-55 to 0.6 percent in 1957-58.

*T. spiralis* larvae were recovered from only 0.2 percent of 861 samples of processed (smoked and/or heat treated) link sausage. The two positive precooked samples contained 20 and one larvae respectively. Thirteen of the 20 larvae in the first sample were dead, as was the single larva in the second. No determination was made of actual ability to infect. Processing may destroy viability of living worms.

The number of trichinae recovered from the 45 g (0.1 lb.) samples was generally small with average recovery of 12.6 from bulk and 19.5 from fresh link. The maximum for bulk was 244 while that for link was 314.
Trichinae were found in 12 of 21 brand types of bulk and five of 18 brands of fresh link. The maximum incidences by brand was 16.7 percent in bulk and 19.3 percent in link. Both maximums were the same brand.

The incidence of trichinae in pork sausage has varied with different areas of the United States. Peres (4) examined 50 samples in New Orleans and found all negative. Dickman (16) found trichinae in six of 63 samples from Philadelphia. Kerr (12) in California found *T. spiralis* in 21 (1.9 percent) of 1,056 samples of nonprocessed pork sausage while 609 samples of sausage processed to kill trichinae were negative.

**DOGS AND CATS**

An incidence of 9.8 percent was found in 531 Iowa dogs examined (17). A definite age relationship was noted with higher incidences in older dogs. No breed differences in susceptibility were noted with over 20 breeds of dogs being represented in the 52 positive dogs. Sex of dogs was likewise not found to be a factor. The infected dogs came from both rural and urban areas. The infections were generally light with a maximum count of 930 in the 45 g samples. A smaller prevalence was noted for cats, with 5.7 percent being found in 53 cats examined. A maximum larval count of 2,437 was obtained in a cat captured in a heavily wooded area where it had access to rodents and other possible sources of infection.

During the 1959-60 period 93 samples of processed dog food containing meat byproducts were examined. None of the samples yielded *T. spiralis* larvae.

Other studies on the incidence of *T. spiralis* in dogs and cats have shown varying results. Sawitz (18) in New Orleans found 1.3 percent in dogs, 10 percent in cats, while Cross and Allen (19) found 16.0 percent in Chicago dogs and 21.6 percent in cats. DiGiusti and Field (20) reported 4.5 percent in dogs and five percent in cats from Detroit.

**WILDLIFE**

The sylvatic occurrence of *Trichinella spiralis* is an important aspect of the epidemiology and epizootiology of trichiniasis. Increasing attention has been given to the determination of wildlife reservoirs and their role in the trichiniasis cycle. In studies on the sylvatic occurrence of *T. spiralis* in wildlife of Iowa during the 1953-61 period (21), examinations have been made on 7,078 wildlife specimens, representing approximately 44 species. Trichinae have been found in fourteen species, namely, rat, mink, fox, opossum, raccoon, striped skunk, spotted skunk, coyote, badger, beaver, least weasel, wolverine, fox squirrel and horned owl. The wolverine is not considered to be native to Iowa. Infected wildlife are distributed throughout Iowa. Two or more infected species have been obtained from 11 of 12 primary sampling areas with a maximum of seven reservoir species from a central Iowa area.

Studies from Colorado (22) (23) have revealed an incidence of 17.8 percent in 472 rats while low prevalences exist in fox, bobcat and coyote. Trichinae are prevalent in Alaskan mammals. Rausch et al. (21) found *T. spiralis* in 23 of 42 species of wildlife examined.
TRANSMISSION STUDIES

Since numerous species of wildlife have been found to be infected with *T. spiralis*, investigations were made to determine a possible relationship between infected wildlife and grain-fed swine in the trichiniasis cycle (25). Two modes of transmission considered were consumption of infected wildlife carcasses by swine, and fecal transmission from infected animals to susceptible animals. Rats and mice may occasionally be consumed by swine, while other species would only rarely be available. Fecal transmission studies were conducted since many species of wildlife are nocturnal and may contaminate feedlots or pastures with their feces. Rat and mice feces may also be found in swine feed. Wildlife to swine transmission was obtained using foxes, rats, hawks and owls as donors. The following factors were found to affect transmission: (a) time interval between infection and passage of feces by donor, (b) relative immunity of donor, and (c) number of trichinae ingested by the donor. The larvae passed in the feces are either encysted larvae in undigested meat particles or larvae which have been digested free of the cyst but failed to establish in the intestine of the donor. About 95 percent of the larvae passing through the intestinal tract were recovered from feces eliminated within 24 hours after ingestion. In transmission trials, 21 of 22 pigs became infected by consuming fox feces when interval between infection and placement of fox cubs over swine pens was four hours. When the postinfection interval was extended to 24 hours, only three of 12 pigs became infected. Immune animals eliminated a larger percentage of the infective dosage in feces than did nonimmune animals.

The possible spread of trichiniasis through a swine herd through fecal transmission from a single infected pig was also investigated (9). The results varied. In an initial trial all three recipients became infected when kept in a pen with an infected donor. In two later trials no transmission occurred.

Another mode of transmission investigated was tail chewing. In a trial involving serum studies, a noninfected control was placed in a pen with three pigs infected 21 days previously. Examination of diaphragms on day 84 showed the control pig to be infected. Tail chewing was a prominent feature when pigs were placed together. Eight tails from infected pigs have been examined, with six containing trichinae.

Spindler (26) transmitted trichinae to swine by feeding the feces of infected dogs, cats, swine and albino rats. Transmission was obtained during both the maturation period of intestinal trichinae (days 1-4) and during the adult stages (days 4-28). Robinson and Olsen (22) transmitted trichinae by feces from mouse to mouse, rat to rat, mouse to pig, and rat to pig. The greatest number of trichinae and cysts were eliminated during the first 24 hours after infection. Previously infected animals eliminated a higher percentage of infective dose than those animals infected only once.

Discussion

This review indicates that *Trichinella spiralis* is still of public health significance in the United States. There are indications, however, that its
importance may be declining as indicated by a decrease in reported human cases, by the decrease in prevalence in commercial pork sausage available in Iowa, and by the relatively restricted usage of raw garbage as swine feed.

In order to further decrease the importance of *T. spiralis* as a human disease entity the parasite must either be eliminated from swine or the trichinae must be destroyed or made nonviable in pork before human consumption.

Elimination of the parasite from swine would entail elimination of all raw garbage feeding, more thorough cooking of garbage before being used for swine feed, and complete control of rodents and other reservoir species in swine rearing areas. The importance of raw garbage has decreased somewhat due to the advent of regulations controlling garbage feeding after the outbreak of vesicular exanthema in swine. In August, 1961, raw garbage was being fed to 12,392 swine in 24 of 48 States (27), whereas in July, 1960, 21,935 swine were being fed raw garbage (8). Although this number is relatively small, its importance is emphasized by the 11.0 percent incidence cited earlier for swine fed raw garbage. An additional 948,617 swine were reported being fed cooked garbage. Their importance in the trichiniasis problem is indicated by the 2.1 to 6.3 percent incidences cited for swine fed cooked garbage. This would stress the need for more thorough enforcement of existing garbage cooking regulations as well as possible development of more efficient techniques for garbage cooking.

The control of rodents in swine feeding areas is important if trichinae are to be eliminated from swine. An abundance of rats in a garbage feeding area would be conducive to creating a reservoir of infection and thus increase the possible sources of infection for swine. Infections with *T. spiralis* in grain-fed swine are often attributed to rats. Continuous rodent control programs should be maintained in any swine feeding establishment. All rodent carcasses should be promptly removed from feeding areas to prevent consumption by swine. The widespread distribution of trichinae in other wildlife complicates the trichiniasis problem. Since all reservoir species cannot be eliminated, an attempt can only be made to keep them from swine feeding areas.

Another possible means of control in swine is through therapeutic or prophylactic treatment. An ideal drug would be one which could be fed at low level with feed or garbage. No drug is available for this purpose as yet.

Various approaches may be made to destroy or devitalize trichinae in pork. Regulations of the Meat Inspection Division prescribing for the destruction of trichinae in pork and pork products which are to be eaten without further preparation include, heating, refrigeration or curing (82). Heating to a temperature of not lower than 137°F throughout product is required. Time-temperature combinations required for refrigeration are: 5°F, 20 days; —10°F, 10 days; —20°F, six days. Products thicker than six inches require longer periods. Curing procedures include salt curing, smoking and drying. Irradiation of pork (29) is effective in controlling reproduction of trichinae but is not yet used commercially. No accurate diagnostic means has yet been
found for detection of trichiniasis in living swine. Recent trials in Ohio (30) have indicated that the Suessenguth-Kline flocculation test may be of value in this field. Microscopic examination of pork is routinely used in parts of Europe and South America. This method is useful in detecting moderate and heavy infections in swine but since only a one-gram tissue sample is used, light infections may be missed. Current studies are being made to evaluate this diagnostic test. The practicability of this method in the United States may be limited due to the large number of swine raised yearly, the baby pig crop in 1960 being over 88 million pigs (7).

Since most outbreaks of trichiniasis can be traced to farm-slaughtered pigs and home-processed sausage, the farm population should be more thoroughly familiarized with available control methods for trichiniasis. The widespread usage of home freezers and of locker plants along with decrease in home preparation of pork sausage are undoubtedly playing an important role in the decrease and eventual elimination of the trichiniasis problem.

REFERENCES

A REVIEW OF THE TRICHINIASIS PROBLEM


THE ROLE OF THE DIAGNOSTIC LABORATORY IN ESTABLISHING
DIAGNOSES OF INSECTICIDE POISONING

R. D. Radeleff*  
Kerrville, Texas

Insecticide poisoning is frequently suspected when death follows a display of neurological symptoms or no symptoms are observed. In many cases, the death occurs following a recent application of an insecticide to the animal or to its food, either deliberately or accidentally.

Diagnostic laboratories can offer support for a correct diagnosis by veterinarians, physicians, or for other interested persons. In many instances the diagnostic laboratory findings are the basis for settlement of damage claims in or out of court. Such laboratories find that the discharge of their obligation to diagnose properly and fairly is not generally easy. In all too many instances, diagnostic laboratories have made serious erroneous conclusions from limited studies, usually doing so with all sincerity.

Erroneous conclusions may be the result of failure to understand many different phases of the problem, one of the most important being the interpretation of chemical findings.

CHEMISTRY

The Significance of Finding a Poison

It is a long-established custom that investigators submit samples of various tissues and substances collected during a necropsy to diagnostic laboratories, together with the impressions of the investigator as to the toxic substance or condition suspected. When poisons are suspected, the toxicologist usually makes such chemical or biological tests as seem appropriate for the suspected material. On finding qualitative evidence of the presence of the substance, he frequently reports his findings to the original investigator who, in turn, confirms his tentative diagnosis. Generally the press of work in our laboratories is so great that quantitative procedures are not conducted. As a result, there is no factual evidence that a toxic amount of the poison was present.

When the symptoms or lesions of poisoning are reasonably definitive or pathognomonic, qualitative identifications add somewhat to a diagnosis. When the symptoms and lesions are shared with a number of other disease conditions, as is true of most of our present day insecticides, the qualitative identification is highly misleading.

* United States Department of Agriculture, Agricultural Research Service, Animal Disease and Parasite Research Division.
The Confusion in Suspected Insecticide Poisoning

Chlorinated hydrocarbon insecticides are, in most cases, stored as residues in body tissues and excreted in milk of all exposed animals. Organic phosphorus compounds follow the same general pattern, but more of them produce residues of a rather small order of magnitude. This act is well recognized.

Compounds such as benzene hexachloride (BHC), DDT and dieldrin, among others, may accumulate in tissues of animals regardless of the degree of exposure. In our experience, the accumulated residues may be as great as 0.03 percent of the weight of fat (300 parts per million) without in any way producing evidence of a harmful effect. Table 1 illustrates the weight gains of cattle and sheep in tests with B.H.C. and toxaphene, together with the residues found in their fat. No evidence of toxicity existed in these animals.

We have used such vast tonnages of insecticides in the United States during the past 15 years that we have virtually insured the presence of one or more of them as normal in our environment. Animals and people exposed to such insecticides must be expected to accumulate them; hence, their tissues will give a positive response to qualitative tests in the laboratory, even when the individual is in the best of health.

In remote areas where no insect control is practiced and only local feeds and foods are used, we may expect to have consistently negative tests. These areas are not numerous. In most areas not treated with insecticides, feeds and foods are brought in from treated areas, supplying small amounts of insecticides as well.

The presence of residues in tissues of animals is not necessarily significant and certainly does not necessarily result in harmful effects upon the animal. In the same way, residues on feeds or in foods are not always hazardous. The Food and Drug Administration of the United States Department of Health, Education and Welfare has established tolerances for various insecticides in various foods, believing that the established amounts may safely

---

**TABLE 1**

*Weight Gains, Efficiency of Feed Utilization and Insecticide Residues Observed in Cattle and Sheep Fed Toxaphene and Benzene Hexachloride*

<table>
<thead>
<tr>
<th></th>
<th>100 p.p.m. Benzene Hexachloride</th>
<th>100 p.p.m. Technical Toxaphene</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cattle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of initial weight gained</td>
<td>42.3</td>
<td>41.1</td>
<td>42.3</td>
</tr>
<tr>
<td>Feed consumed per pound liveweight gained</td>
<td>7.05</td>
<td>7.31</td>
<td>7.37</td>
</tr>
<tr>
<td>Residue in omental fat, parts per million</td>
<td>250</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td><strong>Sheep</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of initial weight gained</td>
<td>35.3</td>
<td>30.7</td>
<td>32.4</td>
</tr>
<tr>
<td>Feed consumed per pound liveweight gained</td>
<td>10.1</td>
<td>11.6</td>
<td>11.3</td>
</tr>
<tr>
<td>Residue in omental fat, parts per million</td>
<td>117</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>
be present; yet eating of foods so contaminated will result in residues in the consumer.

Because of this normal environmental exposure to insecticides, virtually all animals may be expected to show residues of them in their tissues at some time or other. For this reason, qualitative analyses are misleading and useless unless used as a basis for further chemical study.

The Value of Quantitative Analyses

If an insecticide can be identified and the amount of it in gastrointestinal content can be established as being more than that required to produce death, then a reasonably positive diagnosis can be rendered, assuming that all other considerations are compatible with such a diagnosis. Extreme care must be used in concluding that toxic amounts are present because of the great variation in susceptibility to poisoning and because the physical forms of the compound may strongly influence the probability of it being absorbed.

Chemistry Is of Little Value

Chemical procedures may identify a compound and estimate the quantity present, adding significance to a tentative diagnosis. I feel that the chemists' results are of very little value for diagnostic purposes, for the following reasons:

Residues in Tissues Not a Measure of Dosage or Effect

Table 2, taken from another paper by this writer and others (1) shows the obvious lack of correlation between dosage, effect and residue found. From that data we can see that it is not unusual to kill an animal, leaving a small residue, or to fatten one with very high residues. Similar disparities were noted in our work with dieldrin (2).

We must also remember that some of the organic phosphorus insecticides such as malathion, Bayer 21/199, and diazinon among others do not leave a tissue residue or at most a very small one. The absence of a significant amount thus is very misleading.

A number of organic phosphorus compounds are readily destroyed by the rumen content in ruminants, leaving the possibility that a true poisoning would not be diagnosed because of the lack of finding a quantitative amount.

BIOCHEMISTRY

No readily determined enzyme inhibition has been described in animals poisoned by chlorinated hydrocarbon insecticides. Organic phosphorus and carbamate insecticides inhibit the cholinesterases of nerves and blood. This inhibition in the nervous system has been considered to be one of the basic causes of death from exposure to these compounds in insects and in warm-blooded animals. The quantities of the enzymes in blood have also been considered to reflect the amounts in the nervous system.

Early insecticides conformed rather well to the expectation that 85 percent or greater inhibition of blood cholinesterase activity would be accompanied
TABLE 2

Results of Toxicity Studies With Heptachlor Epoxide and Heptachlor in 1- to 2-Week Old Dairy Calves

<table>
<thead>
<tr>
<th>Ear Tag Number</th>
<th>Dose (mg/kg)</th>
<th>Number of Daily Doses</th>
<th>Weight of Animals (kg)</th>
<th>Total Milligrams Given</th>
<th>Total mg/kg Given</th>
<th>Interval, Initial Treatment to Death</th>
<th>Heptachlor Epoxide in Fat (p.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2234</td>
<td>25.0</td>
<td>1</td>
<td>45.0</td>
<td>1,138</td>
<td>25</td>
<td>3 days&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.7</td>
</tr>
<tr>
<td>2235</td>
<td>25.0</td>
<td>1</td>
<td>41.0</td>
<td>1,025</td>
<td>25</td>
<td>4 hours</td>
<td>2.7</td>
</tr>
<tr>
<td>2239</td>
<td>15.0</td>
<td>1</td>
<td>31.8</td>
<td>480</td>
<td>15</td>
<td>4 hours</td>
<td>2.8</td>
</tr>
<tr>
<td>2238</td>
<td>10.0</td>
<td>1</td>
<td>32.7</td>
<td>327</td>
<td>10</td>
<td>28 hours</td>
<td>250.0</td>
</tr>
<tr>
<td>2243</td>
<td>5.0</td>
<td>1</td>
<td>39.5</td>
<td>198</td>
<td>5</td>
<td>3 hours</td>
<td>7.9</td>
</tr>
<tr>
<td>2267</td>
<td>2.5</td>
<td>1</td>
<td>50.0</td>
<td>125</td>
<td>2.5</td>
<td>Mildly poisoned and recovered</td>
<td>No sample</td>
</tr>
<tr>
<td>2236</td>
<td>15.0</td>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.9</td>
<td>1,255</td>
<td>30.0</td>
<td>6 days</td>
<td>48.3</td>
</tr>
<tr>
<td>2249</td>
<td>2.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4</td>
<td>50.0</td>
<td>550</td>
<td>11.0</td>
<td>7 days</td>
<td>98.0</td>
</tr>
<tr>
<td>2269</td>
<td>1.0</td>
<td>15</td>
<td>33.6</td>
<td>504</td>
<td>15.0</td>
<td>15 days</td>
<td>262.0</td>
</tr>
<tr>
<td>2268</td>
<td>0.2</td>
<td>100</td>
<td>—&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1,205</td>
<td>20.0</td>
<td>No symptoms</td>
<td>14.7&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.6&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

A Calf was treated with chloral hydrate when symptoms appeared the first day.

<sup>b</sup> Doses were given 5 days apart.

<sup>c</sup> The first dose given was 3.5 mg./kg.

<sup>d</sup> Calf weighed 39.1 kg. initially; 95.5 kg. at termination—Avg. daily gain .56 kg.

<sup>e</sup> Two omental fat samples were taken by biopsy; one the last day of treatment, the second 43 days later.

by overt symptoms of poisoning. This was particularly uniform for single exposures, but was not so uniform in the case of multiple small doses. As more and varied compounds were developed, depression of cholinesterase activity in blood of animals exposed to them became more and more confusing to interpret.

Figure 1 portrays the progressive depression of activity experienced in treating a Hereford with parathion five days per week, using daily dosages just under the single toxic dose, and the depression in cattle following multiple spraying with Bayer 21/199. The parathion-treated animal displayed symptoms for about two hours after the 83rd dose; the cattle sprayed with Bayer 21/199 did not show symptoms of poisoning.

Only partial inhibition has been observed in many animals that were poisoned or that died following treatment.

From such illustrations we can readily see that a diagnosis of poisoning is impossible using cholinesterase activity alone as the basis.
Specific analytical methods are not available for many insecticides. There is also a need for a general method to identify the class of poisons to be expected. Bioassays of intestinal contents, urine, feces, and blood have been proposed, which used stable flies and other insects as the test organisms. Bioassay results have been as misleading as those from chemical methods and for the same reason.

Neither gross nor microscopic tissue study has, at this time, revealed specific diagnostic changes that would ease the task of arriving at a diagnosis. This does not imply that abnormalities are not observed, but that those that exist are commonly found in many diseases. I believe that adequate, imaginative research can eventually lead us to entirely new microscopic methods, even gross tissue methods, which will permit us to clearly fix the cause of death as due or not due to insecticides. The accomplishment of such research will undoubtedly require several years.

How a Diagnostic Laboratory Can Perform Its Proper Function

The laboratorian should first obtain all the relevant information in the case-time and nature of exposure, the chemical and its physical form, time from exposure to symptoms or death, symptoms and lesions. With these at hand he should evaluate them carefully to see whether the hypothesis of poisoning is strong or has obvious weaknesses. Much time can be saved if this is done seriously first.

Weaknesses to Look For:

1. Unreasonably large losses from exposure to a compound of known low toxicity.

2. Losses over an unreasonable period after exposure, such as deaths occurring weeks after direct treatment or months after premise treatment.

3. Losses occurring when the chemical could not be expected to be present. Many insecticides volatilize or hydrolyze in a matter of a few days. Losses days after the expected disappearance time should be held in suspicion.

4. All affected animals die. The insecticides rarely kill all animals affected by them. Spontaneous recoveries occur in 50-75 percent of the cases. When only one or two animals are involved, this observation is not valid.

5. Failure to respond to recommended antidotal treatment. Occasional refractory animals are found, but when failure is experienced in several animals, the likelihood of poisoning is reduced.

6. Well-developed lesions such as inflammation, necrosis, abscessation, or neoplasias may exist concomitantly with poisoning, but their consistent presence should alert the investigator to the existence of a disease other than insecticide poisoning.
Findings Suggestive of Other Conditions Which Should Also Be Eliminated:

1. Symptoms of chlorinated hydrocarbon poisoning, exposure to low dosage of low toxicity compound—rabies.
2. Same, with anemia—anaplasmosis.
3. Neural involvement circling—cysticerci in brain (coenurosis).
5. Characteristic chlorinated hydrocarbon symptoms, no exposure known—sodium chloride poisoning.
6. Central nervous system stimulation, convulsions, temperature seven degrees or less above normal (12-15° higher in chlorinated hydrocarbon insecticide poisoning)—febrile affliction of central nervous system.
7. Ill-defined central nervous system symptoms, sporadic losses—listerellosis.
8. Wave of abortions, ill-defined symptoms—leptospirosis, brucellosis, and other causes of abortion.

My True Objective

By now it should be all too obvious that I am urging the staffs of diagnostic laboratories to keep open minds in receiving samples from suspected cases of insecticide poisoning and to be sure that all evidence is in before making confirmations of the proposed diagnosis. Equally obvious should be my repeated suggestion that diagnosticians consider all possibilities. The wide use of insecticides insures that our other diseases are certain to appear coincidentally with one or another of them with reasonable regularity.

A sustainable diagnosis can be made only in the light of intelligent interpretation of the history of treatment, symptoms, lesions, and laboratory findings and not by a single, or even multiple, laboratory finding.

References

REPORT OF COMMITTEE ON PARASITIC DISEASES

Wm. C. Tobin, Chairman, Denver, Colorado; Donald Baker, Ithaca, New York; M. J. Cerosalette, Albany, New York; V. D. Chadwick, Jackson, Mississippi; R. L. Cuff, Kansas City, Misouri; F. D. Enzie, Beltsville, Maryland; J. L. Hourigan, Arlington, Virginia; A. E. Janawicz, Montpelier, Vermont; F. R. Koutz, Columbus, Ohio; F. L. Schneider, Albuquerque, New Mexico; L. E. Swanson, Gainesville, Florida; R. D. Turk, College Station, Texas; F. E. Henderson, San Juan, P. R.

CONTROL OF EXTERNAL PARASITES BY FEEDING MEDICATED FEEDS

Some encouraging results have been obtained by practical farm test in Missouri on control of parasites of pasture cattle by free chlorine feeding of medicated materials.

During 1960, certain tests were conducted using systemic organic phosphates in which results indicated that horn fly counts could be reduced 92.6 percent by this treatment. The average consumption of the systemic drug was two ounces per head per day, demonstrating a very economical and effective way for fly control.

In addition, in summing up the results of feeding systemics in the control of grubs, lice and ticks, grubs were 95 percent controlled by their use; ticks were dead within seven days and dead engorged ticks were found after five days of administering a systemic. Lice were very effectively controlled. It should be borne in mind that treatments of this should produce tissue residue problems which delay slaughter of treated animals.

STATUS OF SCREWWORM ERADICATION PROGRAM

Nearly a half-million in-transit animals were unloaded, inspected and treated for screwworms during fiscal year 1961 at the 13 inspection stations along the eastern boundaries of Arkansas and Louisiana. The inspections revealed infestation in 25 animals which were freed of screwworms before being permitted to continue to their destination.

All livestock entering the southeast from the southwest must be unloaded and inspected. All wounds on the livestock are treated with an approved smear, and an organic phosphorus insecticide is applied to all animals, with certain exceptions, as a precautionary measure.

On June 18, 1961, a farmer in Holmes County, Florida, suspected screwworms in an ear wound of a hog, and after collecting several larval specimens, notified his county agent, who in turn notified an A.D.E. inspector. The inspector picked up the specimens which were identified as screwworms by a taxonomist at Sebring, Florida. State and Federal inspectors were immediately dispatched to the area and located four additional cases within a five-mile radius, the most recent case being confirmed on June 27. These
represented the first native cases of screwworms in Florida since June 17, 1959. However, one case had been found by a veterinary practitioner in October 1960 in a racing dog from Kansas at the time of its arrival in Tampa. On August 30, 1960, an infestation of screwworms was reported by a local veterinary practitioner from a wounded steer at a Geneva County, Alabama stockyards. The animal had been shipped from Texas to Alabama a few days prior to discovery. Identification of the parasite touched off intensive efforts and both infestations were brought quickly under control.

All animals within a 20-mile radius of the outbreak in Holmes County were inspected, and all livestock owners were alerted to the danger of possible spread. Part of the inspection area extended into southern Alabama. Approximately 5,000 directly exposed livestock within a 10-mile radius were sprayed with a pesticide as a precautionary measure.

Beginning June 27, approximately one million sterile flies have been released each week in the area. The flies were reared and sterilized with radioactive Cobalt 60 at a Kerrville, Texas, laboratory where A.D.E.D. maintains an "insurance" colony for such emergencies. The released sterile males mate with native females. Eggs resulting from such matings fail to hatch; thereby eliminating the native population.

The first screwworm infestation found in the western portion of the eradication area, was discovered in a native animal in western Mississippi near Vicksburg on the 15th of August. Numerous cases were found in this area of Warren County, Mississippi, and shortly after, screwworms were reported in De Soto County in the extreme northwestern corner of the State. No screwworms had been reported in this area since the initiation of eradication in the southeast.

After being disclosed in the northwestern area of Mississippi, screwworms were soon found in herds in several counties in western Tennessee near Memphis. The pests increased rapidly in numbers and in many herds in each area were reported numerous infestations and some reinfections. In slightly over two weeks after the first screwworms were found in western Tennessee, they were reported by a veterinary practitioner in Franklin County on the Alabama border in middle Tennessee. Almost immediately after this discovery, Alabama reported screwworms in the northern section of Madison County near Huntsville, Alabama. From this initial foci of infestation in Alabama, the screwworms spread in the immediate area to five counties in Alabama and Floyd County, Georgia. Further extension of the outbreak along livestock movement channels involved an additional eight counties in Alabama and four more in Georgia.

Federal and state livestock inspectors are making a close inspection of all livestock in these areas, applying a pesticide to many of the herds, and are closely watching animal movements to control the spread of screwworms into the southeastern over wintering area. Effective November 1, 1961, the Federal regulations were amended to require treatment and/or inspection of livestock moving into Florida from or through Alabama and Georgia.
REPORT OF PSOROPTIC CATTLE SCABIES ACTIVITIES—FISCAL YEAR 1961

SCABIES OUTBREAKS

During fiscal year 1961, psoroptic scabies was diagnosed in 10 herds—one each in Iowa and Texas, two each in Oklahoma and Illinois, and four in Colorado. A brief summary of these outbreaks follows:

On October 7, 1960, psoroptic scabies was diagnosed at Sioux City stockyards in cattle from a feedlot in Plymouth County, Iowa. Additional infected animals were found in the feedlot. It was placed under State quarantine, all infected and exposed cattle were slaughtered. States contributing cattle to the infected feedlot included Iowa, Oklahoma, Nebraska, Wyoming, South Dakota, North Dakota, Kansas, Montana and Minnesota. Inspections were made of all contributing herds in those states and no additional infected cattle were found as a result of this backtracing except in a Texas County, Oklahoma herd in January 1961. Psoroptic mites were also found on a bull in an adjacent contact herd. The two infected herds and all exposed herds were treated. All known movements into and out of the herd were traced and inspected without finding additional infection. Farm-to-farm inspections were conducted throughout Texas County and other Oklahoma panhandle counties.

Psoroptic scabies was found by regulatory inspectors at Chicago Union Stock Yards involving 53 fat steers in one lot of Hereford cattle during the International Livestock Exposition held in Chicago, Illinois, November 25, through December 3, 1960. The animals had been brought to the exposition from a feedlot in Menard County, Illinois. Examinations at the feedlot revealed infection in an additional 43 steers. Two adjacent contact herds showed no evidence of scabies. Toxaphene was used to spray-dip the infected herd twice and the two contact herds once. Prior purchases included cattle from Colorado, Nebraska, Wyoming and New Mexico. These herds were subsequently inspected and no evidence of scabies found.

During November 1960, a State inspector, while making a routine inspection, found cattle scabies in a Swisher County, Texas feedlot. About three percent of the 2,320 head showed lesions. The premises were quarantined by the Texas Animal Health Commission, and all the cattle were dipped twice in toxaphene. Of 1,352 other cattle exposed, 145 were dipped twice and 1,207 were dipped once. A total of 83,590 heads in 821 herds in 125 Texas counties were inspected as a result of the outbreak. During fiscal year 1961, a total of 3,167,575 cattle were inspected in the State and 73,475 dipped. Ninety percent of the cattle in the infected feedlot had been purchased through the stockyards at Clovis, New Mexico, and 10 percent at auction markets in Abilene and San Angelo, Texas. States contributing cattle, possibly through these sources included Colorado, Oklahoma, Texas, New Mexico, Louisiana, Arkansas, and Kentucky.

During March 1961, an alert inspector following up a rumor heard at a cattle sale, inspected a herd of cattle for scabies near Rockford, Illinois, in Winnebago County. He suspected scabies but could not demonstrate mites. His suspicions and persistence caused him to reinspect the cattle on April 3
at which time psoroptic mites were found. Cattle in a second herd evidenced scabies-like lesions but no mites could be demonstrated. The animals had been previously sprayed by the owners. Both herds were quarantined by state officials and additional treatments under supervision were required. Both herds included cattle from a lot of 323 steers and heifers shipped from a New Mexico ranch on November 2, 1960. The cattle had gone through a concentration point at Byron, Illinois. The 217 steers were trucked to three premises. Illinois officials located and inspected the remainder of the 106 heifers with negative results. Cattle other than those from New Mexico had gone through the concentration point at Byron and other than the New Mexico cattle were in the two affected herds. However, all animals with scabies lesions carried brands of the New Mexico ranch.

In New Mexico, all cattle on the ranch from which the cattle were shipped, including the remaining heifers held back for replacements, were inspected. No evidence of scabies was found.

In Colorado during the fiscal year, four outbreaks of scabies were reported. On November 15, 1960, a veterinary practitioner was called to observe suspected cases of scabies among 23,000 cattle in 48 pens at a Morgan County feedlot. He diagnosed psoroptic scabies and notified State officials who inspected the cattle and confirmed the diagnosis. Infected animals were found in 16 of the 48 pens. The owner quickly constructed a 9,000 gallon dipping vat and all the cattle were dipped twice in lime-sulphur, except those going to immediate slaughter which received one dipping. State contributing cattle to the feedlot included Colorado, New Mexico, Nevada, Utah, Wyoming, Nebraska, Kansas, Texas, Idaho, Oregon, Arizona and Montana.

Psoroptic scabies was found in a feedlot in Adams County and confirmed on February 10. The disease was reported by a veterinary practitioner. Of 3,436 cattle in the Adams County feedlot, lesions could be found on just one animal. All cattle in the feedlot were dipped twice, 10-14 days apart, in toxaphene or were shipped intrastate for immediate slaughter. Incoming shipments were traced, and an attempt made to inspect all herds which contributed cattle. These included herds in Wyoming, Texas, Nebraska, Oregon and Kansas. No additional evidence of scabies was seen.

On April 3, inspectors at the Denver Union Stock Yards diagnosed psoroptic scabies in a lot of fat steers consigned from a feedlot located near Windsor in Weld County. Approximately 50 percent of the steers had scabies lesions. The Weld County feedlot included some 1,100 cattle. The cattle were spray-dipped twice with toxaphene. Shipments into feedlots since July 1, 1960, included those from Colorado, Nebraska, Wyoming and Texas. Regulatory inspectors endeavored to locate and inspect all herds involved. No additional infection was found.

On April 10, inspectors at the Denver Union Stock Yards reported psoroptic scabies in a consignment of cattle from a feedlot near Derby in Adams County. Additional infected cattle were found in the feedlot of approximately 4,100 animals. Scabies mites were demonstrated from just one pen. All cattle in the feedlot were spray-dipped twice with toxaphene. Herds in Colorado, Nebraska, Wyoming, Kansas, Texas and Idaho contributing cattle...
to the feedlot prior to the outbreak are being inspected with negative results to date.

TOTAL INSPECTIONS AND DIPPINGS

The total number of cattle inspected for psoroptic scabies during fiscal year 1961, was 7,660,685 and 234,293 were sprayed or dipped, compared with 6,927,266 inspected, 374,990 sprayed or dipped during fiscal year 1960.

INSPECTIONS AND DIPPINGS AT PUBLIC STOCKYARDS

During fiscal year 1961, at the 59 public stockyards, 21,334,686 cattle were inspected and 46,005 dipped.

SCABIES MITES OF ELK

During the fiscal year, psoroptic mites were again found on elk and an attempt was made to learn if the elk scabies mite *Psoroptes equi* var. *cervinae* could be artificially transferred to domestic cattle and sheep. Elk skin scrapings containing a large number of live mites were affixed to the hair/wool of three calves and three sheep and placed in the ears of two of the calves and two of the sheep. Careful examinations at six and 12-week intervals revealed no live mites and indicated that the mites had failed to transfer. This work was done at Laramie, Wyoming, and is being repeated.

FIELD WORK WITH TOXAPHENE

Field trials were conducted at 10 Florida dipping vats, two vats in Arizona, and one each in New Mexico and Colorado to determine if the vatside test for toxaphene would enable regulatory inspectors to maintain a toxaphene dipping bath at the required .5 percent strength. These trials revealed that the vatside test was unsatisfactory and indicated a replenishment ratio would have to be followed to maintain the concentration of the chemical. This work is continuing.

PSOROPTIC CATTLE SCABIES REPORTED IN MEXICO

For the first time in many years, psoroptic cattle scabies was reported in Mexico. The disease was discovered in March 1961, by a United States Department of Agriculture Animal Inspection and Quarantine Division port veterinarian at Juarez, Chihuahua, Mexico. The infected cattle had been offered for importation into the United States and were from a ranch near Casas Grandes in the State of Chihuahua. Standard eradication methods were followed.

SHEEP SCABIES

*Inspection and Dipping Activities*

Psoroptic scabies was reported in 872 flocks of 70,018 sheep in 296 counties in 24 states, compared to 886 flocks in 280 counties in 25 states in 1960.
One hundred and eighty-seven infected lots were found at public stockyards during fiscal year 1961 and 214 during the previous year. 12,031,249 sheep were inspected during 1961 and 506,745 dipped—a substantial increase over 1960 when 10,836,576 were inspected and 390,958 dipped. Last year at the 59 public stockyards, 12,308,185 sheep were inspected and 350,399 dipped.

**Outbreaks in the Sheep Scabies Free Area**

There were outbreaks in two western states believed to have been free of sheep scabies. The California outbreak involved one flock in San Benito County and was apparently introduced by sheep from New Mexico. The sheep had been dipped prior to shipment to California.

New Mexico officials unearthed interesting information that associated the California outbreak with several of those in New Mexico. It appeared that at least the majority of the outbreaks were traceable back to a Lea County flock found previously, in June, 1959, to be infected. The finding of mites in the Lea County flock by means of the maceration technique illustrates the necessity for its use, particularly in areas in the final stages of eradication. In both California and New Mexico, intensive efforts were made to locate and eradicate the disease.

Sheep scabies was found in western South Dakota in Butte (one flock), Fall River (three flocks), and Gregory (one flock) counties, where active eradication measures were also taken.

**Amendments to 9CFR, Part 74—Scabies in Sheep**

These regulations were amended effective August 1, 1960, designating all or parts of 27 states as free areas and remaining as infected areas. The amendment also provided for dipping of sheep moving interstate from infected into free and eradication areas except those destined for immediate slaughter and designated eastern South Dakota as a sheep scabies eradication area.

More recent amendments to the regulations designated the following as sheep scabies eradication areas: New York State in February 1961; Arkansas, Nebraska, North Dakota, and Tennessee in June; and Illinois in July. In September 1961, western Kansas counties were added to the eradication area.

An amendment effective in July 1961, added 11 counties in western Nebraska to the free area. This was the first area, considered infected in August 1960, to be freed of the disease. In South Dakota, a Federal quarantine placed on Butte County in November 1960 was removed the following month after the disease had been eradicated from the county. Fall River County placed in the infected and eradication areas in March 1961, was returned to free-area status in August 1961.

New Mexico was declared an infected area in September 1960, following scabies outbreaks. Counties were returned to the free area following inspection of all sheep; however, additional outbreaks caused San Miguel (December 1960), Chaves and Eddy (February 1961) and Lea (April 1961) counties to again be placed in the infected and eradication areas. These counties were
PARASITIC DISEASES

returned to the free area following reinspection of all sheep in each county
and in August 1961, New Mexico once again became a sheep-scabies-free area.

Steps are also being taken to designate Hawaii, Wisconsin and New Jersey
as sheep scabies eradication areas.

Scabies Training Courses

Scabies training courses were held in Missouri, New York, and Pennsylvania, where some 180 persons were given the opportunity to learn more
about scabies and eradication procedures. In Colorado, all regulatory
inspectors were given on-the-job training during scabies outbreaks, and the
senior class of the College of Veterinary Medicine, Colorado State University,
observed infected herds of cattle. In Nebraska, regulatory personnel took
advantage of the opportunity afforded at sheep shearing schools to orient
sheep shearers in reporting eradicating procedures.

Goat Ear Mange

The survey of goat herds for evidence of ear mange mentioned last year
was continued. Psoroptic mites were found in 273 goat herds in Texas. Psoroptic mites were also found in 48 lots of goats received at the San
Antonio Union Stock Yards and in 29 lots at the Fort Worth Stock Yards.

Psoroptic mites have also been reported in goats' ears in Arizona, Georgia,
Missouri, Oklahoma and Washington.

Last year Dr. H. O. Peterson reported his efforts at the Albuquerque Animal
Disease and Parasite Research Division Laboratory to transfer psoroptic mites
from goats' ears to sheep. This work is continuing; however, to date a
successful transfer has not been achieved.

Field Tests With Dips

Field trials conducted in Illinois, California, Colorado and in Iowa revealed
that the vatside test for toxaphene was not satisfactory and indicated a
replenishment ratio would have to be established and followed to maintain
the concentration of the chemical at the required .5 percent. The work is
continuing.

Work in Iowa is also continuing to determine if unheated lime-sulphur
dips with a suitable wetting agent can be used to eradicate scabies.

Other Mange Conditions Reported in Sheep and Goats

Sarcoptic mange in goats has been reported at intervals in New York State
and also reported in a herd in Douglas County, Oregon. Chorioptic mange
was reported in a flock of Southdown sheep in McKinley County, New
Mexico; in two Texas flocks, one flock in West Virginia and two Southdown
sheep from the latter flock at the Pittsburgh, Pennsylvania Joint Stockyards,
one flock in New York and in May 1960, chorioptic mites were recovered
from Southdown rams consigned to the California State Ram Sale. Also, in
1960, chorioptic mange was found in two lots of Suffolk sheep and three
lots of angora goats in Oregon. Demodectic mange has been reported in goats
in Arizona and in New Mexico and in sheep in Nevada, New York and Texas.
Chorioptic mange in years past was reported in goats and in sheep in Texas.
REPORT OF COMMITTEE

TICK ERADICATION

CATTLE FEVER TICK ERADICATION IN FLORIDA

The intensive Cattle Fever Tick Eradication Program in Florida continued throughout the year. Sixteen infested herds in the following counties were reported by your Committee last year: Palm Beach—10, Martin—four, Hillsborough—one and Indian River—one. No additional infestations have been reported. All infested herds and exposed herds were dipped regularly for 12 months following last report of infestations. At the final dipping, no cattle fever ticks were found on a hand inspection of all cattle in each of the herds concerned. Following this, Federal and State quarantines previously placed on certain areas in Hillsborough, Indian River, Martin, Osceola and Palm Beach counties were removed and the State tick quarantine along a line crossing Florida at Ocala was discontinued.

During the year, 4,295 lots of 105,719 cattle in Florida were inspected and/or dipped for ticks.

In addition to the inspection and dipping of known infested or exposed herds, the eradication program included inspection of cattle at slaughter houses, inspection and dipping of cattle at auction markets, and inspection of all cattle and horses in the 32-county area south of the State tick quarantine line. This area involves the southern two-thirds of pensular Florida. Approximately 1,200,000 livestock were inspected and are believed to represent approximately 97 percent of the livestock in the area.

CATTLE FEVER TICK ERADICATION IN TEXAS

During fiscal year 1961, the following activities were reported in the buffer area in Texas: 79 Mexican livestock and eight native American livestock straying into Mexico and returning were caught by tick inspectors. Of the Mexican livestock, 61 were horses of which two were found to be tick infested, and 17 were cattle of which eight were infested. Also animals in one United States herd were found to be infested, and nine herds were held for further treatment. During the year, 13,046 certificates were issued covering intrastate movement of 83,982 livestock from the area and 66 certificates were issued for the interstate movement of 12,668 livestock. Also, 50,922 lots of 1,033,789 livestock were inspected for ticks, and 10,911 lots of 63,151 livestock were dipped.

TICK ERADICATION TRAINING SCHOOLS

Special training in tick eradication was provided at Laredo, Texas, during the period July 18 to October 21, 1960.

The sessions were divided into two categories. Thirty-four inspectors in groups of eight or nine participated in a series of two-week programs which provided training in tick identification and in routine inspection and eradication activities. Twenty-seven inspectors, in groups of five or six, attended a second series of one-week training sessions devoted to tick identification.
In addition to the work with toxaphene mentioned under the cattle scabies activities, field trials were conducted in Florida with delnav. It was found that the vatside test was not satisfactory and that a replenishment ratio would have to be established in order to maintain the concentration of the chemical in the dipping bath. This work is continuing.

Your Committee's report of last year mentioned ticks identified as *Rhipicephalus evertsi* (red tick) found at Africa, U.S.A. They had been found on September 7, 1960, by a regulatory inspector. Africa, U.S.A., is located at Boca Raton, Palm Beach County, Florida, and includes about 400 wild animals—giraffes, camels, zebras, asses, elephants, ostriches, and several species of antelopes. These animals run loose in a double-fenced 160-acre compound.

The red tick is known in Africa to be a vector of East Coast fever and other exotic diseases. It is also a vector of bovine piroplasmosis. These ticks had not been reported previously in the United States. They are two-host ticks able to survive on the ground for more than a year and are very difficult to eradicate.

Inspections revealed the tick on the following animals: eland, camel, zebra, abyssinian ass, nilgai, giraffe and zebiahorse (cross between a zebra and a horse). None were found on wild birds nor on the ostriches. Intensive surveys and trapping in adjacent areas failed to reveal the presence of the exotic ticks outside the compound. However, native ticks have been found on the following animals: opossum, squirrel, rabbit, skunk, rat, racoon, and dog. The area was placed under both State and Federal quarantines and appropriate inspections and treatments were carried out to eradicate this exotic tick. Zoos that had sent animals to or received animals from Africa, U.S.A., were inspected. No additional exotic ticks were found.

After a series of premises treatments of Africa, U.S.A., 73 animals were restrained and inspected in February 1961. Seventy-one male and one female *R. evertsi* ticks were found. Following this, additional treatments of the premises were required.

Beginning in September 1961, each host animal at Africa, U.S.A., was restrained, carefully inspected for ticks, treated, placed in a tick-free area, and again inspected and again treated. No ticks of any species have been found to date and unless subsequent inspection disclose ticks and the red tick is believed to have been eradicated.

**EXOTIC TICKS FOUND IN NEW YORK AND FLORIDA**

On October 21, 1960, exotic ticks were found on zebras at Busch Gardens, Tampa, Florida, by the director of the gardens. Inspection by regulatory veterinarians revealed that seven of the 10 zebras were infested. The ticks were identified as *Rhipicephalus evertsi* (red tick) and *R. pulchellus* (ornate tick). The 10 zebras had been delivered to the gardens on October 28. They had been trucked from the Clifton, New Jersey, Quarantine Station where they
had undergone a 30-day quarantine following shipment from Mombasa, Kenya, East Africa, by ocean vessel. Other zebras in the shipment had been trucked to the Catskill Game Farm, Catskill, New York, and one remained at the Quarantine Station. The latter animal was tick-free; however, inspectors from the Quarantine Station and New York State found two of the three zebras shipped to Catskill to be infested with the ticks.

Both premises were placed under State and Federal quarantines, and infested and exposed animals, trucks, and premises were treated with acarides. The truck hauling the infested zebras to Florida had, on the return trip, loaded cattle in Tennessee and Virginia. The truck and cattle were located in New York State prior to unloading. The truck was cleaned and disinfected and the possibly exposed cattle treated twice. The premises in Tennessee and Virginia were treated also. The exposed facilities in the latter State had been used prior to treatment for loading a shipment of cattle to Indiana. These animals and the truck involved were treated in Indiana as a precautionary measure.

Other zoo animals in the African shipment had been shipped by air from the Quarantine Station to San Diego, California, Zoological Gardens; and by truck to the Detroit, Michigan, Zoological Park (enroute 13 llamas were loaded at the Brookfield, Chicago, Illinois, Zoo and taken to California); Woodland Park, Seattle, Washington, Zoological Gardens; and to Thousand Oaks, California. Another truck transported animals in the African shipment to the National Zoological Park, Washington, D. C., no ticks have been found on any of these animals or on those which remained at the Clifton Quarantine Station. However, the majority of the animals in the African shipment and other animals and the trucks and premises involved, were treated as a precautionary measure. The exotic ticks were eradicated from the premises in Florida and New York and the quarantines have been removed.

TRAINING IN ZOO ANIMAL IMMOBILIZATION TECHNIQUES

A zoo animal immobilization training course using "Cap-Chur" equipment commenced at Atlanta, Georgia, on February 20, 1961. The first week of training was divided between Atlanta and the School of Veterinary Medicine, University of Georgia, Athens, Georgia. The second week of the course was at Africa, U.S.A. Nine veterinarians from the states of California, Florida, Georgia, Nebraska, New York, Ohio, and Texas received the training.

ANTHELMINTIC ACTION OF ORGANOPHOSPHATE INSECTICIDES

The anthelmintic action of systemic insecticides, especially the organophosphates, is receiving increased attention from research workers in all parts of the world. Indeed, several of these compounds have been shown to be highly effective against a variety of helminthic parasites in nearly all classes of livestock. By and large, however, their anthelmintic uses, at present, are not clearly defined; and they are beset with toxicity hazards to a greater or lesser degree. We are not prepared to recommend any of them for general
field use, yet we must allow that they are an extremely interesting group and fully deserving of our unrelenting efforts to determine their rightful place, if any, in the ever-increasing anthelmintic armamentarium.

For the most part, the organophosphates have been most satisfactorily employed against gastrointestinal helminths of ruminants, especially cattle. Swine and poultry seem to be considerably less tolerant of their toxic effects, at least insofar as we can judge from available information. These chemicals have received very little attention at potential anthelmintics for horses.

In reviewing available information on the anthelmintic uses of organophosphates in livestock, one is impressed not so much by their broad spectrum of activity (although this is rather striking in some instances) or by their marked toxicity or safety; but, rather, by the extreme variability in these respects. In many instances, equivalent dosages of any given compound, administered by workers in various parts of the world or even sections of the country, may result in substantially different anthelmintic responses, toxicity reactions, or both. This may be explained, perhaps in part, by the method of administration since it has been shown rather clearly that the efficiency of some drugs is influenced by their passage initially into either the rumen or abomasum.

There is some evidence also that toxicity may vary somewhat in accordance with the passage of drugs through the gastrointestinal tract. The watchword then, is caution—particularly when evidence of either efficacy or safety is based upon a comparatively small number of animals. Insofar as the definitive evaluation of organophosphates as anthelmintics is concerned, therefore, it would probably be wise to adopt a policy of “watchful waiting” rather than one of “wishful thinking” which has been altogether evident in some instances.

OX WARBLES AND FACE FLY

Ox warbles, technically known as Hypoderma lineata and H. bovis, have been a major problem among the cattle producers of the United States. Now there is for the first time, chemicals which are easily administered and effective in controlling these pests. The phosphates are effective for grub control when applied or administered to cattle at the right time and dose level as recommended by the manufacturers.

The Face fly, Musca Autumnalis, is an exotic fly from Europe and Asia. The first appearance of this fly on the North American continent was found in Canada in 1952. The pest has increased its area in the United States in the northeastern and central states. The pest has been found in the following states: Ohio, Indiana, Illinois, Wisconsin, Michigan, Delaware, Massachusetts, New Hampshire, New Jersey, Pennsylvania, Vermont, West Virginia, New York, Maine and Virginia. In the year 1960-61, the flies have been found in Wyoming, North Dakota, Nebraska, Kansas, Missouri, Maryland and Georgia.

Control of these flies has been difficult in that they breed in fresh manure and most insecticides are ineffective in preventing repopulation of the fly. The members of the United States Livestock Association should be alerted
yearly on the extensive and rapid occurrence of the pests in the United States. United effort on the part of experimental workers should be supported by all members of the United States Livestock Association.

RECOMMENDATIONS

Your Committee recommends:

1. Sheep Scabies Eradication—Your Committee recognizes the increased interest in eradicating sheep scabies and the considerable progress made during the past year.

   We recommend that the eradication program be further accelerated and that increased efforts be made to provide the necessary State and Federal funds to support a realistic eradication program.

2. Tick Eradication—Your Committee urges that the aggressive tick eradication program be continued to locate and eliminate all possible foci of tick infestation. We also again urge that import requirements and inspections be considerably strengthened to prevent additional importation of exotic ticks on any animal regardless as to the species of the animal being imported.

3. The quiescent or latent phase of scabies in both sheep and cattle presents State and Federal livestock officials with a very serious inspection problem. Sheep and cattle going through a period of latent scabies can easily be passed for noninfested animals. Weeks or months later, when weather conditions are more favorable for mite development, scabies may reappear on certain previously infested animals. If animals going through latent phases of scabies have been shipped to distant parts of the country, and reinfestation occurs, it may result in widespread dissemination of the disease.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY

H. E. GOLDSTEIN, Chairman, Columbus, Ohio; R. A. BANKOWSKI, Davis, California; L. E. BODENWEISER, Salem, Oregon; C. CUNNINGHAM, East Lansing, Michigan; B. S. POMEROY, St. Paul, Minnesota; R. H. SINGER, Frankfort, Kentucky; S. C. SCHMITTLE, Gainesville, Georgia; C. L. VICKERS, Columbia, South Carolina; J. WALKER, Washington, D. C.

Your Committee on Transmissible Diseases of Poultry this year has endeavored to function in a dual capacity. This year’s activities were divided into research education and regulatory programming.

The Committee on Transmissible Diseases of Poultry commends the Agricultural Research Services on the consideration of last year’s report regarding the problem of Salmonella in animal feed-stuffs. It is indeed most gratifying to realize results from the serious efforts of such Committee recommendations.

This past year has been an exceedingly strenuous burden on the economic structure of the poultry industry. In these critical periods, disease control is not only imperative but also must be kept economically sound. Thorough knowledge of these problems, help in this approach, and your Committee has again prepared abstracts of the major poultry problems for publication in the United States Livestock Sanitary Association proceedings.

The broiler industry has once again been plagued by high numbers of condemnations in the packing plant.

A field study of broiler condemnation losses conducted at the Georgia Agricultural Experiment Station concluded:

“(1) Respiratory Diseases are the most important factor related to broiler condemnation.

(2) Environmental (management) influences are related to broiler condemnation.

(3) The combination of adverse environmental (management and weather) conditions, with the presence of disease, resulted in the highest condemnation.

(4) The qualities which lead to good record keeping also produce good poultry and less condemnation.”

Your Committee has given serious consideration to the Proposed Restrictions on Interstate Movement of Poultry and Hatching eggs. This Committee is now in agreement with the proposed regulation, since the revised regulation now includes the section as follows: “The shipper forwards one copy of the statement referred to in subparagraph (3) to the veterinarian in charge of the Division’s local field station in the State of origin of the shipment, one
copy to the responsible State official in the State of destination, retaining
one copy."

Your Committee feels that it should now call attention to the practicality of
complying with section 84:10 regarding cleaning and disinfecting vehicles,
premises, and accessories. Within the last year the poultry industry has been
afforded an aluminum crate which makes compliance with the cleaning and
disinfection section of the regulation practical. In a separate portion of this
report, an evaluation of the aluminum crate is mentioned.

Your Committee has given serious attention to the problems associated
with the Proposed Interstate Movement Regulation. This Committee, in
endorsing the regulation, is cognizant that in any disease control program
involving law, rule, or regulation, a cooperative effort must prevail within
the involved industry. Recognizing the poultry industry's concern for
pullorum disease and fowl typhoid, and in response to requests from certain
segments of the industry, your Committee recommends that Animal Disease
Eradication, Agricultural Research Service give consideration to stimulating
the cooperation of the poultry industry, prior to the enactment of this
regulation.

In an attempt to solicit this cooperative effort, this Committee recommends
the following:

(1) Stimulate an effective reporting program within each State for every
diagnoses of pullorum disease and fowl typhoid.
(2) That when such a diagnosis is reported, steps be taken within each
State to ascertain the true epidemiology involved; and when such
diagnosis involve interstate movement the cooperative efforts prevail
between each State to locate foci of infection, and adequate control
measures be taken.
(3) That information be made available to the poultry industry for all foci
of infection, the problems involved, and the cooperation afforded.
(4) That a central office be established by Animal Disease Eradication to
receive reports of each outbreak from each State, so that these records
may be assembled, affording the results to all interested groups.

This Committee commends the American Association of Avian Pathologists
on the Report of the Committee on Pullorum Disease Eradication of August
21, 1961, at Detroit, Michigan, and are in complete agreement that;
Encouragement must be given to State Livestock Disease Control Agencies to
develop programs for eradication of pullorum disease and fowl typhoid.

The Committee is of the opinion that stimulation must be afforded to have
closer relationship between diagnostic laboratories and State regulatory
officials. Such relationships will bring about a much better understanding of
disease incidence and geographic distribution, as well as affording a measur-
ing stick of economic importance.

This Committee recommends that our Colleges of Veterinary Medicine give
serious consideration to stimulating veterinary students in taking more
active interests in poultry problems, and to afford more short courses, in a
continued educational program for practicing veterinarians as well as for regulatory veterinarians.

The Committee this year has endeavored to keep pace with past discussions concerning control and eradication of pullorum disease and fowl typhoid. The Committee is including a Proposed Uniform Methods and Rules for such a program, to be evaluated by all parties concerned. Suggestions, criticisms, or modifications, should be made to the next year’s Committee.

Recognizing that the programs of National Poultry Improvement Plan has performed an excellent service to the poultry industry, there is a need for supplementary assistance to make eradication a reality and goal.

This Committee further recommends to the Executive Committee of the United States Livestock Sanitary Association that each State regulatory official give serious consideration to declaring pullorum disease and fowl typhoid reportable diseases and these reports be submitted to a central office of Animal Disease Eradication.

Your Committee proposes that the Executive Committee of the United States Livestock Sanitary Association appoint an advisory subcommittee on Transmissible Diseases of Poultry to work with Poultry Diseases, Animal Disease Eradication Division for developing short range and long range planning of its poultry disease activities.

It is felt that the Committee should be composed of people experienced in poultry disease research, poultry disease control and eradication through programming, and poultry industry representatives.

The scope of activity of this Committee should include calling attention to the Animal Disease Eradication Division of the need for specific national poultry disease programs as indicated by financial losses being suffered by the poultry industry due to disease. Further the Committee should consider the need for initiating interstate regulations for the control of poultry and hatching egg movement when conditions warrant.

The Committee should function as an advisory group only however it should also have the responsibility of informing the organizations which each member represents of its activity. In this regard the subcommittee members should seek the approval and assistance of their respective parent organization in the poultry disease work considered essential for maintaining an adequate and continuous supply of poultry and poultry products for the national diet.

The chairman of the Transmissible Diseases of Poultry should serve as chairman of the

(1) Poultry Breeders of America.
(2) American Poultry and Hatchery Federation.
(3) American Poultry Association.
   (American Bantam Association).
(4) American Association of Avian Pathologists.
(5) State Departments of Agriculture.
(6) National Poultry and Turkey Improvement Plans.
(7) American Veterinary Medical Association.
(8) National Turkey Federation.
(9) National Institute of Poultry.

The Committee this year has endeavored to keep pace with the past discussions concerning control and eradication of Pullorum Disease and Fowl Typhoid. The Committee is publishing a Proposed Uniform Methods and Rules for such a program, to be studied by all parties concerned. Suggestions, criticisms, or modifications should be made to the next year's Committee.

CONTENTS

Section

I Introduction.
II Definitions.
III Registered Individual Flock System.
   A. Establishing Flock Registration.
   B. Maintaining Flock Registration.
IV Registered Area System.
   A. Establishing Registered Area.
   B. Maintaining Registered Area.
V Registered Free-Area System.
   A. Establishing Registered Free Area.
   B. Maintaining Registered Free Area.
VI Consideration of Infected Non-Hatchery Supply Flocks in Reckoning Infection Rates.
VII Procedures for Handling Infected Hatchery Supply Flocks and Their Products.
VIII Procedures for Handling Infected Non-Hatchery Supply Flocks and Their Products.
IX Cleaning and Disinfection.
X Reporting of Laboratory Isolation of S. Pullorum or S. Gallinarium.
XI Submission of Positives to a Recognized Laboratory.
XII Laboratory Techniques for the Examination of Tissues from Pullorum Disease or Fowl Typhoid Positives.
XIII Hatchery Licensing and Inspection.
XIV Additions to Registered Flock, Registered Area, or Registered Free Area.
XV Movement of Poultry for Exhibition Purposes.
XVI Licensing and Training of Registered Testing Agents.
XVII Feed Additives or Other Drugs Administered to Poultry Scheduled for Testing.
XVIII Identification of Tested Poultry.
XX Entering Premises.
XXI Supervision.
XXII Services to Owner.
XXIII Random Testing for Hatchery Supply Flocks That Were Tested on Registered-Free Area System.

PROPOSED UNIFORM METHODS AND RULES

Pullorum Disease and Fowl Typhoid of Poultry

Section I Introduction.

It is the intent of this paper to present a broad, over-all guide for the use of States for the eventual eradication of pullorum disease and fowl typhoid in chickens and turkeys. The system provides for periodic testing of individual flocks and for the development of area eradication programs. Basically, this system describes the procedures for testing hatchery supply flocks, supervision and inspection of hatcheries, and the procedures to be followed to identify and control egg or hatchery disseminated or farm contracted outbreaks of pullorum disease and fowl typhoid.

Section II Definitions.

(a) Area—Geographic boundary—County—State—or groups of States.

(b) Testing Results.

(1) Negative: An approved testing procedure in which the serum-antigen mixture remains turbid.

(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.

(c) Poultry—Live chickens and turkeys of all ages.

(d) State Livestock Sanitary Authority—The person(s) responsible in each State government for the control and eradication of poultry diseases.

(e) Recognized Laboratory—A laboratory approved by the State livestock sanitary authority, for performing approved serological testing procedures and bacteriological culture techniques.

(f) Approved Serological Testing Procedures—

(1) Chickens:
   a. Standard tube agglutination test.
   b. Rapid serum test.
   c. Rapid whole blood test.

(2) Turkeys:
   a. Standard tube agglutination test.
   b. Rapid serum test.

(g) Division—Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture.

(h) Hatchery Supply Flock—All poultry housed or ranged as one unit for the production of eggs to be used for hatching purposes.
(i) Non Hatchery Flock—All flocks except those maintained for hatching purposes. Hatchery—Equipment, on one premise operated or controlled by any person or persons, for the hatching of poultry eggs.

(j) Infected Flock—Flock in which one or more poultry has been diagnosed to be infected with pullorum disease or fowl typhoid.

(k) Official Test—Serological testing by approved testing procedure of one or more poultry conducted by an accredited veterinarian, State inspector, Federal inspector, or other registered testing agent.

(l) Products—Poultry or hatching eggs.

(m) Registered Testing Agent—Any person authorized by the State livestock sanitary authority to perform blood collection and testing provided under Sections III, IV, and V.

(n) Flock—All of the chickens or turkeys on one premises that are housed, incubated, or ranged as one unit.

Section III Registered Individual Flock System

A. Establishing Flock Registration.

(1) A flock of poultry may attain this status by serological testing in the following manner all eligible poultry which serve as hatchery supply flocks:

a. Each chicken of five months of age or older and each turkey of four months of age or older will be tested each calendar year by an approved testing procedure conducted by an accredited veterinarian, State inspector, Federal inspector, or registered testing agent.

b. Flocks with no evidence of pullorum disease or fowl typhoid for two consecutive annual tests are eligible for the status of “registered flock” for one year.

c. Registered flocks will be kept segregated from poultry raised for other purposes in such a manner as to prevent the dissemination of disease as prescribed by the State livestock sanitary authority.

B. Maintaining Flock Registration.

(1) A flock of poultry may be re-registered each year under this system if there is no serological or other evidence of pullorum disease of fowl typhoid. The flock shall be serologically tested by an accredited veterinarian, State inspector, Federal inspector, or registered testing agent using an approved testing procedure not less than eleven months nor more than thirteen months since the previous flock test. (Age of poultry to be tested—same as (1) a. above.)
TRANSMISSIBLE DISEASES OF POULTRY

Section IV Registered Area System.

A. Establishing Registered Area.
   (1) When 75 percent or more of the hatchery supply flocks, representing not less than 51 percent of the hatchery supply poultry in an area, place their poultry under the individual flock system, the remaining hatchery supply flock owners shall participate in an area system.
   (2) If as a result of a blood test of all hatchery supply flocks in an area the number of positives does not exceed .5 percent of the poultry tested, the area may be declared "registered" for a period of one year.

B. Maintaining Area Registration.
   An area may be re-registered each year under this system provided that all hatchery supply flocks are tested between the eleventh and thirteenth month of the due date and the rate of infected poultry does not exceed .5 percent. (Age of poultry to be tested—same as Section I (1) a. above.)

Section V Registered Free Area System.

A. Establishing Registered Free Area.
   An area may be established as "registered-free" of pullorum disease and fowl typhoid for a period of two years if it meets the following requirements:
   (1) Classified as a registered area for a period of not less than the previous two consecutive years.
   (2) The infection rate of all pullorum disease and fowl typhoid serological testing has been zero percent and there is no other evidence of pullorum disease and fowl typhoid during the above described two years.

B. Maintaining Registered-Free Area.
   An area may be maintained as registered free of pullorum disease and fowl typhoid for two years by complying with the following provisions in all hatchery supply flocks.
   (1) Hatchery supply flocks that were tested on previous registered-free area system.
      a. Serological testing of all poultry 15 months of age and over.
      b. Consideration must be given to a more economical approach other than 100 percent continuous testing.
      c. Zero percent infection rate as revealed by serological testing in a. and b. above.
      d. No other evidence of pullorum disease or fowl typhoid in a. and b. above.
(2) Hatchery supply flocks that were not tested on previous registered free area test.
   a. Serological testing of all chickens over five months of age.
   b. Serological testing of all turkeys over four months of age.
   c. Zero percent infection rate as revealed by serological testing in a. and b. above.
   d. No other evidence of pullorum disease or fowl typhoid in a. and b. above.

(3) If pullorum disease or fowl typhoid is disclosed by serological testing or other evidence in one or more hatchery supply flocks in a registered-free area, the area is reclassified to a registered area when the infection rate is less than .5 percent of all poultry tested. If the infection rate is greater than .5 percent the area shall be required to re-establish registered status. The reclassified area shall proceed to re-establish itself through testing procedures as outlined under Section III, IV, and V.

Section VI Consideration of Infected Non-Hatchery Flocks in Reckoning Infection Rates.

Non-hatchery flocks found to be infected with pullorum disease or fowl typhoid will not be included in reckoning infection rates for registered flocks, registered areas, and registered free areas, if A. and B. of Section VI is complied with in handling pullorum disease or fowl typhoid infection in these flocks.

Section VII Procedures for Handling Infected Hatchery Supply Flocks and Their Products.

A. The flock shall be quarantined until disposed in one of the following manners:
   (1) The flock may be moved to a State or Federally inspected poultry processing establishment accompanied by a written certificate issued by the State livestock sanitary authority or his representative.
   (2) The flock may be destroyed under supervision of a State or Federal livestock inspector.
   (3) The positives may be removed from the flock, and the flock serologically tested. If as a result of two consecutive negative flock tests, the first not less than 21 days following removal of positives, and the second not less than 21 days later, the flock shall be considered negative.

B. All incubating eggs from flocks found to be infected may be removed from the incubator and destroyed prior to hatching.
Section VIII Procedures for Handling Infected Non-Hatchery Supply Flocks and Their Products.

All non-hatchery flocks in which pullorum disease or fowl typhoid is disclosed will be handled the same as infected hatchery supply flocks as outlined in Section VII.

Section IX Cleaning and Disinfection.

Premises found to have housed, incubated, brooded, or ranged poultry infected with pullorum disease or fowl typhoid shall be cleansed and disinfected under regulatory supervision within 15 days following removal of positives or infected flocks, unless an extension of time is granted. No infected premise shall be restocked with poultry or eggs for hatching purposes until the above cleaning and disinfecting requirement is complete.

Section X Reporting of Laboratory Isolation of Salmonella Pullorum and/or Salmonella Gallinarium.

All public, commercial, industrial, and private laboratories shall report the name and address of the flock owner from whose poultry they have isolated Salmonella pullorum and/or Salmonella gallinarium to the State livestock sanitary authority within three days of the diagnosis.

Section XI Submission of Positives to a Recognized Laboratory.

A. Each flock owner of infected poultry found by serological testing may submit positive birds from that flock to a recognized laboratory for laboratory culture examination confirmation. If laboratory examination fails to reveal S. pullorum or S. gallinarium organisms, the flock shall be considered negative.

B. The number of poultry to be submitted for laboratory confirmation of serological tests shall be determined by the State livestock authority or his representative.

Section XII Laboratory Techniques for the Examination of Tissues from Pullorum Disease or Fowl Typhoid Positives.

The bacteriological examination procedure shall be the same as that outlined in (9CFR Part 146, sub-part B, Section 147.11).

Section XIII Hatchery Licensing and Inspection.

A. The State livestock sanitary authority shall require that each commercial hatchery be licensed and subject to periodic inspection. The inspection should consider:

(1) Hatchery sanitary practices.
(2) Identification of all hatching eggs.
(3) Disposal of hatchery waste.
(4) Cleaning, disinfecting, and fumigation practices as recommended by the State livestock sanitary authority.

B. Only eggs from registered hatchery supply flocks or hatchery supply flocks located in registered or registered-free areas shall be used for hatching purposes.

Section XIV Additions to Registered Flocks, Registered Area, or Registered-Free Area.

A. Poultry from a registered-free area may enter another registered-free area, registered area, an area in the process of such registration, or registered flock without restriction.

B. Poultry from a registered flock or registered area may enter a registered-free area if the flock of origin was tested negative within 60 days of entry. Individual poultry to be moved shall be negative to an official test within 30 days of entry. Poultry from a registered flock may enter a registered area, an area in the process of such registration, or registered flock if the flock of origin was tested negative within 60 days prior to movement.

C. Poultry from all other sources may enter registered-free area, registered area, an area in the process of such registration or registered flock, if it originated from a flock which has been subjected to two negative official tests within 60 days prior to entry. In addition, individual poultry to be moved must be negative to an official test within 10 days of entry.

Section XV Movement of Poultry for Exhibition Purposes.

Poultry for exhibition purposes may be moved intrastate if each bird has been tested by an approved testing procedure and found negative within 60 days prior to movement. Each bird shall be identified by a numbered wing band acceptable to State livestock sanitary authority.

Section XVI Licensing and Training of Registered Testing Agents

It shall be the duty of the State livestock sanitary authority to provide adequate training and examination of registered testing agents. These agents shall be issued a license subject to review the first of each calendar year.

Section XVII Feed Additives or Other Drugs Administered to Poultry Scheduled for testing.

All food or water drug additives or preparations given by injection or other routes which have been proved by scientific research to cause interference with approved testing procedures for pullorum disease and fowl typhoid shall be withheld from all flocks scheduled for testing not less than 10 to (15) days prior to the date of bleeding.
Section XVIII Identification of Tested Poultry.

All poultry serologically tested for pullorum disease and fowl typhoid shall be identified in a manner prescribed by the State livestock sanitary authority.

Section XIX Report of Tests.

All tests conducted for the detection of pullorum disease and fowl typhoid shall be reported to the State livestock sanitary authority in the State tested within ten days following the date of bleeding.

Section XX Entering Premises.

Persons engaged in pullorum disease and fowl typhoid detection and eradication should be authorized to enter premises to carry out their duties.

Section XXI Supervision.

The pullorum disease and fowl typhoid eradication system shall be supervised by full time State and/or Federal employed veterinarians.

RESPIRATORY DISEASES

In spite of the urgency and attention given to respiratory diseases in chickens by poultry pathologists, chronic infections of the respiratory tract which contribute to heavy condemnation rates of poultry remains to be one of the most important fields of investigation. Earlier studies on the etiology of the air-sac syndrome perhaps over-emphasized the role played by the then newly described agent of *Mycoplasma gallisepticum* in the respiratory disease complex in chickens. Recognition that other agents, when present as multiple infections, may result in severe respiratory disease in gaining momentum. Gross (1), continuing his studies, demonstrated that chicks recently vaccinated with the B-1 strain of Newcastle disease (ND) and exposed two days later with a pathogenic strain of *E. coli* administered by the aerosol route resulted in a mortality as high as 10 percent. He found that susceptibility in chicks was greatest when vaccinated one-four days of age, and exposed to *E. coli* eight days later. The synergistic action was evidenced for at least 22 days after vaccination. Similar experiences were reported by Bankowski (2) in experiments conducted in the laboratory and the field with a number of agents in the United States, and by Hoekstra (3) with infectious bronchitis (IB) and N.D. in the Netherlands. Dardiri, *et al.* (4) also observed lesions of the air sacs following administration of ND virus and IB vaccines to two-week-old chicks in the absence of mycoplasma infection.

*Newcastle disease.* Studies of the physical characteristics to obtain more information on the nature, properties and differentiation of the various strains of virus are continuing. Dutcher, *et al.* (5) studied the effect of inactivating ND virus at 175 C within 0.033 seconds which had little or no
effect on the level of HI or SN antibody response when given once or twice at three-day intervals. Pursuing the observation that nitrous acid effect ed mutation of virus, Granoff (6) studied its action on the RO strain of ND virus. Mutations by this procedure were not regularly produced and of the eight nitrous acid-induced clones, isolated from tissue cultures, five of the mutants lost some virulence for embryonating eggs. The mutants, how ever, were not tested in chickens. Gellenczei and Bordt (7) showed that strains B-1, MK107, Roaken, and GB. Texas differed considerably in their growth characteristics in various cell cultures relative to their retention of virulence and antigenicity. Although the more virulent strains grew well and retained their antigenicity in most cultures, the B-1 strain lost its antigenicity and failed to stimulate an immunity when administered to one-day-old chicks. Dardiri, et al. (4) demonstrated that a single dose of killed virus or live virus vaccine, did not provide adequate protection against intratracheal challenge with the GB strain of ND virus. Revaccination with the killed or live virus vaccine, however, produced an anamnestic response which protected 100 percent of the birds for a period of 22 weeks.

Other complicating factors in establishing an immunity of a high order to ND was substantiated by Cole and Hutt (8) who have clearly demonstrated marked differences in mortality and reaction of two genetic strains of chickens vaccinated with the Roaken strain of ND given by the wing-web route. These authors believed that more resistant stock can be developed by a controlled breeding program. Winterfield and Hitchner (9) demonstrated, by serological means, that strain N-47 of ND virus when administered by the vent-drop method was capable of boosting an immunity level in chicks previously vaccinated with the B-1 strain in the drinking water. On the other hand the Roaken strain given intramuscularly or by the wing-web route failed to stimulate a significant rise in the serological response. The only criterion used for interpreting immunity was based upon the number of deaths following intramuscular challenge. With a better understanding of immunity per se to ND, a single criteria is not sufficient for evaluation of immunity. Resistance of the reproductive tract, and the systemic reactions including duration of such protection, must be included in the evaluation of the quality of immunity.

Osteen and Mott (10) discussed the need for re-evaluating the ND control program in the United States. These authors indicated that an effective control measure was not successful with the modified live virus vaccines, therefore, favored a killed-ND vaccine, which would be safer, would not perpetuate the disease nor stimulate latent infections. Proof is still wanting that the presently proposed killed-ND vaccine is capable of protecting chickens against the respiratory form of the disease and that laying flocks can be protected against a drop in egg production. The proposal did not discuss the progress which has been made with a live virus vaccine, such as TCND which was shown to be innocuous to unvaccinated penmates for as long as two years, absence of stress and ability to produce an immunity of a high level sufficient to protect chickens against a drop in egg production.
A program of eradicating the disease with the killed product from the United States where the virus is almost ubiquitous merits considerable discussion, evaluation and modification before it is considered. The improvements of killed-vaccine which would offer poultrymen a protection against a pause in egg production in laying flocks and protection of the respiratory epithelium to the natural disease as complicating factors in a respiratory disease complex should be thoroughly studied and sought before this step is undertaken.

During the past year an official ND study team from Great Britain visited representatives of the poultry industry, State Regulatory officials and diagnostic laboratories to evaluate our programs and methods for controlling ND. Britain has eradicated the peri-acute type of the disease in accordance with the 1935 regulations of the Disease of Animals Act which empowers the Ministry of Agriculture to slaughter affected poultry and indemnify the poultryman. The appearance of the subacute form of the disease which occurred in increasing numbers concurrent with the rapid expansion of their broiler industry has resulted in a cost which prompted a review of the policy by the Committee of Inquiry (11). The results of their findings and decisions are pending.

The importance of multiple infections in poultry and presence of heretofore undescribed agents was observed by Sharpless and Jungherr (12) when they discovered that two viruses transmitted in chicken to chicken passages with an inoculum composed of a lymphomatous liver from a chick infected with the RPL-12 strain of lymphomatosis. The virus was described as polygonal, having a diameter of 86 μm which produced areas of necrosis in the liver accompanied by cells with large basophilic intranuclear inclusion bodies. Bankowski, et al. (13) isolated a myxo-virus, immunologically and serologically distinct from ND, from the respiratory tract of chickens of a young flock having an unusually high rate of deaths following laryngotracheitis vaccination. Hwang, et al. (14) investigating the enteroviruses of chickens isolated an agent which they believed was avian encephalomyelitis, however, upon further investigation proved that the agent was actually the CELO virus. Chomiak, et al. (15) later demonstrated that the CELO virus was readily propagated in tissue culture, produced plaques in kidney cell cultures and the technique was applicable for adaptation to the plaque neutralization test technique. They also found that the virus produced macroscopic and histopathological lesions on the CAM which were indistinguishable from infectious laryngotracheitis (ILT), but differed in that it does not produce intranuclear inclusion bodies. Yates, et al. (16) found that the CELO virus can be carried in embryos and chicks hatched from eggs infected with the virus, present signs of leg weakness and ataxia in spite of the fact that the eggs were obtained from chickens known to carry antibodies to the CELO agent. It is possible from these reports that agents of apparently low virulence may be more widespread than previously realized, and points to the possibility of still other agents heretofore unrecognized.

What role these agents may play as synergists in diseases of poultry must be determined and evaluated.
**Laryngotracheitis.** Numerous reports of “breaks”, seven to 14 days following vaccination with commercial products has stimulated research with this infection. Cover, Benton, and Krauss (17) showed that chickens vaccinated eight, 14, and 21 days of age obtained from immune or nonimmune parents showed only minor differences in their ability to react to vaccination by the vent drop method. Chickens vaccinated two weeks of age were found to be susceptible at five and 10 weeks of age to an intrasinus method of challenge. Benton, Cover and Krauss (18) conclude from their studies that antibodies were detected in yolks and serum of progeny of immune parents, but the SN antibody was not a critical criterion for evaluating immunity levels. They have also shown that the level of antibodies was only slightly greater in the yolks and in chicks from ILT vaccinated parents than from nonvaccinated dams, and that the antibodies had little influence upon infection with the ILT virus.

Hitchner and Winterfield (19) showed that chicks four and eight weeks of age vaccinated by the brush or drop method were highly susceptible when challenged 16 weeks of age by the infraorbital sinus route. Revaccination by the various routes showed that dropping the vaccine in the eye showed greatest promise for an immune response as determined serologically and by challenge. The authors failed to show a correlation between the SN serum titers and resistance to challenge with the virus. Raggi and Armstrong (20) investigating outbreaks of LT following vaccination demonstrated that hemorrhagic conjunctivitis was associated with the sequela and that the virus isolated from the lacrimal fluid was antigenically similar to the agent used in vaccination. Furthermore the agent was not dissimilar from any other strains of virus used for vaccination in the area. In conjunction with the increased incidence of the disease Sevoian (21) described a rapid method for diagnosis of fowl pox and ILT, which can be accomplished in 48 hours with ILT and in four days with fowl pox. The procedure incorporates a method of fixation and staining of the inclusions within three hours and ability to demonstrate inclusions as early as two days after ILT inoculation and four days following pox inoculation. Goldhaft (22) compared the stability of fowl pox and ILT vaccines prepared in chickens and in embryonating eggs. Whereas embryo propagated fowl pox vaccine held 21 years at 40 F. protected chickens against challenge, four batches of fowl pox vaccine made from scabs in live birds and packed in bottles or vials sealed with rubber stoppers, were inactive after 22-28 years of storage. Material containing live virus preserved in a vacuum was a far more effective means of maintaining stability of fowl pox, pigeon pox and ILT vaccines than material propagated in chickens held in a dried form in bottles or vials sealed with rubber stoppers or corks. Chomiak, et al. (23) and Chang, et al. (24) were able to propagate and demonstrate plaques in chicken kidney cultures using a number of strains of the ILT virus. The former authors successfully propagated six strains obtained from commercial vaccines in relatively high titers. The tissue culture propagated virus was found to be infective for chicken embryos when inoculated on the CAM, and produced intranuclear
inclusions and was infective for chickens after 10 serial passages. The tissue culture method was adaptable to a virus neutralization test, however, the cell culture plaque method appeared to be less sensitive than embryonating eggs.

Pulsford (1960) (25) cultivated three strains of LTV in serial passage in trypsinized chicken embryo lung. Titration of “free” virus and cell-bound virus was performed using pock-forming units on the chorioallantoic membrane of embryonating chicken eggs as the criterion of viral infectivity. It was not possible to demonstrate a one-step type of growth curve, but the eclipse phase was about 18 hours followed by a rapid production and release of virus. During the early increase of virus, the “free” virus lagged four to six hours behind the cell-bound virus. Comparisons were made of the various strains of virus employed.

Pulsford (1961) (26) proposed that LTV be used as the model for study of variation among viruses.

Croghan (1961) (27) studied LTV cultivated in chicken embryo liver cells and those from the chicken embryo lung and kidney. The CPE of the virus as determined by microscopy was neutralized by anti-LTV serum. Growth curve studies showed that within 15 minutes more than 98 percent of the virus was absorbed to the cells. The extracellular virus titer rose rapidly during the next 90 minutes, and then gradually increased to a maximum at 48 hours. Following this, the titer gradually decreased during the next 48 hours. Infective intracellular virus was present in the ground cellular material in amounts too small to be quantitatively measured. Intranuclear inclusion bodies were demonstrable 18 hours post inoculation period. Three passages in Chang’s liver cells, and two passages in bovine and monkey kidney cells failed to show infection as evidenced by CPE.

Infectious bronchitis. Hofstad (28) compared three antigenically different strains of infectious bronchitis with five additional strains of virus by cross immunity studies in chickens. Although differences could be demonstrated by the cross immunity test in chickens, the results did not correlate with the SN tests. The pathogenic isolates were more effective as immunizing agents than the less virulent strains and the most pathogenic strain produced the best immunity against all strains. Raggi (29) compared two strains of the IB virus obtained from commercial vaccines which were used within the same area and found them to differ immunologically. Chickens vaccinated with one vaccine were resistant to its homologous virus but presented a poor immunity (20 to 40 percent) to the heterologous strain. Both authors, Hofstad and Raggi, showed a total lack of correlation between SN titers and immunity as determined by cross immunity challenge. Larose and Van Roekel (30) serially passed the Beaudette and Massachusetts strains of IB in embryonating eggs at 12 hour intervals. The authors demonstrated that a maximum titer in embryonating eggs was obtained within the first few passages whether the virus was propagated in embryonating eggs obtained from susceptible or IB immune dams. The Massachusetts strain of virus passed through 300 embryos failed to produce a completely embryo-adapted virus without completely losing some of its virulence for chickens.
REPORT OF THE COMMITTEE

AVIAN INFECTIOUS HEPATITIS

The incidence of avian hepatitis during the past few years has been decreasing according to the reports of the Committee on Nomenclature and Reporting of Diseases of the Northeastern Conference on Avian Diseases (31, 32, 33, 34). Of the total consignments received in the States of the Northeastern Conference the incidence has been 1.5 percent in 1957, 3.1 percent in 1958, 2.4 percent in 1959, and 1.2 percent in 1960. Reports from other areas also indicate a decline although the condition is still present sporadically (35).

Recent research during the past few years has differentiated several types of hepatitis and for this reason the following nomenclature is being adopted:

- Avain infectious hepatitis (vibrionic)
- Duck virus hepatitis
- Turkey virus hepatitis
- Avain hepatitis (unidentified)

Because of the similarities between the various types of hepatitis the unidentified form is reserved for those cases in which an etiologic diagnosis has not been established. Each of the specific types of hepatitis is a distinct entity and the agent involved appears to be species specific. Investigational work with the specific agents of the various types of hepatitis show that embryonating chicken eggs provide a suitable medium for cultivation of the agents.

Sevoian and Calnek (36) report that the vibrio of avian infectious hepatitis can be most consistently demonstrated in the bile of affected birds. They also report that in addition to furazolidone medication, dihydrostreptomycin sulfate gave good control of infectious hepatitis in laying chickens. Narotsky and Taylor (37) reported on clinical evidence which suggested that avian hepatitis of chickens is egg transmitted. In virus hepatitis of turkeys, Snoeyenbos and Basch (38) suggest that egg transmission is one means of dissemination of the virus. An excellent study in turkey virus hepatitis is also reported by Mongeau (39).

Immunological characteristics and other properties of the duck virus hepatitis have been reported (40, 41). Rahn and Hanson (42) have reported turkey poults to be susceptible to the duck hepatitis virus. Murty and Hanson (43) reported on the usefulness of the microgel diffusion method in the study of the duck hepatitis virus.

COCCIDIOSIS

In recognition of the serious economic losses caused the poultry industry by coccidiosis, research and other workers, both foreign and domestic, continue their investigations in the field of prevention and control. Anticoccidial drugs, new and old (44, 45, 49, 50, 52, 53, 54, 55, 56, 57, 59, 64, 65, 68, 69, 70, 71, 74) were studied. Contributions were made concerning the incidence of various coccidial species (48, 58, 66, 67). The effect of coccidiostats on growth, feed conversion, and the development of flock
immunity received consideration (60, 71) and one study (46) concerned an appraisal of coccidiosis vaccination for replacements. One worker (47) reported on the effect of cecal coccidiosis infection upon adrenal ascorbic acid levels and another (51) compared various sources and levels of vitamin K activity using chicks with cecal coccidiosis.

Reid (71) studied the relationship between coccidiostats and poultry flock immunity in coccidiosis control programs. His studies showed that several coccidiostats caused delay in development of flock immunity. Coccidiostats inhibit oocyst production which may interfere with immunity development due to lack of sufficient exposure under field conditions according to him. Flock immunity was developed most rapidly by artificial exposure at three days of age using the Coccivac program.

Lawatsch et al. (60) conducted an experiment evaluating coccidiostats for growth, feed conversion and the establishment of immunity. The data indicated that when coccidiosis is not a problem, best growth and feed conversion can be obtained when no coccidiostat is fed. The difference between various coccidiostats with respect to the degree of immunity established varied from no immunity to complete protection. These two studies would indicate that if coccidiostats are used, the possibility of delay in the development of flock immunity needs to be recognized in planning a control program.

Lotze and Leek (61) did basic research studies that may explain the hatching mechanism of coccidia. This could help immeasurably in developing an effective coccidiosis control program. They found that something in the bile arouses the coccidial parasite to activity and excystment and a previous incubation of the sporulated oocysts for some time in contact with an enzyme-containing substance prepares the oocyst so that the parasites become quickly activated when the oocysts come in contact with bile. Lotze (62) states that most of the work has involved the use of coccidial parasites of sheep, but limited studies were carried on with sporulated oocysts of avian coccidia and he feels that the results of the studies indicate that the fundamental principles for excystation are similar in avian and ovine coccidia.

A new method for the primary evaluation of anti-coccidial activity of chemicals was developed by Lynch (63). He describes a new direct-dosing technique which was developed to carry out a large scale anti-coccidial program in a limited space having the advantage of saving time, space and drugs.

Reports of the discovery of a new species of coccidia, named *E. Mivati*, by Dr. S. A. Edgar of Auburn University were not confirmed at the time this report was written.

It can be concluded that (44) coccidiosis continues to be a problem in the poultry industry, (45) considerable research is being done on the problem, (46) some, if not all coccidiostats, influence the development of immunity, (47) coccidiostats have a definite place in control and treatment of coccidiosis and (48) research must continue in this field.
HEMORRHAGIC DISEASE

If the volume of literature on a disease were any criteria as to incidence or importance, then it could be assumed that hemorrhagic disease was unimportant. A review of the literature for the last 12 months since the previous report by this Committee reveals only one report on this disease. Schumaier et al. (76) reported on mycotoxins as a possible cause of the hemorrhagic syndrome. Since mycotoxins had been reported to produce lesions indistinguishable from hemorrhagic disease they tested this hypothesis. They obtained pure mold cultures and grew them on autoclaved wheat. Hemorrhages, bone marrow changes and diarrhea were produced from oral ingestion of ether extract or substrate plus mycelial growth of certain molds.

Skamser (77) sent a questionnaire to pathologists throughout the country inquiring as to the incidence and importance of the hemorrhagic syndrome. At the time of this report he had received answers from about 50 pathologists and while he had not made a statistical compilation pending return of more of the questionnaires, he stated that a perusal of the reports indicated: “(1) that hemorrhagic disease occurs in chickens and turkeys; (2) the incidence varies from zero percent to 10 percent of the flocks examined; (3) most pathologists agree that it is not an important disease; (4) the etiology is unknown and (5) no specific treatment is known.”

Davis (75) who is presently working on the disease believes it to be a separate disease entity, as distinguished from several allied conditions, which might under given circumstances produce a hemorrhagic syndrome. In true hemorrhagic disease, as distinguished from the so-called hemorrhagic syndrome, he feels there will be a consistently decreased erythrocyte count (below 1,000,000), a low hemoglobin (often below five grams), a normal clotting time and platelet count. He feels there is capillary fragility. He has obtained response from treating this condition by the addition of vitamin C in the drinking water at the rate of two ounces (62½ grams) to each 50 gallons. This treatment is continued for four or five days. Since vitamin C is relatively instable, precautions must be taken in administration of this drug.

While the over-all economic importance of this disease may be subject to question, it is seen in many diagnostic laboratories with sufficient regularity to warrant continuing or additional investigation as to cause and treatment.

Report on the Findings of the Survey of the Presence of Salmonella Organisms in Animal By-Products

Resolutions were received by the Agricultural Research Service of the United States Department of Agriculture to the effect that action should be taken by a proper Federal agency to review the problem of Salmonella organisms in animal by-products that are incorporated into rations for poultry and livestock. The resolutions were received from the North Central States Poultry Disease Conference, the Committee on Salmonellosis and Related Enteric Diseases of the National Plans Conference and American
TRANSMISSIBLE DISEASES OF POULTRY

Association of Avian Pathologists. The responsibility for this study was assigned to the Animal Disease Eradication Division and in November, 1960, the study was undertaken. The purpose of the study was to develop information on the occurrence of the Salmonella organisms in animal by-products, factors accounting for their presence, their real or potential disease threat to the country's poultry and domestic livestock, and to determine what measures could be taken to assist in eliminating the problem.

Working through staff veterinarians of this Division, some 1,100 personal contacts were made in all 50 States. The contacts included the following groups: (a) laboratory and extension services of colleges and universities, (b) public health and private industry laboratories, (c) regulatory agencies, (d) veterinary practitioner groups, (e) rendering groups, (f) animal by-products processors, (g) feed manufacturing and processing groups, (h) meat packers and allied groups, (i) fur bearing animal raisers, (j) biological and drug manufacturers. Meetings also were held with leaders of national organizations representing interested industries.

Evidence was documented which indicated that a wide variety of animal by-products and rations may become contaminated with organisms of the Salmonella sp. Specifically, a total of 5,712 samples were recorded from which isolation attempts had been made with 718 samples yielding Salmonella. The reports were received from 31 States, and 14 different animal by-products were involved. Isolates from 379 of the 718 samples were reported by serotype and 62 different serotypes were recorded. S. montevideo, S. senftenberg, S. typhimurium, S. cubana, S. infantis and S. oranienburg occurred most frequently. The consensus was that recontamination of the product was the most important single factor accounting for Salmonella sp. in animal by-products, and that rodents were the most possible source of the contamination although other animals, wild birds and human handlers of the products were considered as possibilities. Definitive evidence pointing to animal by-products or rations as sources of organisms responsible for field outbreaks of disease was lacking. Because of the lack of such evidence, the study group concluded there was no cause for alarm concerning this problem, although causes for concern were apparent. These included the potential disease problem arising from the "cycling" of a number of Salmonella serotypes from farms to processing plants and back to farms via animal by-products incorporated in rations as well as indications that the known pathogenic Salmonella serotypes were among those most frequently recovered.

While granting that much additional work is desirable concerning uniform methods of recovery of the Salmonella organisms from animal by-products, relative pathogenicity of various Salmonella serotypes, and number of organisms in by-products necessary to create a disease threat or establish a carrier state in so-called "healthy" populations, the study group concluded that realistic, workable measures to minimize the chance for exposure of livestock and poultry to Salmonella organisms that may be present in animal by-products should be undertaken. The cooperation of the interested industries throughout this study was heartening, and it was apparent that many
processors of these products are endeavoring to assure a product free of these organisms when it leaves their plants. Research in the area is being carried out by the industries to study current procedures and environmental problems associated with possible contamination of some products.

Further meetings are scheduled between members of this study group and the interested industries for consideration of development and adoption of a sanitary code to assure industry practices designed to present a product free from Salmonella and to prevent recontamination with these organisms. The industries themselves are willing to place into effect whatever measures are necessary once it is shown that such methods are effective.

The study group is hopeful that with cooperation of the industries and animal disease workers interested in the control of salmonellosis this problem will be minimized, if not eliminated.

**PARASITIC DISEASES**

The problem of gapeworm control in pheasants was studied by McGregor et al. (81) who found chemical control of earthworms (intermediate hosts of Sygamous trachea) to be highly effective in controlling outbreaks of synergismosis.

The histopathological lesions in domestic pigeons infected with Trichomonas gallinae were found by Mesa et al. (82) to consist primarily of ulcerations in the pharynx together with progressively enlarging non-liquefying abscesses in the liver.

Severe proventriculitis in pigeons caused by Dispharynx nasuta was reported by Hwang et al. (83). They described the histopathology, life history and control of this parasite.

Current control measures recommended for the intestinal hairworm (Capillaria obsignata) in poultry were evaluated by Payne et al. (84) who found that Hygromycin B significantly decreased the number of adult capillaria although it did cause a marked drop in egg production of the birds.

Evidence of acquired resistance of chickens to capillaria obsignata was reported by Frazier (86) who also found an increase of gamma globulin levels in infected birds as compared to controls.

A comprehensive study of the life cycle of Hexamita meleagridis was reported by Slavin et al. (82) and which included many photomicrographs of the various stages of development of this organism.

The antihelmintic value of Hygromycin B in broiler rations was studied by Day et al. (78). Supplementation of the ration containing adequate Vitamin A with hygromycin B resulted in almost complete suppression of roundworm infestation.

Tower and Floyd (79) studied the effect of infestation of the chicken body louse (Eomenacanthus stramineus) on egg production in pullets. The lice free birds had a slightly higher average egg production than the lice infested birds although the differences were not statistically significant.
Farr (85) determined the length of time of survival of *Histomonas meleagridis* as well as eggs of other poultry nematodes in the feces of infected birds.

**AVIAN ENCEPHALOMYELITIS**

Calnek *et al.* (89) studied the epizootology of avian encephalomyelitis. They found that (a) susceptible birds placed in a house previously occupied by A.E. infected birds developed positive serology within three to seven weeks (b) chicks could be infected by the intestinal route (c) oral (drinking water) administration of AE virus to adult breeders produced a syndrome indistinguishable from that observed in field outbreaks and (d) transmission of AE from egg infected chicks to susceptible chicks took place following exposure either within the incubator during hatching or in batteries following hatching and that clinical signs appeared in 11-16 days following contact.

A virus of the CELO group was noted by Hwang *et al.* (91) which was capable of producing ataxia and histological lesions and which might be confused with those produced by AEV.

Jungherr (88) reviewed the principal developments in the study of Avian Encephalomyelitis which have led to our present concept of the disease.

The resistance of breeding flocks to Avian Encephalomyelitis was studied by Gentry *et al.* (92) who noted that some hens may lose their immunity over a period of time.

Calnek *et al.* (71) tested the safety and efficacy of a live-oral vaccine for avian encephalomyelitis. They found that the embryopropagated vaccine when administered via drinking water resulted in immunity of all birds within a flock. It was recommended that vaccination be done between the eighth and 20th weeks of age.

An in vitro neutralization test against avian encephalomyelitis virus was evaluated by Hwang *et al.* (87). The results suggested the usefulness of the technique for the assay of serum potency against AEV.

**LEUKOSIS**

Sharpless *et al.* (93) studied two viruses obtained from a cell free filtrate of lymphomatous liver from chicks infected with the RPL12 strain of “lymphomatosis” virus. One produced typical erythroblastosis in chicks and in chick embryos while the other was antigenically unrelated to that of lymphomatosis, although it did produce mild necrosis of the liver and some basophilic inclusion bodies.

**MISCELLANEOUS DISEASES**

Peckham (94) reported a Gram positive, spore forming rod shaped organism which was isolated from naturally occurring cases of ulcerative enteritis in chickens, turkeys and quail. He was able to reproduce the disease by feeding yolk sac cultures of this organism to quail.
Two species of Aspergilli were noted by Schumaier et al. (95) to produce lesions indistinguishable from typical hemorrhagic syndrome when fed orally to chickens.

Smart et al. (96) found that the use of Novobiocin in the feed consistently reduced losses from Staphylococcal synovitis in turkeys.

**PRELIMINARY REPORT ON INVESTIGATIONS COMPARING ALUMINUM AND WOODEN POULTRY CRATES AS REPORTED BY CORRESPONDENCE FROM DR. STADELMAN**

The objective of the first study was to determine relative microbiological loads on an aluminum crate as compared to a wooden crate with a plywood bottom.

The procedure followed was to place dirty peanut hull litter, containing chicken excreta, in each crate to a depth of one inch. The pH of the litter was 8.2 as determined using a pH meter with glass electrodes. The dry litter was completely mixed in a small concrete mixer prior to its application in the crates. The litter was then soaked with tap water. The wetting was repeated every 48 hours. The crates were placed on experiment August 11. On August 19 the crates were rinsed with tap water to remove most adhering dirt. A cotton swab smear was then taken on a 1.13 square inch surface from each of two areas on each crate floor. The two cotton swabs were placed in a tube containing 10 ml of 0.1 percent peptone broth. Previous work has shown this solution to be superior to standard saline in preserving cells during dilution.

After the swabbing was completed the crates were thoroughly washed with tap water. A second collection of swab smears was made. Each crate was then rinsed with one gallon of 1:600 phenol solution and allowed to drain for five minutes. A third sampling of organisms was collected. All samples were then diluted by standard techniques in 0.1 percent peptone broth to a $10^8$ dilution. Each dilution was then plated on brain-heart infusion agar and incubated at either 22° or 37° C. The 22° C. plates were counted after 48 hours and the 37° C. plates were counted after 34 hours incubation.

The results of the microbiological examination are summarized in table 1. The data were not subjected to statistical analysis as the magnitudes of the differences were so great. The aluminum crate was as clean from a microbiological viewpoint after the first rinse as it was possible to get the wood by the further cleaning treatments. From these results the value of an aluminum crate for ease of sanitation is obvious. The counts obtained indicated a slight reduction based on additional cleaning effort. With aluminum this was about a tenfold decrease whereas, with wood it was about 100 times fewer colonies. The increase in count of the 1:600 phenol rinsed wood incubated at 22° C. suggests that some undetected dirt high in mesophylic organisms was washed onto the surface by the rinse solution. This could also account for the slight benefit resulting from phenol at 37° C. incubation of cultures from wood or aluminum.
The second study was to determine the effectiveness of several coatings on aluminum to reduce corrosion resulting from the high pH of poultry excreta. The test sheets were subjected to the same conditions as the crates in the first experiment. The panels were observed on August 19 but no obvious corrosion was evident. The unprotected aluminum of the crate showed some discoloration. A report on this study will be submitted later.

| TABLE 1 |
| Colonies Per Square Inch of Surface Examined |
|-----------------|-----------------|
|                | Aluminum        | Plywood        |
| 37°C incubation:|                 |                |
| Rinsed          | $3.6 \times 10^5$ | $1.8 \times 10^7$ |
| Washed          | $6.2 \times 10^4$ | $3.2 \times 10^6$ |
| 1:600 phenol    | $2.8 \times 10^4$ | $3.5 \times 10^5$ |
| 22°C incubation:|                 |                |
| Rinsed          | $7.5 \times 10^4$ | $4.9 \times 10^6$ |
| Washed          | $2.5 \times 10^4$ | $1.5 \times 10^6$ |
| 1:600 phenol    | $1.0 \times 10^4$ | $2.9 \times 10^6$ |

Note: The 37°C incubated plates were counted after 34 hours incubation. The 22°C incubated plates were counted after 48 hours incubation. All plates were brain-heart infusion agar.

SALMONELLOSIS

Progress continues in the reduction of pullorum disease and fowl typhoid under the National Plans. The number of hatcheries participating in the National Poultry Improvement Plan in 1960-61 was 59.7 percent of the total number of hatcheries in M. S. (97). This represents 71.7 percent of the hatching egg capacity. The number of hatcheries participating in the National Turkey Improvement Plan in 1960-61 was 73.3 percent representing 97.0 percent of the hatching egg capacity. The percentage of reactors on the first test of birds to be used as breeders reached a new low of .13 percent in chickens and 0.003 percent in turkeys. Only one classification, U. S. Pullorum-Typhoid Clean, is now recognized in the Turkey Plan and in the Poultry Plan 95.1 percent of the hatching egg capacity qualified as U. S. Pullorum-Typhoid Clean and 98.1 percent of the chickens. The results of the Pullorum-Typhoid Control Programs under the National Plans are given in Table 1.

A Committee on Pullorum Disease Eradication re-emphasized the importance of five areas to be considered by each State in the development of a program to eradicate pullorum disease:

1. 100 percent participation of all chicken and turkey breeding flocks and hatcheries under an official Pullorum-Typhoid Control Program.
2. Pullorum disease and fowl typhoid be made reportable diseases.
(3) Infected flocks be quarantined and the marketing of such flocks be under the supervision of the disease control agency.

(4) A Federal regulation be adopted to control the interstate movement of poultry from the standpoint of pullorum disease and fowl typhoid.

(5) Poultry consigned to public exhibition originate from a flock under an official Pullorum-Typhoid Control Program.

The National Plans are voluntary programs, but there is need for the development of a unified official Pullorum-Typhoid Control Program on a State and Federal level recognizing the goal of eradication of these diseases and not merely voluntary control program.

During the past few years increasing reports from the field have indicated the isolation of both variant and standard strains of *S. pullorum* from joint lesions of broiler chickens. (Ferguson *et al.* (98) and Cosgrove and Lindenmaier (99).)

Paratyphoid.

Paratyphoid infections continue to be a problem in turkeys and chickens. Increasing number of Salmonellas were found in chickens (Moran (100)). Certain chemotherapeutic agents as sulfonamides, antibiotics and nitrofurans are used in the treatment of outbreaks. Recently (Mitrovic *et al.* (101)) demonstrated the effectiveness of 3,5-dinitrobenzamide in paratyphoid infections in turkeys. Bierer *et al.* (102) found furaltadone effective in the treatment of pullorum disease, fowl typhoid and paratyphoid infections. Yamamoto *et al.* (103, 104, 105) have conducted studies on the incidence of Salmonella infections in market fowl and cultural procedures for the isolation of Salmonella.

Contamination of animal feedstuffs with Salmonella organisms and other microorganisms have been extensively reviewed by a task force of the U.S.D.A. and a preliminary report (105) of this study was reported at the Fourth Annual Meeting, Conference of Veterinary Laboratory Diagnosticians at Minneapolis, 1961.

**TABLE 1**

*Chickens and Turkeys, Officially Tested for Pullorum Disease, Number and Percent of Reactors, 1958-61*

<table>
<thead>
<tr>
<th>Year</th>
<th>Chickens Tested Number</th>
<th>Chickens Reactors Number</th>
<th>Chickens Reactors Percent</th>
<th>Turkeys Tested Number</th>
<th>Turkeys Reactors Number</th>
<th>Turkeys Reactors Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1958</td>
<td>36,756,760</td>
<td>11,423</td>
<td>0.03</td>
<td>3,509,580</td>
<td>640</td>
<td>0.018</td>
</tr>
<tr>
<td>1959</td>
<td>40,716,266</td>
<td>9,920</td>
<td>0.024</td>
<td>3,940,033</td>
<td>437</td>
<td>0.01</td>
</tr>
<tr>
<td>1960</td>
<td>37,030,568</td>
<td>6,812</td>
<td>0.018</td>
<td>3,510,154</td>
<td>243</td>
<td>0.006</td>
</tr>
<tr>
<td>1961</td>
<td>36,599,927</td>
<td>4,892</td>
<td>0.013</td>
<td>4,500,090</td>
<td>132</td>
<td>0.003</td>
</tr>
</tbody>
</table>
REFERENCES


42. Rahn, Dennis P., and Hanson, Lyle E.: Susceptibility of turkey poult's to duck hepatitis virus and serologic comparison to hepatitis virus of turkeys. Abstracts of papers, 98th annual meeting of the American Veterinary Medical Association, August 20-24, 1961, Detroit, Michigan: 100.

44. Aycardi, J.: Activite' coccidiostatique d'un complexe sulfate de framcyetine-
47. Challey, J. R.: * The effect of cecal coccidiosis infections and experimental
hemorrhage upon adrenal ascorbic acid levels in the chicken. J. Parasitol. 46 (6)
pp. 727-731.
48. Chute, H. L., Kalvaitis, A., Frazier, J., O'Meara, D. C., Payne, D. D., and Waller,
49. Cuckler, Ashton C., Cobb, Walter R., McManus, Edward C., and Ott, Walter
drugs for the control of coccidiosis in chickens and turkeys. Abs. of papers, 50th
51. Harms, R. H., Waldroup, P. W., Cox, D. D.: Comparison of various sources and
52. Horton-Smith, C., Long, P. L., and Collier, H. O. J.: Potentiation of sulphadimino-
dine by 2,4-diamino-6,7-di-isopropylpteridine and other 6,7-di-substituted 2,4
diaminopteridines against Eimeria infections of chicks. Brit. J. Pharmacol. and
for chickens. Down Earth 16 (1) pp. 2-6, Summer 1960.
56. Joyner, L. P.: The relationship between toxicity and coccidiostatic efficacy of
pyrimethamine and sulphonamides and their relative reversal by folic acid. Res.
57. Joyner, L. P.: The coccidiostatic activity of 3,5 dinitroortho-toluamide against
Eimeria necatrix among Georgia broilers as determined by challenge techniques.
59. Kocan, J. M.: Effect of sulfa drugs administered to chickens on the capacity to
(7) pp. 978-983.
60. Lawatsch, M. F., Erner, Eugene, and Zosel, James R.: Evaluation of coccidiostats
for growth, feed conversion and the establishment of immunity. Abs. of papers,
61. Lotze, John C., and Leek, R. G.: Some factors involved in Exxystation of the
sporozoites of three species of sheep coccidia. J. of Parasitol. 46 (5) Sec. 2,


A SIX MONTHS SURVEY OF STAPHYLOCOCCAL FLORA IN THE MILK FROM A LARGE DAIRY HERD


The role of coagulase positive staphylococci as a cause of bovine mastitis is well established (1, 2, 3). Evidence further indicates that coagulase positive staphylococci may also inhabit the normal udder (4, 5). These facts assume greater importance when staphylococcal food poisoning in man associated with fluid milk, dried milk, and cheese are considered. The widespread use of some antibiotics in the treatment of mastitis no doubt has favored the development of resistant strains of staphylococci. As pointed out by Dauer (6), many aspects of the whole problem of staphylococci in milk and milk products need to be investigated more thoroughly.

In September 1959, an opportunity was presented to study the staphylococcal flora in a large dairy herd in which a mastitis control program has been underway since 1950. This control program included physical examination of the cattle and application of the California Mastitis Test (7) and the Hotis Test (8), as well as monthly bacteriological examination of the milk from each cow. All staphylococci isolated during the study were tested for coagulase and antibiotic sensitivity. The coagulase positive strains were examined for susceptibility to 24 human staphylococcal phages. The findings on composite milk samples from each cow for six monthly surveys are described in this report.

METHODS

Collection of Milk Samples:

1. The udders were washed first with water, then with two separate solutions of Novalson (chlorhexidine), and, finally, rinsed with chlorine solution containing 200 ppm.

2. The first few streams of milk from each quarter were discarded, then four to five ml. of milk from each quarter were collected in a Hotis tube containing one ml. of 1:300 aqueous solution of bromocresol purple.

From the Department of Health, Education and Welfare, Public Health Service, C.D.C., Atlantic, Georgia: ¹Chief, Veterinary Public Health Laboratory Unit; ²Formerly Epidemic Intelligence Service Officer, Epidemiology Branch, presently at Boston City Hospital, Boston, Massachusetts; ⁴Chief, Staphylococcus and Streptococcus Unit; ⁵Scientist (R), Staphylococcus and Streptococcus Unit; ⁶Analytical Statistician, Epidemiology Branch.

³Disease Investigation Supervisor from the County of Los Angeles Livestock Department, South Gate, California.

The technical assistance of Mrs. Catherine R. Sulzer, Medical Biology Technician, Veterinary Public Health Laboratory Unit, Epidemiology Branch, C.D.C., Atlanta, Georgia, is gratefully acknowledged.

251
3. All samples were iced and delivered to the local mastitis control laboratory. It usually required one to two hours for the samples to reach the laboratory.

Procedure in Mastitis Control Laboratory:

In general, the culture procedures followed in this laboratory are those outlined by Schalm (9).

1. Upon arrival in the laboratory, the California Mastitis Test for inflammatory products was performed on all samples. The Hotis tubes were then incubated at 37 C overnight.

2. After incubation, the Hotis tubes were read* and recorded, then .01 ml. of milk from each tube was streaked onto one-half of a blood agar plate, prepared from Blood Agar Base (Difco) with three percent defibrinated bovine blood and 0.1 percent aesculin added.

3. After the plates were streaked, 0.1 to 0.2 ml. from each Hotis tube was inoculated into 10 ml. of Trypticase Soy Salt Broth (TSSB) in 16 x 125 mm. screwcapped test tubes.

4. Both plates and broth tubes were incubated at 37 C for 24 hours.

5. The T.S.S.B. cultures were then packed and shipped via air express to the Communicable Disease Center in Atlanta.

6. The blood agar plates were examined for the presence of streptococci, coliforms, pseudomonas, corynebacteriae, staphylococci, and yeasts.

Procedure in Veterinary Public Health Laboratory:

Although several different media were used for comparative purposes at various times during this study, the method described below proved most effective. The results of the comparative laboratory studies have been described in detail in another report (10).

1. The T.S.S.B. cultures were enroute to the Communicable Disease Center laboratory between two and five days. Either the day of arrival or the following morning, a three mm. loop was used to streak the broths to Trypticase Soy Agar (TSA) plates, with the exception of two occasions when the cultures arrived during a weekend, they were refrigerated until Monday.

2. The T.S.A. plates were incubated for 24 hours at 37 C and examined for the presence of adequately isolated colonies. If a plate was overgrown with contaminants, the original broth culture was restreaked onto another T.S.A. plate containing sodium azide (0.1 percent). If colonies were well isolated, the plates were reincubated overnight and then held at room temperature for 48 hours.

3. Each colony picked from the plates was inoculated to an agar slant, and into broth for tube coagulase and antibiotic sensitivity tests. In addition, the slide clumping test and a Gram stain was performed with growth from the same colony. During the early part of the study, all slide tests were

* Only 18 to 20 hour readings were made on Hotis tubes as more dependence could be placed on the blood agar plate to indicate bacterial flora.
confirmed by the tube coagulase test. Later, only colonies negative by the slide clumping test were confirmed. Both pigmented and non-pigmented colonies were tested.

4. At least one coagulase positive staphylococcal colony per specimen was phage typed. If coagulase positive staphylococcal colonies had different pigmentation and proved to have a different antibiogram, both varieties were phage typed although it is recognized that neither characteristic is definitely associated with phage susceptibility. The standard phages in routine use in the National Staphylococcal Reference Laboratory were used. These included: 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 73, 75, 77, 42D, 187, 83(VA4), 44A, 81.

The October isolates were tested against these phages at both routine test dilution (RTD) and at 10 times R.T.D. The latter concentration was used for all subsequent typing since results could be interpreted more readily.

FINDINGS

During the six-months period, October, 1959 through March, 1960, composite milk samples were obtained from one to six times from a total of 262 cows. Of these, 214 were in the herd during the entire period, 25 were slaughtered and 23 were replaced. The number of cows sampled each month varied from 161 to 176, as shown in Table 1. Coagulase positive staphylococci isolated from these cows ranged from a low of 55 percent in October to a high of 84 percent in January and March. In addition, three to 17 percent of the cows were found to have more than one strain of coagulase positive staphylococci in their milk, as indicated by difference in antibiotic sensitivity pattern.

The resistance of coagulase positive strains of staphylococci when tested against discs impregnated with penicillin, dihydrostreptomycin, terramycin,
chloramphenicol and erythromycin, is shown in figure 1. Throughout the period the greatest number of cultures were resistant to penicillin and dihydrostreptomycin. An increase in resistance to dihydrostreptomycin appeared in December and continued through March, with more than 50 percent of the cultures falling into this category.

It was noted that coagulase positive staphylococci were isolated from the milk of 55 to 100 percent of the cows that were treated each month. Seventy-two percent of the cultures from these treated animals were resistant to one or more of the antibiotics. Of these, 35 percent were resistant to dihydrostreptomycin only, and 17 percent to penicillin only.

When phage typing was performed at the routine test dilution (RTD) on the initial strains of coagulase positive staphylococci isolated from the October samples, many of the reactions were weak and relationship among cultures could not be determined with any degree of reliability. However, when the same cultures were retested with phages at 10 times R.T.D., the reactions were sharper and could be interpreted more easily. For this reason, all subsequent typing during the study was done with phages at 10 times R.T.D.

By this modified procedure, typable strains of coagulase positive staphylococci for the six months period ranged from 59 percent to 85 percent. Of these typable strains, seven to 14 per month, or an average of 11 percent, proved to be phage type 80/81 and 89 percent were lysed by other phages as shown in Table 2.
A SIX MONTHS SURVEY OF STAPH.

255

TABLE 2

Findings on 801 Coagulase Positive Staphylococcal Isolates From Bovine Milk Samples When Tested Against 24 Human Phages

<table>
<thead>
<tr>
<th>Month</th>
<th>Coagulase Positive Isolates</th>
<th>Type Typable</th>
<th>Phage Typing* on Coagulase Positive Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Percent</td>
<td>Type 80/81</td>
</tr>
<tr>
<td>October</td>
<td>105</td>
<td>84</td>
<td>80</td>
</tr>
<tr>
<td>November</td>
<td>107</td>
<td>91</td>
<td>85</td>
</tr>
<tr>
<td>December</td>
<td>116</td>
<td>88</td>
<td>75</td>
</tr>
<tr>
<td>January</td>
<td>161</td>
<td>102</td>
<td>63</td>
</tr>
<tr>
<td>February</td>
<td>147</td>
<td>88</td>
<td>59</td>
</tr>
<tr>
<td>March</td>
<td>165</td>
<td>98</td>
<td>59</td>
</tr>
<tr>
<td>Totals</td>
<td>801</td>
<td>551</td>
<td>68</td>
</tr>
</tbody>
</table>

* Ten times R.T.D. of phages was used on all cultures.

In an attempt to determine whether there was any relationship between clinical mastitis in the animals and the presence of coagulase positive staphylococci in the milk, the results of the California Mastitis Tests (CMT) and the Hotis tests were compared. In addition, these findings were compared with the number of animals treated each month. The C.M.T. is designed to screen cows for mastitis due to trauma or an infectious agent before they reach the clinical stage of the disease. Those cows showing a persistent C.M.T. reaction were treated in the dry stage and only those cows showing visible

TABLE 3

Monthly California Mastitis Test and Hotis Test Findings on 1,010 Bovine Milk Samples Compared to the Number of Animals Treated

<table>
<thead>
<tr>
<th>Month</th>
<th>No. Milk Samples Tested*</th>
<th>C.M.T. Reaction 2 and 3</th>
<th>C.M.T. Reaction 1</th>
<th>Hotis Positive†</th>
<th>Treatment Number Animals Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
<td></td>
</tr>
<tr>
<td>October</td>
<td>168</td>
<td>27</td>
<td>16</td>
<td>31</td>
<td>18</td>
</tr>
<tr>
<td>November</td>
<td>176</td>
<td>21</td>
<td>12</td>
<td>32</td>
<td>18</td>
</tr>
<tr>
<td>December</td>
<td>173</td>
<td>36</td>
<td>21</td>
<td>30</td>
<td>17</td>
</tr>
<tr>
<td>January</td>
<td>166</td>
<td>16</td>
<td>10</td>
<td>24</td>
<td>14</td>
</tr>
<tr>
<td>February</td>
<td>166</td>
<td>22</td>
<td>13</td>
<td>27</td>
<td>16</td>
</tr>
<tr>
<td>March</td>
<td>161</td>
<td>15</td>
<td>9</td>
<td>59</td>
<td>36</td>
</tr>
<tr>
<td>Totals</td>
<td>1,010</td>
<td>137</td>
<td>14</td>
<td>203</td>
<td>20</td>
</tr>
</tbody>
</table>

C.M.T. = California Mastitis Test.

N.R. = No record.

† 14 cows treated during each of 2 months; 6 animals treated during each of 3 months.
clinical evidence of mastitis were treated while in the milking line. Those cows that showed a negative or trace reaction to the C.M.T. or did not show a persistent reaction were considered normal cows. The Hotis test has been used as a valuable aid for the detection of streptococcus infections and certain reactions have been found to be indicative of some strains of staphylococci (10). All clinical cases were treated in the affected quarter with 1,000,000 units of penicillin and one gram of streptomycin.

The results of the C.M.T. and Hotis tests on all samples by month are presented in Table 3. In addition, the number of treated animals are shown for comparison. In the mastitis control laboratory, only two and three C.M.T. reactions were considered of real significance. A one reaction, interpreted as a weak positive, was considered only if persistent or if a two or three reaction developed on repeated examination. For this reason, all two and three reactors have been shown separately. There were 137 (14 percent) of the total of 1,010 samples with two and three C.M.T. reactions and 292, excluding November (35 percent) positive to the Hotis test.

As may be noted, there was no great difference in the percent of positive C.M.T. reactions with the exception of December with 21 percent positive. Similarly, there was a slight increase in the percent of positive Hotis tests during December. It is apparent that these tests do not indicate any marked increase in mastitis during the period. However, the number of animals treated each month during January, February and March, was more than double the numbers treated in the previous three months.

When the type of organisms isolated were compared with the C.M.T. findings and the number of treated animals, a positive C.M.T. (two and three reactors) was observed in 99 (16 percent) of 609 samples that were found to contain coagulase positive staphylococci, as shown in Table 4. The

<table>
<thead>
<tr>
<th>Type of Organism</th>
<th>C.M.T. No.</th>
<th>C.M.T. Pos.</th>
<th>C.M.T. Percent</th>
<th>Hotis No.</th>
<th>Hotis Pos.</th>
<th>Hotis Percent</th>
<th>Treated No.</th>
<th>Treated Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase positive</td>
<td>609</td>
<td>99</td>
<td>16</td>
<td>607</td>
<td>228</td>
<td>38</td>
<td>65</td>
<td>11</td>
</tr>
<tr>
<td>Coagulase negative</td>
<td>212</td>
<td>15</td>
<td>7</td>
<td>211</td>
<td>42</td>
<td>20</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Streptococci</td>
<td>72</td>
<td>31</td>
<td>43</td>
<td>72</td>
<td>32</td>
<td>44</td>
<td>13</td>
<td>18</td>
</tr>
</tbody>
</table>

* November results excluded as Hotis results were not available.
† C.M.T. reactions 2 and 3's only.

November results were excluded as Hotis test results, were not available for that month. Sixty-five (11 percent) of these animals were treated. On the other hand, a positive C.M.T. was observed in only 15 (seven percent) of 212
samples with coagulase negative staphylococci. In contrast 43 percent of the 72 samples with streptococci were positive in the C.M.T. and 18 percent of the animals were treated during the month of sample. However, 20 of the 48 animals shedding streptococci were treated at some time during the study period. The number of samples by month found to contain streptococci and coagulase positive staphylococci is shown in Table 5. Both organisms were found in 74 (87 percent) of the samples.

TABLE 5

Comparative Findings on 85 Bovine Milk Samples Positive for Streptococci That Also Contained Coagulase Positive Staphylococci

<table>
<thead>
<tr>
<th>Month</th>
<th>Number Specimens with Streptococci</th>
<th>Coag. Pos. Staph.</th>
<th>C.M.T. Reaction 2 and 3</th>
<th>Cows Positive</th>
<th>Animals Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>November</td>
<td>13</td>
<td>11</td>
<td>7</td>
<td>6</td>
<td>N.R.</td>
</tr>
<tr>
<td>December</td>
<td>22</td>
<td>19</td>
<td>10</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>January</td>
<td>14</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>February</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>March</td>
<td>21</td>
<td>17</td>
<td>5</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Totals</td>
<td>85</td>
<td>74</td>
<td>38</td>
<td>47</td>
<td>35</td>
</tr>
</tbody>
</table>

N.R. = No record.

The frequency with which coagulase positive staphylococci were isolated from the milk of individual cows during the six months period is given in Table 6, excluding cows slaughtered and replacements. From one to six samples were obtained from 214 cows and 200 of these cows contained coagulase positive staphylococci one or more times. Of the 55 animals with six specimens, coagulase positive staphylococci were found one or more
times from 54, and in each of the six samples from 20 (36 percent) of the cows. Of these 20 cows, 10 (50 percent) were treated during the study and in 13 (65 percent) the C.M.T. was positive in one or more specimens. Multiple strains of coagulase positive staphylococci were isolated from nearly half of these 20 cows. Of a total of 135 isolations from 120 samples, 112 (83 percent) were typable. There was only one cow in which all coagulase positive staphylococci isolated failed to react with any of the staphylococcal phages. The phage patterns obtained from the milk of these cows were numerous and varied, as may be seen in the examples of some of the patterns in Table 7. There appeared to be no predominance of any particular type.

**TABLE 7**

*Examples of Staphylococcal Phage Patterns Among Cultures Isolated Repeatedly From Bovine Milk*

| Pattern | 80/81 | 53 | 53/83 | 71 | 71/54 | 71/54/81 | 71/54/73/81 | 44A | 77 | 77/44A | 77/44A/42E | 6/42E/47/53/83 | 6/42E/53/42D/83 | 6/7/42E/47/53/54/75/77/83/44A/81 | 7 | 7/47/53/54/83/44A/81 | 7/42E/47/53/54/75/77/83/44A/81 |
|---------|-------|-----|-------|-----|-------|---------|-----------|-----|-----|-------|----------|----------------|----------------|----------------|----------------|-----|----------------|---------------|

**TABLE 8**

*The Occurrence of Type 80/81 Staphylococci in Multiple Milk Samples From 40 Cows During a 6-Month Period*

<table>
<thead>
<tr>
<th>No. Specimens Per Cow</th>
<th>No. of Cows Positive</th>
<th>Cows With 80/81 One or More Times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>31</td>
</tr>
</tbody>
</table>
### TABLE 9

**Summary Findings on Milk Samples From 40 Cows Found Positive for Type 80/81 Staphylococci Together With Clinical Observations on These Animals During a 6-Month Period**

<table>
<thead>
<tr>
<th>Month</th>
<th>No. of Samples</th>
<th>No. Positive for 80/81 Treated</th>
<th>80/81 Treated</th>
<th>Positive Cows Clinical Obs.</th>
<th>Coag. Positive Staph. Not 80/81 Treated</th>
<th>Clinical Obs.</th>
<th>Neg. Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>18</td>
<td>8</td>
<td>1</td>
<td>Rx cow has smashed teat. Flares up periodically</td>
<td>4</td>
<td>0</td>
<td>— No mastitis</td>
</tr>
<tr>
<td>November</td>
<td>24</td>
<td>7</td>
<td>0</td>
<td>No mastitis</td>
<td>10</td>
<td>2</td>
<td>— 2 with mastitis</td>
</tr>
<tr>
<td>December</td>
<td>24</td>
<td>10</td>
<td>2</td>
<td>2 Rx cows and 1 other had clinical mastitis</td>
<td>11</td>
<td>1</td>
<td>— 1 with mastitis</td>
</tr>
<tr>
<td>January</td>
<td>29</td>
<td>9</td>
<td>0</td>
<td>No mastitis</td>
<td>19</td>
<td>4</td>
<td>— 2 with mastitis</td>
</tr>
<tr>
<td>February</td>
<td>28</td>
<td>14</td>
<td>4</td>
<td>3 with mastitis in Feb., 2 in Jan.</td>
<td>11</td>
<td>1</td>
<td>— No mastitis</td>
</tr>
<tr>
<td>March</td>
<td>28</td>
<td>10</td>
<td>1</td>
<td>Mastitis in Rx cow</td>
<td>13</td>
<td>2</td>
<td>— 2 with mastitis</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>151</strong></td>
<td><strong>58</strong></td>
<td><strong>8</strong></td>
<td></td>
<td><strong>68</strong></td>
<td><strong>10</strong></td>
<td></td>
</tr>
</tbody>
</table>

---

A SIX MONTHS SURVEY OF STAPH.
Some animals continued to shed the same or a similar type during the entire period. In others, several types were found. Two phage types were obtained from the same specimens on two occasions from one cow and on three from another.

The frequency with which phage type 80/81 staphylococci were isolated from the milk of 40 cows found to have this type at some time during the six months is presented in Table 8. A total of 151 samples were obtained from these animals and type 80/81 was isolated from 58 (38 percent). Single isolations were obtained from 31 of these 40 cows, two from four cows, three from two cows, four from two cows, and five from one cow. Staphylococci of other phage types were isolated simultaneously from six of the 80/81 positive samples. Only eight of these cows were treated during the same month that type 80/81 was isolated and visible evidence of mastitis was observed in eight, as shown in Table 9. Of the remaining 93 samples, 68 (73 percent) contained coagulase positive staphylococci that proved to be other phage types or non-reactive. Ten of these cows were treated during the same month that the positive sample was obtained. In June and in July, 1960, milk samples were again obtained from 16 of the cows that had been found to shed staphylococci type 80/81 at some time during the study period. Although coagulase positive staphylococci were isolated from 20 (63 percent) of the 32 samples, none of these cultures were phage type 80/81.

That other phage types occurred repeatedly in monthly samples from the same cow is shown in Table 10. Staphylococci of phage type 77/44A, or a

<table>
<thead>
<tr>
<th>No. Specimens</th>
<th>No. of Cows</th>
<th>Cows With 77/44A* One or More Times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>126</td>
<td>71</td>
</tr>
</tbody>
</table>

* Or a similar phage pattern.

similar pattern, were isolated one or more times from 126 cows. Multiple isolations were obtained from 55 (44 percent) of these animals. There were 14 animals treated during the month that the positive sample was obtained.
DISCUSSION

The high prevalence of coagulase positive staphylococci detected in this herd compares closely to the findings of other workers (1, 5, 12). The average incidence of shedding of staphylococci in one herd was found by Schalm (1) to vary from 43 percent to 62 percent during a six-year period. He attributed the increase in isolations, in part, to improvements in sampling procedure and laboratory methods. Clinical mastitis due to staphylococci appeared to be related to the rate of persistency of shedding of these organisms in the milk.

White and McDonald (4) recently reported their observations on an outbreak of staphylococcal mastitis in a large dairy herd in Scotland. The prevalence of coagulase positive staphylococci in the milk samples remained relatively constant during a two and one-half year period but the incidence of clinical cases decreased markedly after the peak of the outbreak was passed. However, they were able to establish a relationship between clinical disease and high counts of coagulase positive staphylococci in the milk.

At the time that the present investigation was undertaken, the prevalence of clinical mastitis in the herd was not considered abnormal. In previous years, usually during the winter, a slight increase in mastitis was observed which was attributed primarily to changes in weather conditions. This is borne out by the fact that of the 62 cows treated during the six months’ period, 67 percent were treated in January, February and March. It is interesting to note that the apparent increase in clinical mastitis during this three months was accompanied by an increase in isolations of coagulase positive staphylococci.

Since penicillin and streptomycin were used in treatment of animals, it is not surprising that the greatest number of coagulase positive staphylococcal isolates were resistant to these two antibiotics. The increase in strains resistant to dihydrostreptomycin observed from December through March would suggest an increase in the amount used in treatment, but insofar as it could be determined, there was no change in dosage of streptomycin during the period of the study.

In considering the relation of the C.M.T. and Hotis test findings to the reported occurrence of clinical mastitis in the herd, it should be pointed out again that regardless of the C.M.T. or Hotis results, only cows showing visible evidence of mastitis were treated while in the milking line. In order to evaluate these findings critically, it would be necessary to follow the complete history on each individual animal.

This closed herd is a member of the Dairy Herd Improvement Association. The herd was free from *Streptococcus agalactiae* at the beginning of this survey and has remained so to the present. It is noteworthy that during the past four years milk production has gradually increased more than 3,000 pounds to an annual rate of 15,969 pounds per cow. Similarly, butterfat production has risen over 100 pounds to an annual figure of 578 pounds per cow. These figures were based on the annual average of monthly tests from August 1 each year. During this same four-year period, significant
C.M.T. reactions ranged between four and 10 percent, however there was an average of 14 percent for the six months that the study was in progress. This low rate of C.M.T. reactions and a 24 percent increase in both milk and butterfat production serve to point out the excellent performance of equipment and the competence of the operator in this dairy.

The use of 24 human staphylococcal phages in a concentrated form (10 times R.T.D.) to characterize the coagulase positive staphylococci isolated from these cows indicates that this method may provide a worthwhile tool to study and compare isolates from different herds in widely separated areas. The fact that these coagulase positive isolates from bovine milk were lysed by human bacteriophages does not establish that such strains are of human origin or that they are necessarily identical. There appeared to be no difference with particular phage types isolated from cows with mastitis or from milk from normal udders. This observation concurs with the findings of Williams Smith (13) more than a decade ago. A great deal more work will be needed to further elucidate the possible relationship between strains from human and animal sources.

SUMMARY

Composite milk samples obtained from one to six times at monthly intervals from a herd of 262 dairy cows (including replacements) were examined for the presence of coagulase positive staphylococci. A total of 1,010 milk samples were examined and coagulase positive staphylococci were isolated from 713 (71 percent). Difference in antibiotic sensitivity pattern revealed the presence of more than one strain of coagulase positive staphylococci in 85 (eight percent) of the samples. All coagulase positive staphylococcal isolates were tested for sensitivity to five antibiotics. During October and November, strains resistant to both penicillin and dihydrostreptomycin ranged between 30 and 40 percent. This level continued against penicillin but an increase in strains resistant to dihydrostreptomycin occurred in December and continued through March. Possible reasons for this increase are discussed.

Phage typing was performed on all coagulase positive staphylococcal isolates against 24 human phages at 10 times R.T.D. A total of 801 strains were tested and 551 (68 percent) were typable. Phage type 80/81 was recovered from 40 (15 percent) of the cows and phage type 77/44A (or a similar pattern) from 126 (48 percent). The remaining typable strains proved to be a variety of other patterns. Type 42D alone was not found and only rarely was it detected in combination with other phages.

Clinical evidence of mastitis as indicated by treatment of the animal was compared with the results of C.M.T. and Hotis tests and the presence of coagulase positive staphylococci. Although an increase in coagulase positive staphylococci was observed in January, February and March, and the number of cows treated increased during these months, the occurrence of clinical mastitis could not be attributed to the presence of any particular staphylococcal phage pattern.
REFERENCES


The World Health Organization defines Veterinary Public Health as "the field of activity which protects and advances human well-being by utilizing the combined knowledge and resources of all those concerned with human and animal health and their interrelationships."

Admittedly this is an extremely broad definition and when analyzed designates every phase of veterinary medicine as working in the field of public health.

There has been a great need to formally clarify the position of veterinary medicine in this respect.

In August 1957, the Institute on Veterinary Public Health Practice outlined as its purpose and objectives:

1. to explore, document, and expand upon the present and future contributions of all veterinarians to human health.
2. To collect and evaluate source material on veterinary public health practices and administration.
3. To compile a publication on veterinary public health practice which reflects the thinking of the Institute, stimulated by the formal presentations and committee studies.

The First Institute presented by the University of Michigan, School of Public Health, was an interprofessional approach to the utilization of veterinary medical resources in public health. It was held October 6-10, 1958, in Ann Arbor, Michigan. Many agencies, including the American Veterinary Medical Association, American Public Health Association, United States Public Health Service, and the Food and Drug Administration, to mention only a few, assisted in the project.

The result is an excellent report entitled "Comparative Medicine in Transition"(1). This book was published in 1960 by the University of Michigan, School of Public Health.

The Public Health Committee has reviewed this publication and recommends it as an extensive contribution to better understanding of Veterinary Public Health practices.

**Motivations Affecting Acceptance of Disease Control Programs**

The fact that disease control techniques are often more advanced than the public's acceptance of them is common knowledge to all who attempt to
implement control programs, whether it is brucellosis in animals or polio-
myelitis in man. For this reason we feel that it is worthwhile for us to ask
for help from experts in the field of the behavioral sciences. We need to
know more about why stockmen accept or reject control activities. Are we
offering our programs in the most desirable form? Has the livestock industry
been conditioned to ask for and accept the services we wish to supply?

The advertising business is built on conditioning the consumer to accept
a product or service: the situation with regard to regulatory medicine is
markedly similar. We have a product to sell and the consumer often has
certain resistance to using our product. It is of interest that a study (2) of
farm safety regarding eye injuries has pointed out that Iowa farmers have
different standards of personal safety than has the general public. It may
be that farmer acceptance of health programs is also different from that
expected of the general public. It will probably be possible to do a better
job of "selling" disease control if we understood more about the stockman
as a consumer of disease control. Does our approach cause resentment and
further urges to not cooperate? If so, why and how should our program
be offered?

We feel that more information about this subject will be beneficial and
suggest that the attention of research groups working in the field of human
behavior be called to this situation and their assistance sought.

MASTITIS

Mastitis is considered to be the most costly livestock disease in the United
States today. As such, it has received much attention in the form of con-
ferences and symposiums throughout the country.

The International Association of Milk and Food Sanitarians, Inc., through
its Mastitis Action Committee, sponsored a mastitis conference in October,
1960, in Chicago, Illinois (3). This meeting was developed as a result of
consideration by the executive board of the Association in an effort to deal
with the disease on a collective basis.

Reports of five task groups were presented on (1) Research Needs, (2)
Education in Mastitis Control, (3) Regulatory Aspects of Mastitis, (4)
Organization of National Effort Toward Mastitis Control and (5) Organiza-
tion Support for Mastitis Control.

W. D. Knox, Editor of Hoard's Dairyman, gave the keynote address and
very capably outlined "Where We Stand—What Can We Do?" I quote from
his text: "Who is going to bell the cat? Where is the directing and co-
ordinating agency? While all agricultural agencies can be expected to
cooperate, or at least give moral support, there must be somewhere a catalyst
group to spark and guide the energies of such diversified interests."

It is the opinion of the Public Health Committee that the United States
Livestock Sanitary Association can and should provide that "catalyst group."

This Association has been imminently successful as the coordinating agency
in other diseases of public health significance—notably tuberculosis and
brucellosis.
It is the recommendation of the Public Health Committee that the Association appoint a mastitis committee of national stature to undertake this extremely difficult task.

TRICHINOSIS

The Chief of the Veterinary Public Health Section, Department of Health, Commonwealth of Pennsylvania, reports (4) that they have reviewed 529 cases of trichinosis during the period 1930-59 inclusive. The cases are almost evenly divided between males and females with a slightly higher number reported in females. The vast majority occurred in the white race between the ages of 20 and 39. In recent years rural outbreaks tended to primarily involve persons of German extraction while in the urban areas individuals of Italian extraction were most frequently involved.

The most common vehicle of transmission was presumed to be pork sausage.

The Ohio Department of Health reports (5) on 34 investigations of cases of trichinosis in Northwestern Ohio between 1955 and 1960. Thirty-two (32) of these were confirmed through laboratory tests, clinical symptoms, or biopsy.

Raw or insufficiently cooked hamburger was considered the source in seven cases; raw smoked or insufficiently cooked pork sausage in 21 cases; and raw, precooked or insufficiently cooked ham in four cases.

These reports indicate that trichinosis is still a problem even though its incidence is generally considered to have been greatly reduced by the cooking of garbage fed to swine.

This points up the need for increased enforcement of garbage cooking regulations throughout the United States.

UNIFORM STATE POULTRY PRODUCTS INSPECTION ACT

The Public Health Committee recommends that State, county, and municipal authorities develop legislation covering the slaughtering and processing of poultry for the protection of the consuming public.

To aid in the formation of regulations it is further recommended that the proposals outlined in the “Uniform State Poultry Products Inspection Act” be utilized as the guide. Copies of this Act are available on request from the Inspection Branch, Poultry Division, Agricultural Marketing Service, United States Department of Agriculture, Washington 25, D. C.

EQUINE ENCEPHALOMYELITIS

It is becoming increasingly apparent that the majority of veterinarians and others specializing in the encephalitides that the term “equine encephalomylitis” is no longer a description of the disease to which it is applied.

Eastern encephalitis and Western encephalitis have been suggested as more descriptive of the two diseases. This terminology would be more in keeping with the diseases St. Louis encephalitis and Venezuelan encephalitis.

The Public Health Committee recommends that consideration be given to a change in the terminology of equine encephalomylitis.
LISTERIOSIS

While the epizootiology and the epidemiology of listeriosis is still unknown, the diagnosis of human listeriosis is increasing (6, 7). Most frequently it is recognized as a cause of meningitis, often of infants. Other clinical syndromes of the infection in man include conjunctivitis, septicemia, abortions, sterility, influenza, urethritis and infectious mononucleosis may be simulated (7).

This increased recognition of Listeria monocytogenes as a cause of disease in man may be a result of a better understanding of the culturing requirements of this organism (8, 9).

Veterinary medicine recognizes Listeria monocytogenes as a pathogen for a wide range of species of animals with many different symptoms (6, 10). In several cases, the disease has been reported in animals and man on the same premises (7).

In view of these facts the Committee suggests that the practicing veterinarian can make a valuable public health contribution and render a public relations service for the profession by reporting diagnosed cases to public health agencies for investigation and also should advise the clients of the human disease potential of the infection.

RECOMMENDATIONS

(1) The assistance of research groups working in the field of human behavior should be obtained in an attempt to improve acceptance of disease control programs.

(2) The United States Livestock Sanitary Association should appoint a mastitis committee of national stature to undertake nationwide coordination of mastitis control activities.

(3) The various states should increase garbage cooking enforcement activities to aid in control of trichinosis as well as other diseases.

(4) State, county and municipal authorities are encouraged to use the Uniform State Poultry Products Inspection Act as a guide in developing legislation covering the slaughtering and processing of poultry for the protection of the consuming public.

(5) Consideration should be given to change the term "equine encephalomyelitis" to one which is more correctly descriptive of the disease.

(6) Practicing veterinarians should report cases of Listeriosis to public health agencies for investigation and advise clients of the human disease potential of the infection.

REFERENCES


PRE-EXPOSURE IMMUNOPROPHYLACTIC PROTECTION
OF LABORATORY PERSONNEL AGAINST RABIES

Ernest S. Tierkel, A.B., V.M.D., M.P.H.*

For a long time, there has existed the problem of repeated exposures to rabies in certain high risk groups such as laboratory workers, veterinarians, dog shelter personnel and field naturalists. Repeated post-exposure treatments necessary in these groups increases the possibility of severe reactions to the vaccine. For this reason, schedules of immunization before exposures are now being continued to protect these groups and obviate the necessity for prolonged vaccine treatments after exposure occurs. This study was designed to determine the specific serum neutralizing antibody response to two vaccines used in a reduced schedule of pre-exposure immunoprophylaxis. The two vaccines used were duck-embryo vaccine and the high egg passage (HEP), Flury chicken-embryo vaccine, both of which are practically devoid of encephalitogenic properties.

Materials and Methods

Volunteer veterinary students in the Schools of Veterinary Medicine at the University of Georgia and Texas A & M College, respectively, were inoculated with either chicken-embryo rabies vaccine (CEV) or duck-embryo rabies vaccine (DEV). The vaccination program at each school was carried out as a separate study; however, the vaccine and methods used were the same.

The chicken-embryo vaccine was high egg passage modified live virus Flury strain supplied by Lederle Laboratories and the duck-embryo vaccine was P.V.S. strain, single passage in duck-embryo, inactivated with beta propiolactone and supplied by Eli Lilly Company.

All inoculations consisted of 0.2 ml. of vaccine administered intradermally on the lateral aspect of the upper arm (deltoid region). For previously unvaccinated volunteers, who comprised the bulk of the group, a regimen was carried out consisting of a primary series of three inoculations one week apart, followed by a booster six weeks after the third inoculation of the primary series. Blood samples were collected just before the first inoculation (pre-vaccination) and four weeks after the booster dose. Volunteers were vaccinated with either C.E.V. or D.E.V. on an alternating basis. The small group of volunteers with a history of previous rabies vaccination received only a single inoculation and they are considered separately in the results.

The serum neutralization tests were done according to W.H.O. standards with fivefold serum dilutions against constant dose of C.V.S. rabies virus. The amount of rabies virus used in the tests varied between 21 and 56 LD₅₀ for the Georgia study and between 32 and 63 LD₅₀ for the Texas study.

* Communicable Disease Center, Public Health Service, United States Department of Health, Education and Welfare, Atlanta, Ga.
RESULTS

The results are given in terms of the number and proportion of volunteers showing a positive serum antibody response. Those serum samples were considered positive which protected 50 percent of the mice at a dilution of 1:2 or greater against 21 LD$_{50}$ to 63 LD$_{50}$ of fixed rabies virus.

**TABLE I**

*Individuals Showing Positive Serum Antibody Titers One Month Following Completion of Four Inoculations of Duck or Chicken Embryo Rabies Vaccines*

<table>
<thead>
<tr>
<th>Vaccinated</th>
<th>No.</th>
<th>Percent</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick Embryo Vaccine</td>
<td>42</td>
<td>15</td>
<td>35.7</td>
<td>56</td>
</tr>
<tr>
<td>Duck Embryo Vaccine</td>
<td>42</td>
<td>20</td>
<td>47.6</td>
<td>61</td>
</tr>
</tbody>
</table>

*Positive Titer: A serum which protected 50 percent of the mice at a dilution of 1:2 or greater against 21 to 63 LD$_{50}$ of fixed rabies virus.*

Table I gives results obtained on serum samples collected one month after the fourth (booster) inoculation in the vaccinated volunteers at the University of Georgia and Texas A & M College. In the Georgia group, 35.7 percent of the volunteers who received chicken-embryo vaccine responded with positive titers as compared with 47.6 percent positive responders in those receiving duck-embryo vaccine. In the Texas group, the proportion of positive responders was higher. Here the results showed 50.0 percent positive in those receiving the chicken-embryo vaccine and 80.3 percent responders in the duck-embryo vaccinated group. Thus, the duck-embryo vaccine in the Texas volunteers elicited a greater proportion of positive responders than the chicken-embryo vaccine. This difference was shown to be statistically significant, whereas the difference between the two vaccine groups in Georgia was not.

In order to check the relative persistence of demonstrable serum antibody one year later, blood specimens were collected from those volunteers who had reacted positively the previous year and who were still available for study. The results of these tests are given in Table II. In Georgia, the pro-

**TABLE II**

*Relative Persistence of Previously Positive Serum Antibody Titers One Year Following Completion of Four Inoculations of Rabies Vaccines*

<table>
<thead>
<tr>
<th>Tested</th>
<th>No.</th>
<th>Percent</th>
<th>Tested</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick Embryo Vaccine</td>
<td>11</td>
<td>2</td>
<td>18.2</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Duck Embryo Vaccine</td>
<td>17</td>
<td>4</td>
<td>23.5</td>
<td>28</td>
<td>14</td>
</tr>
</tbody>
</table>
portion still positive after one year in the chicken-embryo groups was 18.2 percent compared with 23.5 percent in the duck-embryo group and those in Texas showed persistence of antibodies in 31.2 percent of the chicken-embryo vaccine group compared with 50.0 percent in the duck-embryo vaccine group. That there was a general fall-off of positive antibody responders in all groups after one year had elapsed since the immunization series. The pattern of differences in both schools remained about the same as they had the previous year; however, neither of the differences between the two vaccine groups in the antibody persistence test was found to be statistically significant.

A booster inoculation of the same dose and route was administered one year later to those individuals who had reacted negatively one month after the immunization series. The proportion of conversions from negative to positive following this booster is given in Table III. Here the percentage of converters were shown to be of a relatively high order with 81.2 percent and 60.0 percent in Georgia and 56.2 percent and 33.3 percent in Texas in the chicken-embryo vaccine group and the duck-embryo vaccine group, respectively. The differences in the antibody conversion rates between the two vaccines were not significant in either the Georgia or Texas portions of the study.

A number of the volunteers in both schools were individuals who had a previous history of antirabies immunization at least one time in their lives. These individuals received only a single intradermal inoculation. The results of the antibody tests in this group are shown in Table IV. It will be noted

**TABLE III**

*Conversion Rates in Negative Individuals Given a Booster One Year After Completion of Four Inoculations*  

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Georgia Group</th>
<th>Texas Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boosted</td>
<td>Converted</td>
</tr>
<tr>
<td>Chick Embryo Vaccine</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Duck Embryo Vaccine</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

* Serum collected one month following booster.

**TABLE IV**

*Antibody Response of Individuals With a History of Previous Antirabies Immunization to One Booster Dose of Vaccine*  

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Georgia Group</th>
<th>Texas Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time Following Booster</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 Month No. Tested</td>
<td>1 Year* No. Positive</td>
</tr>
<tr>
<td>Chicken Embryo Vaccine</td>
<td>6/6</td>
<td>2/3</td>
</tr>
<tr>
<td>Duck Embryo Vaccine</td>
<td>2/3</td>
<td>1/1</td>
</tr>
<tr>
<td>Total</td>
<td>8/9</td>
<td>3/4</td>
</tr>
</tbody>
</table>

* All persons tested at one year had been positive at one month.
that the proportion of positive responders among these was of a high order regardless of the type of vaccine used as a single booster. This has been the rather consistent experience of previous work we have done and of the observations of others.

**DISCUSSION**

The results of this study have shown some superiority in antibody response of duck-embryo over H.E.P. (Flury) chicken-embryo vaccine in the comparative vaccination series carried out in student volunteers in Texas A & M College. None of the other differences in the proportion of responders between the two vaccine groups was statistically significant. On the basis of previous experience and the generally high conversion rates after one year in this study, it would appear that administration of the booster dose for a longer period of time than six weeks following the primary series would give a higher proportion of positive responders than those shown in the results in Table I. We are currently conducting studies to determine optimum spacing of the primary series and of boosters and a comparison between intradermal and subcutaneous routes of administration.

In the meantime, it is recommended that for high risk groups such as laboratory personnel, a schedule of immunization consisting of a primary series of three doses of non-nervous tissue vaccine be administered at weekly intervals followed by a booster inoculation six months after completion of the primary series. If the individual continues to work under risk, he should be revaccinated with a similar booster dose every two to three years. It should be pointed out, however, that antibody response does not occur in all vaccinated individuals. A serum neutralization test should be carried out on a serum sample collected 30 to 60 days after the immunization regimen to test for the presence of detectable antibody. Booster doses can be repeated until antibody is detectable. For post-exposure treatment in those individuals who have demonstrated consistent antibody responses to antirabies vaccinations received in the past, one to three doses of vaccine are recommended in cases of mild to moderate exposure.

**ACKNOWLEDGMENTS**

The author wishes to express his appreciation to Dr. R. Keith Sikes and James B. Thomas for their assistance in conducting the serum neutralization tests; to Dr. Joe R. Held and Dr. Paul Arnstein for their assistance in experimental design; and to Dr. John Foster and Dr. F. P. Jaggi for co-ordinating the student immunization programs at the University of Georgia and the A. and M. College of Texas, respectively.
RABIES IN SKUNKS IN THE NORTH-CENTRAL STATES

RICHARD L. PARKER, B.S., D.V.M.*

Rabies is a disease which for centuries has struck terror into the hearts of those exposed and their families. Even today, there are few diseases which create such hysteria in the general populace as the report of a mad dog on the loose. Rabies has, for the greater part of its history, been connected with dogs and with other classes of domestic animals. However, since 1956, wild animals have contributed more laboratory diagnosed cases of rabies than have dogs (1). Wildlife rabies in the continental United States appears to have fairly well defined geographic areas of species involvement. The fox, both red and grey, contributes the bulk of wildlife cases in the Appalachian Chain from New York south to Georgia and west across the coastal plains into Texas. On the other hand, skunks contribute the greatest number of wildlife cases in the central valley of California and over the broad expanse of the middle part of the country north of a line from Kansas and Missouri east to Ohio (2).

Two years ago, Dr. H. N. Johnson discussed the history of rabies in skunks at this meeting (3). According to his search of the literature, the first case of skunk rabies was reported from lower California in 1826, and rabies appears to have been fairly common during the Gold Rush period in California. In the 1870's, rabies was well recognized in the Plains States; but, for a period of some years, skunk rabies virtually disappeared from the Midwest.

Lantz reported in 1923, "The popular belief that hydrophobia will result from a skunk bite is in error. There is no more danger from this source than there is in handling cats or dogs" (4). This statement could not have been made in good faith either 50 years earlier or 30 years later. The current epizootic of skunk rabies, which hopefully has passed its peak in the north-central States, appears to have had its onset in the early 1950's, with a steady build-up of cases until the peak of 782 laboratory cases were reported in a 12-State area in 1958.

The Communicable Disease Center of the United States Public Health Service, has become increasingly interested in the role of wildlife dissemination of rabies and has accordingly developed three field stations situated in geographically strategic locations to study the three principal wildlife vectors of the disease, i.e., the fox in the Southeast, the bat in the Southwest, and the skunk in the Midwest. If we may limit our discussion to the terrestrial mammal rabies problem, some of the first questions that appear in need of answering are those concerning the rather unique geographic distribution of species involved in wildlife rabies. A year ago at this meeting, Dr. R. K.

* Midwest Rabies Investigations Station, Communicable Disease Center Activities, United States Public Health Service, Poyntette, Wisconsin.
Sikes of the C.D.C. Southeast Rabies Investigations Station presented data which indicated that skunks were not as susceptible to rabies virus isolated from foxes in the southeast as were foxes themselves, which may well account for the lower incidence of skunk rabies at least in the southeastern part of the United States (5).

Using a previously described technique to compare fox and skunk abundance (6), it appears that there are at least as many foxes in certain areas of the skunk rabies region of the United States as there are in some of the highly enzootic fox areas; and, conversely, there are just as many or more skunks in some of the high fox rabies areas as there are in parts of the Midwest. It is difficult to say whether the depression of the species involved is a reflection on the incidence of rabies in this species or not. However, let it suffice to say at this stage that there are plenty of susceptible animals of the other species in areas where they do not seem at the moment to be contributing materially to the rabies problem. While on the theme of relative populations and of susceptibility, I would like to point out that work completed within the past year at the C.D.C. Poynette Station indicates that both foxes and skunks are highly susceptible to a skunk rabies virus isolated in the northern Midwest. At least some foxes and skunks can be infected by intramuscular inoculation with less than 10 mouse intracerebral LD$_{50}$’s of virus. Skunks receiving low doses of virus have on three occasions survived over 100 days; the longest period observed to date was 177 days. This work will be reported in detail at a later date.

Skunks are primarily crepuscular or nocturnal animals which accounts for their being seldom seen abroad during daylight hours. Indeed, in very many areas where skunk rabies is prevalent, sighting a skunk abroad during daylight hours is reasonable grounds to suspect the animal of being infected with the disease. Skunks are rather ubiquitous animals adapting readily to a wide variety of habitats. Since they are classed as carnivores, their feeding habits govern somewhat their choice of habitat; but since they are actually nearly omnivorous, they are quite adaptable to a wide range of habitats. Hamilton, in a fall and winter food study of skunks, reported that nearly one-third of the animals examined had fruit of some sort in their stomachs and over one-fifth had grasses, buds, leaves and other plant material. Mammal remains in stomach contents occurred in nearly one-fourth of the animals examined. Insects increased in percent of occurrence at the expense of fruit and mammals during the summer; however, the latter occurred in one-fourth and one-sixth of the stomachs (7).

While skunks are not true hibernating animals, at least in the northern part of their range, they are known to den up for varying periods of time during the extremely cold weather in the winter time. Skunk dens in general are dug to or below the frost line and may be occupied by one or more animals. Durwood Allen has reported that in no case has he ever observed more than one male in a communal den but he has observed as many as 10 females denning together in the same hole (8). Hamilton has indicated that denning activity of the animals is directly related to extremes of cold weather
and that females and young appear to den earlier and stay in the den later than do adult males (9). In northern latitudes, skunks appear to breed shortly after the coldest weather of the year, from late February well into March. The young are born approximately 60 to 65 days after breeding and average about five per litter (10). The family groups appear to remain together until late summer, breaking up sometime in late August or early September. Various skunk trappers have reported to me personally that during the cold months when there is snow on the ground, it is not uncommon to find skunk tracks leading from hole to hole, often stopping and entering the hole apparently to investigate, and then emerging to continue on to another hole. It is also apparently fairly common when following such a wandering skunk to find blood in the tracks after the animal has emerged from a hole, indicating perhaps that a fight has taken place with the inhabitants of a skunk den which suggests to me the possibility of a wandering "bachelor" skunk being driven from a harem by a single resident male.

As a part of our work at the C.D.C. Rabies Station at Poynette, we have been for the past two years trapping and marking skunks for further study, and in certain areas we examine all skunks trapped for rabies virus. In one area where all skunks taken have been examined for rabies, for two consecutive winters skunks taken in the months of January and February have shown relatively high incidences of rabies. In Wisconsin, temperatures during these months are apt to be quite low (average January temperature at Portage, Wisconsin, the county seat closest to Poynette, +17.5° F. (11)) and one would suspect that skunk movement would be at a minimum. However, at this time of year, we have had fair success in trapping skunks. This may be a reflection of the high incidence of rabies in these animals at this time of year or it may be that the animals, as has been previously reported, are not true hibernators and that some remain active even in the coldest weather. Working on a unit of land approximately 320 acres in size, one winter we obtained eight animals. Six of these were rabid at the time of capture. However, only about half were showing clinical signs of the disease. No attempt was made to obtain animals from this area for the next twelve months. However, at approximately the same time of year the following winter, a sample of six animals was taken, two of which were rabid. The summer following the second winter's trapping in this area, skunks appeared to be non-existent, suggesting that possibly this area is more desirable as a winter denning and feeding area than as a summer feeding area and that the introduction of rabies virus occurs in this area during the winter months when the animals may be living in closer proximity than at any other time of year. One cannot discount entirely the possibility that trapping and disease have virtually eliminated skunks from this area; however, the trapping in this area has been fairly restricted and work in other areas indicates that the natural movement of skunks is such that they would tend to repopulate such a small area rather quickly. Returns of a limited number of marked animals in other areas indicate that skunks may travel as much as three-fourths of a mile and possibly further. In an area where rabies virus has not been isolated and
where animals are being marked and released, a Lincoln Index calculation
(12) indicates skunk population may be as high as 33 per square mile, and
although the small number (three) of recaptures tends to make this a
maximum estimate, it is similar to the 31 per square mile reported by
Jones (13). In still a third much smaller area of study, one of two skunks
captured the first winter was rabid. Of four more skunks taken from this
farm, none were rabid. In addition, a number of animals in this area have
been marked and released and to date no recaptures of marked animals have
been made.

To summarize the skunk and rabies situation in these three areas that
were chosen quite randomly, it appears that we have by chance selected an
area where skunk populations are highest during the winter months when
activity is usually at its lowest and which may well represent a winter
denning area. In this area, skunk rabies appears to be at a high incidence
at least at the time of year the animals are present. In another or inter-
mediate area, it would seem that we have an area quite often frequented
by skunks, but lacking recaptures of marked animals; it may represent a
hunting and feeding area where the skunks merely pass through. Rabies
has been demonstrated once in this area. A third area appears to have a
somewhat stable population with animals being recaptured with some degree
of regularity. As yet, rabies virus has not been isolated from this particular
area.

What is the seasonal incidence of rabies in skunks in the north-central
States? Data collected over the past three years from twelve north-central
States and data from the State of Wisconsin over the five-year period, 1953-
1957, indicates that the highest incidence of rabies in skunks occurs from
January through July, with peaks in April and July.

While the facts at hand are not sufficient to draw firm conclusions, it
appears that they strongly support the following hypothesis which may
explain at least a portion of the epizootiology of skunk rabies. If rabies
virus is introduced into a skunk population at a time when the adult animals
appear to be somewhat concentrated, the virus may spread rapidly through
the population because of the close contact in communal dens and the
apparent seeking out of other skunks by solitary wandering animals. Those
animals receiving relatively large amounts of virus would have incubation
periods that would culminate in frank clinical signs of the disease about the
time of the April peak. Those animals receiving somewhat less virus might
have delayed incubation periods resulting in the projection of a high incidence
of rabies well into the summer. Those animals receiving even less virus might
account for the continued cases of rabies reported throughout the summer,
and, indeed, might serve as the reservoir for re-infection the following
winter, either directly or through one or two additional skunk passages
throughout the year. While such a hypothesis is based on a collection of
information from various sources, I feel that it gives the necessary toe hold
for future investigations.
Let us look further for an explanation of the explosive outbreak of skunk rabies beginning in the early '50's and persisting to date. Figure 1 graphically presents information on the reported skunk fur harvest from four representative States in the north-central region, combined with the average pelt price.

**Figure 1.** 1930-1960 Annual averages for four states (Iowa, Ohio, Minnesota and Wisconsin).

* Average prices paid for skunk pelts in terms of 1947-1949 average dollars.
prices received for skunk pels in this area and the reported incidence of rabies from the same States. The average pelt prices as footnoted in the figure, are computed on a 1947-1949 dollar average (14). Since certainly it is not an accurate representative of trapping incentive to present fur prices from 1930 in 1960 dollars, the line depicting the average value of the pelts is calculated from a dollar average base line and more accurately reflects the “barter price” of a pelt or the purchasing power of a skunk pelt. It is noted in this graph that a sharp increase in rabies cases in skunks occurred shortly after a sharp decrease in the harvest of skunks, a situation which might have occurred with any disease which is spread by close contact of animals. Whenever rudimentary checks on populations are removed and an animal population is allowed to expand freely, the stage is set for an epizootic.

It was mentioned earlier that skunks were ubiquitous in habitat requirements. However, it appears that skunks are especially abundant in and around abandoned or active farm buildings since these areas afford many temporary and/or winter den sights in the forms of rock piles, decayed building foundations, unoccupied buildings, post and lumber piles, etc. An increase in cover seems to favor most species of wild animals and the return of much marginal or sub-marginal land to timber production, Christmas tree production, and the soil bank in place of clean farming operations appears to have favored skunk population build-ups. Figure 2 graphically presents the total acreage harvested in the State of Wisconsin over a period of 39 years.

Having spent some time presenting what is factual about skunk rabies in the north-central States and developing a hypothesis which may in part explain the epizootiology of the disease, let us look into our crystal ball and perhaps predict what the future of skunk rabies in the north-central States may be. It has been my experience that in areas where rabies suddenly assumes prominence, reporting is excellent. As rabies becomes “old hat,” interest in the disease dies. Submitting material to distant laboratories, particularly when the material is apt to be offensive both to the persons wishing the report and to laboratory personnel, seems to present problems
RABIES IN SKUNGS IN THE NORTH-CENTRAL STATES

279
easier to avoid than overcome. If it behaves like it is rabid, it's shot and considered rabid without being submitted for diagnosis. For this reason, and because it appears that skunk populations sharply declined in certain areas of the north-central States from a high in the late middle '50's to the present, it may well be that skunk rabies cases will continue to go down as they have since the peak year in 1958. I do not think that this will signal an end to the skunk rabies problem in the north-central States but perhaps it will assume enzootic rather than epizootic proportions and may flare up in isolated areas as skunk populations build up in these areas.

Today skunk rabies poses many problems both to human health and to agriculture. With the increase in leisure time brought on by increased productivity and income, many people are turning to outdoor recreation such as camping, hunting, fishing, etc. This materially increases the population at risk to wildlife rabies; and, indeed, there have been several instances where people fishing and camping have been exposed to wildlife rabies over the past few years. Every indication today is that this will become more of a problem rather than a lessening problem, not only with rabies but with other zoonoses, which may find reservoirs or vectors in our wildlife. With the intensification of agricultural practices in certain areas, the individual value of domestic animals has been raised appreciably. Rabies in wildlife today presents the greatest single domestic animal rabies hazard; and, in terms of dollars and cents, this promises to become an increasing rather than decreasing problem.

REFERENCES


REPORT OF THE COMMITTEE ON RABIES


Rabies Incidence and Trends—Calendar Year 1960

The total number of laboratory confirmed animal rabies cases reported for calendar year 1960 was 3,427, representing a decrease of 656 cases from the total number of confirmed cases reported during the previous calendar year.

The numbers of confirmed cases reported for 1960 by type of animal included 697 dogs; 288 cats; 910 foxes; 722 skunks; 47 raccoons; 65 bats; 563 cattle, and 72 cases in other types of farm animals. The greatest decline occurred in dogs, with a drop of 422 cases between 1959 and 1960. On the other hand, the total number of fox and skunk cases for the country in 1960 remained about the same as the number in 1959. Most impressive in this 1960 report is the all-time low of 697 canine cases reported for the entire country.

The New England States remained rabies-free in 1960. There were 14 states in which there was an increased incidence in total rabies over the previous year: New Jersey, Ohio, Illinois, Michigan, Missouri, Nebraska, Virginia, West Virginia, Arkansas, Oklahoma, Colorado, New Mexico, Washington and Oregon. In Virginia, the only State in which the increase was by more than 50 cases, the principal problem has been one of fox rabies in the southwestern part of the State. In Oregon, which has been rabies-free for many years, nine cases (one dog and eight bats) of rabies were reported for calendar year 1960.

In 26 states there was a decrease in the number of cases reported and in seven states the decrease was by more than 50 cases: Alabama, Wisconsin, Minnesota, Kentucky, Georgia, South Dakota and Texas. Much of the decrease in Georgia can be attributed to dog vaccination and fox trapping programs. Although Texas had a decrease of more than 50 cases; that State reported 558 cases, more than any other State. The Texas State Health Department reported that the local areas of high incidence in Texas during 1960 were in foxes in the Brazos River Valley or east-central Texas, and dog rabies in the Brownsville-Matamoros Border area. New York reported 455 cases, the second largest number to be reported by any one State. This high incidence was largely due to a continuation of an epidemic of fox rabies which began in 1959 in the western counties of the State.

Rabies Trends by U.S.P.H.S. Reporting Regions—1960

(See outline map of the U.S. showing distribution of cases by State.)
New England
(Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, and Connecticut.)
Remained completely rabies-free during 1960.

Middle Atlantic
(New York, New Jersey, Pennsylvania.)
New Jersey continued to enjoy rabies-free status. Pennsylvania dropped from 34 cases in 1959 to 18 cases in 1960. New York showed the second highest incidence in the country with 455 cases. This is attributed to the epizootic fox rabies situation in the western New York counties, with 186 cases reported in foxes, resulting in 152 cattle deaths; there were 23 cases reported in dogs for the entire State.

East North Central
(Ohio, Indiana, Illinois, Michigan, Wisconsin.)
Slight decline from 415 to 371 for this reporting region. Wisconsin's decrease was offset by an increase in Illinois.

West North Central
(Minnesota, Iowa, Missouri, North Dakota, South Dakota, Nebraska, Kansas.)
This reporting region showed a healthy decline of almost 300 cases in 1960 with every State showing a decrease except Iowa and Missouri which remained essentially the same as 1959.

South Atlantic
(Delaware, Maryland, District of Columbia, Virginia, West Virginia, North Carolina, South Carolina, Georgia, Florida.)
The decrease here was from 587 cases in 1959 to 467 in 1960. Substantial decreases in the southernmost states accounted for the regional decline. Virginia and West Virginia showed increases due to epizootics in foxes.

East South Central
(Kentucky, Tennessee, Alabama, Mississippi.)
Alabama's drop from 271 in 1959 to 91 in 1960, and Kentucky's decline from 208 to 120 were mainly responsible for the over-all decrease from 625 to 396 cases in this area. Mississippi continues its remarkably low number of cases (seven in 1959 and five in 1960).

West South Central
(Arkansas, Louisiana, Oklahoma, Texas.)
The incidence in this reporting region remained about the same as the previous year. Texas contributed the bulk of the cases, and leads the nation in the number of reported cases. Of interest in Texas is the steadily increasing
number of reported cases in skunks, which in 1960 surpassed the number of fox rabies cases for the first time.

**Mountain**

(Montana, Idaho, Wyoming, Colorado, New Mexico, Arizona, Utah, Nevada.)

Continued at low ebb with northern Mountain States essentially rabies-free, except for two cases in bats reported from Montana.

**Pacific**

(Washington, Oregon, California, Alaska, Hawaii.)

In previous years California contributed the only cases in this region; however, in 1960 Oregon reported nine cases, eight of which were in bats and one in a dog. The single case of canine rabies reported from Washington was not indigenously exposed; it had been travelling through several states and had come originally from California.

**Progress Report—Over the Years**

The progress that has been made in our total national rabies control effort since we organized in this direction in 1946 is eloquently illustrated by the following table:

**Reported Cases of Rabies in the United States**

(ARS-USDA: CDC-USPHS)

<table>
<thead>
<tr>
<th>Year</th>
<th>Dogs</th>
<th>Wildlife</th>
<th>Cats</th>
<th>Livestock</th>
<th>Man</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1946</td>
<td>8,384</td>
<td>956</td>
<td>452</td>
<td>1,055</td>
<td>22</td>
<td>10,872</td>
</tr>
<tr>
<td>1956</td>
<td>2,592</td>
<td>2,079</td>
<td>371</td>
<td>794</td>
<td>10</td>
<td>5,846</td>
</tr>
<tr>
<td>1960</td>
<td>697</td>
<td>1,805</td>
<td>288</td>
<td>635</td>
<td>2</td>
<td>3,427</td>
</tr>
</tbody>
</table>

**Human Rabies Deaths—1960**

Only two human rabies deaths were reported in the United States for calendar year 1960, and only one of these resulted from indigenous exposure. The first case was in a nine-year-old colored male in Atlanta, Georgia, who died on May 21, 1960. This was a particularly sad case complicated by mistaken identity of the biting dog. It seems that the wrong dog was incarcerated for clinical observation and immunoprophylactic treatment of the victim—who was one of seven children bitten—was stopped after he had received hyperimmune serum and seven daily inoculations of vaccine. He received multiple bites on the nose, lip, elbow and back, and the incubation period in this case was 56 days.

The only other human rabies death occurred in Cleveland, Ohio, in a 19-year-old girl who was bitten by a cat during a visit to Guatemala City, Guatemala. The cat was later proved rabid, but the biting incident was not reported to the public health authorities in Guatemala and there was no treatment administered.
The total number of animal rabies cases during the first nine months of 1961 is 2,604. These are provisional figures and are based on cumulative totals reported on a weekly basis to the Communicable Disease Center, United States Public Health Service. This represents a decrease of 124 cases as compared with the same period in 1960. In this period a rise in incidence was noted in Illinois, Iowa, Minnesota, South Dakota, Florida, Texas and California. Notable declines in incidence were reported from New York, Ohio, Missouri, Kentucky, Arkansas and Georgia.

Human Rabies Deaths—1961 to Date

At this writing (October 28, 1961) there have been three human rabies deaths reported for calendar year 1961. A 53-year-old woman died of rabies in Harlan County, Kentucky, on January 6, 1961, 59 days after being bitten on the leg by a gray fox. She kicked at the animal to scare it away from her puppy. The fox was killed by the woman’s husband. The next day she saw a physician and immediately began a series of 14 doses of duck-embryo rabies vaccine.

The second case of human rabies for the United States this year occurred in a 76-year-old man who died on January 20, 44 days following the bite of a rabid dog near the Imperial Dam, Imperial County, California. The patient received a five-inch bite wound on the right wrist when the dog jumped from a bank above and attacked him on December 7, 1960. The wound was washed
following the attack and a series of 14 doses of duck-embryo rabies vaccine was begun six days after the biting episode. Onset of illness was 31 days after the bite and death occurred on the 13th day after onset of illness.

The third human rabies death was also reported from Kentucky due to a bite of a rabid fox. On May 15, a 74-year-old resident of Powell County investigated a commotion in his chicken house and found a fox under the shed. On attempting to chase the fox away, he was bitten on his left thumb. The fox was killed and discarded. The man refused rabies vaccination initially, but after two calves had died of apparent rabies, within the following two weeks, he consented to vaccination. He received 14 doses of duck-embryo vaccine beginning on June 7, two and one-half weeks after the exposure. The last dose was administered a day before onset of symptoms. The patient died on June 27.

Bat Rabies

During 1960 six new states were added to the list of states reporting bat rabies, which brought the total up to 30 states reporting the disease in bats up to the end of the calendar year. These states were Oregon, Indiana, Missouri, Iowa, New Jersey and Kansas.

Thus far in 1961, five additional states have reported bat rabies: Tennessee, Washington, Arkansas, South Dakota and Massachusetts. This now makes a total of 35 states reporting bat rabies since the first reported case in Florida in 1953. Thus far, the record shows five human rabies deaths in the United States which have been attributed to rabid bats. Epidemiological and viro-
logical investigations on rabies in bats and other wild animal vectors are continuing in the three regional field stations of the Communicable Disease Center.

**Human Rabies Treatments in the U.S.—1951 and 1960**

In a survey conducted by the C.D.C. Rabies Control Program, data was obtained on the number of 14-daily dose human rabies treatments distributed for use in the United States during the past two years. During 1959, 30,223 14-dose treatments of human rabies vaccine were produced. Of this number 13,400 doses were duck-embryo vaccine. In 1960, 30,771 treatments of human vaccine were produced for the country, of which 15,500 (over 50 percent) were duck-embryo vaccine.

**W.H.O. Global Survey of Rabies Made Available**

In December 1959 the World Health Organization's Expert Committee on Rabies met in Geneva and reviewed the world rabies situation. A preliminary report based on information collected by W.H.O. in a survey-questionnaire from 170 services in 75 countries disclosed that rabies is present in 52 countries and not present in 23 countries.

Rabies was reported present in animals in Algeria, Bechuanaland Protectorate, Brazil, Bulgaria, Burma, Cambodia, Canada, Chile, Czechoslovakia, Dominican Republic, Egypt, Ethiopia, Finland, France, French Equatorial Africa, French West Africa, Germany, Ghana, Greece, Guatemala, British Guiana, French Guiana, Honduras, India, Indonesia, Iran, Israel, Italy, Kenya, Libya, Madagascar, Mexico, Morocco, Mozambique, Nigeria, Nyasaland, Pakistan, Philippines, Poland, Northern and Southern Rhodesia, Somaliland Protectorate, Sudan, Tanganyika, Thailand, Tunisia, Turkey, Uganda, Union of South Africa, United States, Viet Nam and Yugoslavia.

The following countries reported rabies not present: Australia, Basutoland, Belgium, North Borneo, China (Taiwan), Denmark, Guadeloupe, Hong Kong, Iceland, Ireland, Japan, Luxembourg, Federation of Malaya, Martinique, Netherlands, New Zealand, Norway, Portugal, Singapore, Sweden, Switzerland, United Kingdom, and Uruguay.

For 1958, reports of more than 10 human rabies deaths were received from the following: Pasteur Institute of Southern India, Coonoor (250), Haffkine Institute, Bombay, India (205), Philippines (217), Egypt (30), and Turkey (12).

The chief reservoir of rabies in Germany is the fox; in 1958, 1,017 cases were recorded in the Federal Republic. In Canada and Czechoslovakia the fox also plays a predominant role. In Iran, rabid wolves are the greatest source of rabies. The Iran Pasteur Institute reports, from 1949 to 1958, 443 persons were bitten by wolves with 39 deaths.

At this meeting the W.H.O. Expert Committee on Rabies was concerned with bringing its previous recommendations on diagnosis, treatment and prevention up-to-date in the light of recent findings. It also discussed
epidemiology, statistics and laboratory techniques, and made recommendations on future rabies research. Its report will be published in the near future.

**Rabies in the Americas**

Included in the Report of the Zoonoses Technical Advisory Group (Zotag) of the Pan American Health Organization (PAHO) is current information on the incidence of rabies in the Americas. Incidence data for 1958 were received from 22 areas in the Western Hemisphere. From these areas 7,724 cases of rabies in animals were diagnosed and reported. More than 100 cases were reported in each of the following countries: Argentina, Brazil, Canada, Chile, Peru and the United States. Guatemala, Nicaragua and Venezuela reported more than 50 cases each. Although no information was provided for Mexico, a report sent to the World Health Organization indicated that in 1958, 747 captured animals in the Federal District were positive for rabies. Similarly 81 positive animal cases were reported by the National Institute of Hygiene of Ecuador. Dogs have been most often incriminated as the source of rabies in other animals and man. The other animal most often reported with this disease in the Americas is the cow. Uruguay reports that it has been rabies-free for more than 20 years. Brazil, Mexico, the United States, French Guiana, and Trinidad have reported incidents involving the transmission of rabies by bats.

In 1958, 186 human cases of rabies were reported in countries of the Western Hemisphere. In five countries, 10 or more human rabies deaths were reported, namely, Brazil, Columbia, Mexico, Peru and Venezuela.

**Rabies Transmission by Nonbite Route in a Bat Cave**

A study carried out this summer by Dr. Denny Constantine and co-workers at the C.D.C. Southwest Rabies Station indicated evidence of airborne transmission of rabies to wild carnivorous animals held in a bat cave. Native carnivores developed rabies after they had been kept in animal-proof and arthropod-proofed cages for about four weeks in Frio Cave, a large limestone bat cave near Uvalde, Texas.

A number of animals, including dogs, cats, foxes, coyotes, ringtails and skunks, were placed in cages in Frio Cave. Four types of cages were used, one being constructed with plastic mesh and glycerinated spun-glass pad “moats” in such a way that neither mammal nor arthropod could penetrate it. Coyotes and foxes housed in all four cages became ill and died with clinical signs of rabies. Mouse inoculation, fluorescent antibody and serum neutralization identification tests have confirmed the diagnosis of rabies in these animals.

**Fourth Rabies Report of W.H.O. Expert Committee Available**

The Fourth Report of the Expert Committee on Rabies, World Health Organization was published during 1960. The Committee has, in this report, brought up-to-date its previous recommendations on diagnosis, treatment and prevention. Some of the newer items in this latest report were recommendations regarding the use of the fluorescent antibody technique in
diagnosis, the administration of two boosters of vaccine 10 and 20 days following the 14 daily-dose treatment in exposed persons, administration of hyperimmune serum to obviate possibility of interference between serum and vaccine, contraindication of L.E.P. Flury vaccine in cats and wild animal pets and schedules for human pre-exposure immunoprophylaxis with non-nervous tissue vaccines in high risk groups.

Interstate Shipment of Foxes

Many states have been confronted with the problem of the release of foxes imported from other parts of the country. Ecologists have long stated that this is poor conservation practice and it constitutes an effective method for the introduction of rabies and other diseases into noninfected areas. The last report of the W.H.O. Expert Committee on Rabies (1960) condemns this practice.

Very often these wild animals are introduced with permits from State game commissions and occasionally they are accompanied by veterinary health certificates. Since the complete prohibition of these wildlife transfers does not seem possible at this time, it is felt that the Public Health Veterinarian and the State Veterinarian in each State should be given firmer control of the situation when they occur. Dr. Martin Marx, State Public Health of Virginia suggests that any approval of veterinary health certificates for the introduction of foxes or other wildlife should be qualified as follows:

(1) That the signature of the veterinarian and/or State veterinarian attests only to the apparent state of health of the fox when examined and carries no guarantee that said animal is not in the incubative stages of rabies.

(2) That until a satisfactory rabies vaccine for foxes is marketed there can be no artificial or real protection assumed.

(3) That unless the foxes are acceptable to the Public Health Veterinarian and the State Veterinarian of the State receiving the animals, the health certificate becomes null and void.
### U. S. HUMAN RABIES DEATHS

#### 1960

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date Died</th>
<th>Age</th>
<th>Sex</th>
<th>Nature of Exposure</th>
<th>Incubation Period</th>
<th>Length of Illness</th>
<th>Treatment</th>
<th>Biting Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Atlanta, Fulton County, Georgia</td>
<td>5/21/60</td>
<td>9</td>
<td>M</td>
<td>Bitten through nose and lip, elbow and lower back</td>
<td>56 days</td>
<td>3 days</td>
<td>Serum + 7 doses vaccine</td>
<td>Dog or dogs running amuck</td>
</tr>
<tr>
<td>2. Cleveland, Cuyahoga County, Ohio (exposed in Guatemala City, Guatemala)</td>
<td>9/5/60</td>
<td>19</td>
<td>F</td>
<td>Nip on the right hand</td>
<td>50 days</td>
<td>5 days</td>
<td>None</td>
<td>Cat</td>
</tr>
</tbody>
</table>

### U. S. HUMAN RABIES DEATHS

#### January-October 1961

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date Died</th>
<th>Age</th>
<th>Sex</th>
<th>Nature of Exposure</th>
<th>Incubation Period</th>
<th>Length of Illness</th>
<th>Treatment</th>
<th>Biting Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Harlan County, Kentucky</td>
<td>1/6/61</td>
<td>53</td>
<td>F</td>
<td>Bite on leg</td>
<td>52 days</td>
<td>7 days</td>
<td>14 doses duck-embryo vaccine begun the day following bite</td>
<td>Fox</td>
</tr>
<tr>
<td>2. Imperial County, California</td>
<td>1/20/61</td>
<td>76</td>
<td>M</td>
<td>Bite on right wrist</td>
<td>31 days</td>
<td>13 days</td>
<td>14 doses duck-embryo vaccine begun 6 days after bite</td>
<td>Dog</td>
</tr>
<tr>
<td>3. Powell County, Kentucky</td>
<td>6/27/61</td>
<td>74</td>
<td>M</td>
<td>Bite on left thumb</td>
<td>38 days</td>
<td>5 days</td>
<td>14 doses duck-embryo vaccine begun 2 1/2 weeks after bite</td>
<td>Fox</td>
</tr>
</tbody>
</table>
RABIES IN ANIMALS
First Nine Months—1960-1961
(CDC—United States Public Health Service)

<table>
<thead>
<tr>
<th>State</th>
<th>1960 (First Nine Months)</th>
<th>1961 (First Nine Months)</th>
<th>State</th>
<th>1960</th>
<th>1961</th>
</tr>
</thead>
<tbody>
<tr>
<td>New England</td>
<td></td>
<td></td>
<td>West Virginia</td>
<td>91</td>
<td>96</td>
</tr>
<tr>
<td>Maine</td>
<td>0</td>
<td>0</td>
<td>North Carolina</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>New Hampshire</td>
<td>0</td>
<td>0</td>
<td>South Carolina</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Vermont</td>
<td>0</td>
<td>0</td>
<td>Georgia</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>0</td>
<td>0</td>
<td>Florida</td>
<td>16</td>
<td>56</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connecticut</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>East South Central</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle Atlantic</td>
<td></td>
<td></td>
<td>Kentucky</td>
<td>101</td>
<td>84</td>
</tr>
<tr>
<td>New York</td>
<td>393</td>
<td>64</td>
<td>Tennessee</td>
<td>145</td>
<td>156</td>
</tr>
<tr>
<td>New Jersey</td>
<td>0</td>
<td>0</td>
<td>Alabama</td>
<td>59</td>
<td>46</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>18</td>
<td>12</td>
<td>Mississippi</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>West South Central</td>
<td></td>
<td></td>
</tr>
<tr>
<td>East North Central</td>
<td></td>
<td></td>
<td>Arkansas</td>
<td>264</td>
<td>165</td>
</tr>
<tr>
<td>Ohio</td>
<td>95</td>
<td>61</td>
<td>Louisiana</td>
<td>33</td>
<td>58</td>
</tr>
<tr>
<td>Indiana</td>
<td>73</td>
<td>76</td>
<td>Oklahoma</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Illinois</td>
<td>43</td>
<td>79</td>
<td>Texas</td>
<td>406</td>
<td>460</td>
</tr>
<tr>
<td>Michigan</td>
<td>60</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wisconsin</td>
<td>19</td>
<td>18</td>
<td>Mountain</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Montana</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Idaho</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wyoming</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>West North Central</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minnesota</td>
<td>92</td>
<td>104</td>
<td>Colorado</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Iowa</td>
<td>133</td>
<td>290</td>
<td>New Mexico</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>Missouri</td>
<td>193</td>
<td>172</td>
<td>Arizona</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>North Dakota</td>
<td>34</td>
<td>25</td>
<td>Utah</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>South Dakota</td>
<td>48</td>
<td>87</td>
<td>Nevada</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nebraska</td>
<td>37</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kansas</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pacific</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Atlantic</td>
<td></td>
<td></td>
<td>Washington</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Delaware</td>
<td>0</td>
<td>0</td>
<td>Oregon</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Maryland</td>
<td>0</td>
<td>0</td>
<td>California</td>
<td>96</td>
<td>172</td>
</tr>
<tr>
<td>District of Columbia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virginia</td>
<td>177</td>
<td>167</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Totals</td>
<td>2,728</td>
<td>2,604</td>
</tr>
</tbody>
</table>
EPIDIDYMITIS IN RAMS: THE EFFECT OF THE RAM EPIDIDYMITIS ORGANISM (REO) ON THE PREGNANT EWE*


Davis, California

Introduction

Previous work reported from this station (1) (2) (5) (6) has dealt with various ramifications of this disease in the ram.

The high percentage of California rams which are infected (5) and shedding the organism in their ejaculate would seem to pose a potential problem if it were found that R.E.O. is capable of producing abortion, as has been reported from Australia and New Zealand (3) (5).

The purpose of this paper, therefore, is to report the results of infecting ewes at various stages of pregnancy with R.E.O.

Procedure

For this experiment 35 eighteen-month-old first lambing grade Corriedale ewes, with known breeding dates, were arranged in five lots of 7 animals each. Lot I was infected just prior to breeding and Lots II to V were infected at the start of the second, third, fourth and fifth month of gestation, respectively. Infection was accomplished by intra-ocular instillation of approximately one cc. of four-day culture of R.E.O. washed from tryptose blood agar slants and suspended in saline to give a density of McFarland three. Cultures for infecting the ewes were isolated monthly from the semen of the same shedder ram. Prior to infection the ewes, and the rams to which they were bred, were determined to be negative to R.E.O. by the complement-fixation test described previously (1).

During the experimental period, lung, liver, spleen and stomach contents from all aborted fetuses and dead lambs, plus all placentas, were harvested and cultured for R.E.O. In addition, some weak lambs were killed and cultured shortly after birth. Placental cultures were selected from necrotic areas when present, or at random if absent. Placental tissue was seeded in replicate on tryptose agar with five percent defibrinated bovine blood added, and incubated under ten percent CO₂.

Fetal and lamb tissues were handled as above but, additionally, were seeded in ten percent serum broth for 48 to 72 hours as an enrichment procedure, and then plated on blood agar.

All plates were incubated for two weeks before discarded as negative.

From the School of Veterinary Medicine, University of California, Davis.

* This investigation was supported in part by United States Public Health Service. Grant E-3071.

291
Whey was tested for complement-fixing antibodies for a period of four months after lambing. Complement-fixing titers on sera of all surviving lambs were determined at approximately two-week intervals for a five-month period.

Results

The effect of R.E.O. on gestation in ewes inoculated just prior to conception (Group I) is shown in Table I. It can be seen that infection at this time had little effect on the ensuing pregnancy. Evidence that these ewes were successfully infected is demonstrated by a positive complement-fixing test on six out of seven of their sera, as well as by a positive whey test of three of the seven ewes.

Of the ewes in Group V, which were infected at the beginning of the fifth month of gestation (Table I), it can only be concluded that infection did not occur. Only one of seven ewes demonstrated a positive complement-fixing test and none a positive whey test. One dead single, one weak single, and one weak twin birth occurred in this group; but all placental and lamb tissues cultured were negative for R.E.O.

Table II shows the effect on gestation when ewes were infected with R.E.O. at the start of the second, third or fourth month of pregnancy. It is evident that infection established at these periods is considerably more detrimental than if accomplished earlier. It can also be seen that abortion occurred only in the group infected at the start of the second month of pregnancy. Two abortions occurred at 144, and one at 149 days. There was no evidence of decomposition of the aborted lambs which would indicate they died shortly before expulsion. The aborted lambs appeared fully mature, but somewhat smaller than normal, non infected lambs. Lambs born from any of the groups which were alive, but weak, and infected with R.E.O. were also small. Ewes which aborted or which produced weak, infected lambs had small udders and very little milk.

Grossly affected placentas appeared with abortions, with weak, infected lambs, and with apparently normal lambs. Five grossly normal, but R.E.O. positive placentas, were also produced.

The most constant gross changes in the fetal membranes consisted of multiple, discrete, yellow foci of necrosis of the cotyledons, accompanied by variable degrees of hyperemia and pale yellow edema of the chorio-allantois. In more advanced cases, large areas of the chorio-allantoic membrane had developed a leather-like appearance as a result of extensive necrosis and fibrosis.

Within limits, whey titers reflected the severity of infection among groups. Thus we see a higher percentage of positives in Groups II, III and IV, as compared to Groups I and V.

Lamb sera titers similarly followed the infection pattern established in the ewe but with less agreement than the whey titers. As the ewes and lambs from all groups were pastured together following lambing, there was ample opportunity for cross-infection to occur among lambs, or from ewes to lambs.
Conclusions regarding the significance of whey titers and lamb sera titers are beyond the design of this experiment. Additional experimental procedures are under way to elucidate these observations, and particularly to attempt to determine the source of the antibody response in the lamb.

Discussion

The results of this experiment are generally in accordance with those of Hartley et al. (4) and Buddle (3), who described experimental abortion induced in ewes inoculated with their agent at one stage of gestation. The course of the disease, placental pathology, and the time at which abortion occurred are all in agreement.

Additionally, however, the data presented here indicate that the stage of gestation at which infection occurs dictates the success or failure of R.E.O. to produce abortion or diseased lambs. Thus we see that R.E.O. inoculated into ewes just prior to conception did not produce abortion, gross placental pathology, or diseased lambs, even though infection was established in these ewes as evidenced by positive complement-fixing tests.

Furthermore, abortion occurred only in those ewes infected at the start of the second month of gestation. It is interesting to note that the New Zealand workers infected their ewes 21 days after the rams were introduced, at which time most of the ewes had been served once (4).

Even though weak lambs and infected placentas were produced by ewes in Groups III and IV the infection presumably did not have sufficient time to kill the developing fetus. Even though the number of ewes in each treatment group was small, it appears that R.E.O. needs a gravid uterus in which to flourish, and from three to four months of activity within the uterus in order to kill the fetus.

This is further substantiated by the term or near-term occurrence of the abortions.
### TABLE I

*Results of Infecting Ewes With R.E.O. Just Before Conception (Group I) and at the Start of the Start of the Fifth (Group V) Month of Gestation*

<table>
<thead>
<tr>
<th>Group I</th>
<th>Ewe Serum</th>
<th>Lamb</th>
<th>Lamb Tissues</th>
<th>Fetal Membranes</th>
<th>Days Gestation</th>
<th>Whey</th>
<th>Lamb Serum&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2035</td>
<td>+</td>
<td>Normal Twins</td>
<td>-</td>
<td>-</td>
<td>146</td>
<td>+</td>
<td>&gt;13</td>
</tr>
<tr>
<td>2049</td>
<td>+</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>147</td>
<td>+</td>
<td>&gt;10</td>
</tr>
<tr>
<td>2060</td>
<td>+</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>152</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2079</td>
<td>+</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>149</td>
<td>-</td>
<td>&gt;14 &lt;30</td>
</tr>
<tr>
<td>2115</td>
<td>+</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>148</td>
<td>-</td>
<td>&gt;63 &lt;81</td>
</tr>
<tr>
<td>2119</td>
<td>-</td>
<td>Dead Dystocia</td>
<td>-</td>
<td>-</td>
<td>151</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2138</td>
<td>+</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>147</td>
<td>-</td>
<td>&gt;2 &lt;24</td>
</tr>
<tr>
<td>Group V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1299</td>
<td>-</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>160</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2076</td>
<td>-</td>
<td>Weak</td>
<td>-</td>
<td>-</td>
<td>147</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2080</td>
<td>-</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>150</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2081</td>
<td>-</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>146</td>
<td>-</td>
<td>&gt;41 &lt;57</td>
</tr>
<tr>
<td>2101</td>
<td>+</td>
<td>Weak Twins</td>
<td>-</td>
<td>-</td>
<td>157</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2103</td>
<td>-</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>149</td>
<td>-</td>
<td>1:20 at 14 days only</td>
</tr>
<tr>
<td>2137</td>
<td>-</td>
<td>Dead</td>
<td>-</td>
<td>-</td>
<td>158</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>1</sup> Figures indicate age in days at which lamb demonstrated last positive and first negative C.F. test.
### TABLE II

Results of Injecting Ewes With R.E.O. at the Start of the Second (Group II), Third (Group III) and Fourth (Group IV) Month of Gestation

<table>
<thead>
<tr>
<th>Group II</th>
<th>Ewe</th>
<th>Ewe Serum</th>
<th>Lamb</th>
<th>Lamb Tissues</th>
<th>Lamb Gross Lesions</th>
<th>Lamb Culture</th>
<th>Days Gestation</th>
<th>Whey</th>
<th>Lamb Serum1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1119</td>
<td>+</td>
<td>Normal</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>150</td>
<td>+</td>
<td>&gt;96 &lt;150</td>
<td></td>
</tr>
<tr>
<td>1186</td>
<td>+</td>
<td>Weak/Killed</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>147</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1298</td>
<td>+</td>
<td>Normal*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>161</td>
<td>A. C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>+</td>
<td>Normal</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>151</td>
<td>—</td>
<td>&gt;51 &lt; 67</td>
<td></td>
</tr>
<tr>
<td>2086</td>
<td>+</td>
<td>Dead Twins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>149</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2118</td>
<td>+</td>
<td>Dead Twins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>144</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2143</td>
<td>+</td>
<td>Dead</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>144</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>1123</td>
<td>+</td>
<td>Normal</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>144</td>
<td>+</td>
<td>&gt;60 &lt;140</td>
</tr>
<tr>
<td>1300</td>
<td>+</td>
<td>Weak Twins 1 Killed</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>149</td>
<td>+</td>
<td>&gt;31 &lt; 46</td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>+</td>
<td>Weak</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>148</td>
<td>+</td>
<td>&gt;97 &lt;149</td>
<td></td>
</tr>
<tr>
<td>2052</td>
<td>+</td>
<td>Weak/Killed at age 30 days</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>144</td>
<td>+</td>
<td>&gt;30</td>
<td></td>
</tr>
<tr>
<td>2095</td>
<td>+</td>
<td>Normal</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>151</td>
<td>—</td>
<td>&gt;90 &lt;142</td>
<td></td>
</tr>
<tr>
<td>2133</td>
<td>+</td>
<td>Normal†</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>150</td>
<td>+</td>
<td>&gt;87 &lt;138</td>
<td></td>
</tr>
<tr>
<td>2147</td>
<td>+</td>
<td>Normal†</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>149</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>2016</td>
<td>+</td>
<td>Normal</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>146</td>
<td>+</td>
<td>&gt;92 &lt;161</td>
</tr>
<tr>
<td>2054</td>
<td>+</td>
<td>Normal Twins 1 Killed</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>147</td>
<td>+</td>
<td>&gt;14</td>
<td></td>
</tr>
<tr>
<td>2073</td>
<td>+</td>
<td>Weak Twins Killed</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>136</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2091</td>
<td>+</td>
<td>Normal</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>148</td>
<td>+</td>
<td>&gt;41 &lt; 57</td>
<td></td>
</tr>
<tr>
<td>2120</td>
<td>+</td>
<td>Normal</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>149</td>
<td>+</td>
<td>&gt;87 &lt;140</td>
<td></td>
</tr>
<tr>
<td>2123</td>
<td>+</td>
<td>Weak</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>150</td>
<td>+</td>
<td>&gt;62 &lt; 75</td>
<td></td>
</tr>
<tr>
<td>2140</td>
<td>+</td>
<td>Normal</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>150</td>
<td>+</td>
<td>&gt;87 &lt;140</td>
<td></td>
</tr>
</tbody>
</table>

1 Figures indicate age in days at which lamb demonstrated last positive and first negative C.F. test.

* Died at 1 day of age. Gut contents and all tissues positive for *Escherichia coli*.

† Died at 1 day of age. Gut contents positive for *Clostridium perfringens* type D toxin.
REFERENCES


REPORT OF THE COMMITTEE ON DISEASES OF SHEEP AND GOATS


Your Committee has given special consideration to scrapie and, with your indulgence, we desire to comment thereon as follows:

Some criticism has been registered with the Committee that the Association has not followed through with the Committee's recommendation presented at the 1959 meeting, which was re-emphasized in the 1960 report, that the Association designate a committee to review the history of the scrapie eradication program through 1959 and make definite proposals as to possible modifications of the program.

This Committee finds and accepts the fact that in 1959 the President of the Association did not appoint a scrapie committee as recommended for several reasons; principally that such an effort in his opinion would result in a duplication of the efforts of the Secretary of Agriculture's Committee appointed in 1958; that funds were not available for such a study, and research proposed and under way might throw new light on the problem, which would then justify further evaluation of the program at a later time. This Committee has studied the progress of both scrapie eradication and research efforts and, in this report, we are recording the information obtained which we regard as being impressive and encouraging. The Committee recommends that the responsible officials in the Agricultural Research Service of the United States Department of Agriculture continually evaluate both research findings and progress of the program in this country, Canada, Britain, and elsewhere and make recommendations as they deem appropriate in light of additional knowledge; further that they keep the Committee informed of new pertinent information as it becomes available.

The Committee feels that it is most unfortunate that there is some difference of opinion in the industry regarding some features of the program as followed in this country. We urge officials of the Agricultural Research Service and the industry people opposing the program to give this situation their serious consideration and endeavor to come to some common understanding.

The Committee commends the Agricultural Research Service for arranging the allotment of $299,432 to Compton Station and the Moredun Laboratory for research on scrapie. We urge that progress of the studies be watched carefully and that additional funds be allotted as necessary to avoid any interruption of the projects.
Scabies—Your Committee commends the Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture on the progressive steps they have taken to institute State-Federal eradication programs in states where psoroptic scabies is known to exist. We believe, however, that the amount of Federal funds allotted to this program is inadequate to eradicate the disease in time to receive maximum benefits. We therefore urge the Secretary of Agriculture to request an increase in these funds to approximately $1,450,000 during each of the fiscal years 1963, 1964, and 1965. All states, where scabies is known to exist, are urged to enter into the cooperative eradication program with the United States Department of Agriculture.

Your Committee requests that the Association recommend to each of the states, the Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture that regulations be adopted on or before January 1, 1963, prohibiting shipment of sheep from any State where sheep scabies is known to exist into any other State, except for immediate slaughter, until the State where sheep scabies is known to exist has been designated by the Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture as a Sheep Scabies Eradication Area.

The Committee further requests that the Secretary of the United States Livestock Sanitary Association furnish copies of this report to the Secretary, Director, or Commissioner of Agriculture of each of the 50 states, the Secretary of the United States Department of Agriculture, and the Secretary of the National Wool Growers Association.

This year we have endeavored to have certain authorities present situation reports on some new and some old diseases and programs. A special effort has also been made to review and catalogue research programs now under way. Our reports are as follows:

**ARTIFICIAL INSEMINATION**

Dr. Gordon Shultz, sheep disease specialist, California Department of Agriculture, reports that during the past summer two large California flocks of ewes, involving about 1,750 animals, were artificially inseminated by two California practicing veterinarians. The semen used was from both Hampshire and Suffolk rams supplied by a breeders’ association in Logan, Utah. Results of these first attempts of large-scale artificial insemination of ewes in California will not be known until after the lambing period is over. The Committee recommends that Agricultural Research Service officials take cognizance of this development as to any possibility it may have on the transmission of diseases, especially scrapie.

**FOOT ROT**

This Committee recommends that the Association recognize and support the activities of the National Foot Rot Committee and reaffirms a 1959 recommendation suggesting that State regulatory officials consider quarantine
of flocks infected with contagious ovine foot rot and further consider official supervision of treatment. Further, the Committee requests that the Animal Disease Eradication Division of the United States Department of Agriculture prepare and distribute to sheep growers and other interested parties an informative circular on the recognition, treatment, and control of infectious ovine foot rot.

REGULATORY CONTROL OF FOOT ROT

In the past, foot rot in sheep was a particularly costly disease in range band sheep in Montana. As a result of an intensive program of quarantining, treatment (as prescribed in Newsom’s Sheep Diseases by Hadleigh Marsh) and then reinspection and release of quarantine if found free of infection, the range sheep operation in this State has remained remarkably free of foot rot for the past 15 or 20 years.

The inspection of sheep for the detection of foot rot consists of “setting up” every sheep in the flock and carefully inspecting each foot in detail, trimming the foot if necessary, to detect evidence of infection. It is a lot of work, but we find it pays off in making sure that no infection is left in the band.

The foot rot problem for the past five years in Montana has been one of farm flocks. The same procedure is applied to farm flocks as just mentioned. About six to eight flocks have been found infected each year with foot rot the last six years. It is more difficult to handle farm flocks because of lack of owner concern of the infection in a small band. We know the application of strict quarantine measures on the farm flock has played a most important part in preventing a wide dissemination of the disease and its attendant economical loss. (Report submitted by John W. Safford, D.V.M., State Veterinarian, Helena, Montana.)

REGULATORY CONTROL OF LICE

Lice were not reported in sheep in Montana until 1948. From 1948 to 1958 infestations were reported with continual increasing frequency. In 1958 the problem caused considerable concern to producers, buyers, and feeders. As a result, the Livestock Sanitary Board consulting with the Montana Woolgrowers adopted a quarantine-dipping regulation June 1, 1959. The first year this regulation was in effect, about 21 flocks and 5,000 sheep were quarantined and dipped. This past year approximately 12 flocks and 100 sheep have been so treated. All lice identified to date have been Bovicola ovis.

We have no program of area inspection of flocks of sheep for lice infestation. The program consists of quarantining sheep found infested on health inspection, auction market inspection, show and sale inspection, and other incidental inspections that may be made on sheep, and then following up to locate the flocks of origin of infested sheep. The Livestock Sanitary Board hoped that the knowledge that lice infestation was a quarantinable condition would tend to stimulate sheep owners to be more alert to this problem and do something about it on their own. So far it seems to be working quite well.
We are hopeful that the expense of flock-by-flock inspection on an area basis will not be necessary.

We have found that lice can be most difficult to find on sheep, particularly sheep not showing extremely advanced infestation. Sheep might have a quite heavy infestation during the winter. By late spring, lice are most difficult to find on the very same untreated sheep. More information is needed on the life cycle of sheep lice, particularly under different seasonal conditions.

The main complaints received on lice-infested sheep were from feeders because lice-infested sheep failed to gain. A few complaints were also received on loss of wool. Buyer discrimination against lice-infested sheep possibly was the most influential thing in getting the wool growers to request the regulation be enacted. (Report submitted by John W. Safford, D.V.M., State Veterinarian, Helena, Montana.)

**Bluetongue**

During 1960, bluetongue in sheep was reported from eight states; namely—Arizona, California, Missouri, Nebraska, Nevada, New Mexico, Texas, and Utah. Bluetongue virus was isolated from sheep in each of the states prior to 1960. It is logical to assume, on the basis of the history of the disease, that many cases occurred which were not officially reported. The virus was isolated from a Jersey calf eight months of age, in Idaho, which was not in contact with sheep, and from each of three calves in Utah which were being grazed on the same pasture with proven bluetongue-infected sheep.

The Animal Disease and Parasite Research Division and the Entomology Research Division, Agricultural Research Service, United States Department of Agriculture, are cooperating in extensive and controlled investigations on the transmission of bluetongue virus to sheep by vectors.

Investigations on bluetongue are being made to determine reservoirs of infection, other hosts, the viremia period in animals, procedures for improving methods for laboratory diagnosis, screening virus isolates to determine whether other antigenic types exist in this country, and studies on immunity and duration of immunity in artificially infected animals. (Report submitted by Bryce R. McCrory, D.V.M., Director Animal Disease Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Denver, Colorado.)

**Selenium in White Muscle Disease**

The use of selenium has proved highly effective in the prevention and treatment of white muscle disease (WMD). In original trials 0.1 ppm Se (dry weight basis) was added to the ration of the pregnant ewes, but this method of administration, as well as its use in mineral supplements, has been discouraged by the Food and Drug Administration since selenium is classified as a carcinogen. The Food and Drug Administration has approved injectable solutions containing selenium for use in sheep, and these have been successfully used in thousands of lambs. Experimental results indicate that treatment
of pregnant ewes will forestall congenital muscle degeneration in lambs. There is some evidence that, in the absence of clinical W.M.D., body weight gains may be improved with selenium administration. (Report submitted by O. H. Muth, D.V.M., Oregon State University, Corvallis, Oregon.)

SCRAPIE ERADICATION ACTIVITIES

(A Situation Report)

During the past fiscal year there were nine scrapie-infected flocks reported in six states. The infected flocks, source flocks, exposed sheep and their immediate progeny slaughtered as a result of the nine scrapie outbreaks, involved the payment of Federal indemnity on 156 claims for 2,373 sheep slaughtered from 21 states.

Illinois—Three of the infected flocks were in Illinois in Livingston, McLean, and Macaupin counties and involved one Cheviot and two Suffolk flocks. The infected Cheviot ewe was from the same Cheviot flock in Illinois believed responsible for a previous Columbia County, Oregon, outbreak. The source flock in Illinois is under quarantine pending slaughter. The infected Suffolk ewe in the Livingston County outbreak would have been slaughtered previously as an exposed animal had her existence been known. The infected Suffolk ewe in the Macaupin flock was from a flock from which exposed sheep had previously been removed for slaughter.

Virginia—Scrapie was diagnosed in three Suffolk ewes in a Virginia flock. A previous flock on the same Augusta County farm had been considered a source flock and, as such, was slaughtered in 1956.

Pennsylvania—The Pennsylvania outbreak was found when the infected Suffolk ram was being appraised for slaughter as an exposed sheep moved from a source flock in the State.

South Dakota—The infected ram in the South Dakota flock was reported by a veterinary practitioner. Regulatory veterinarians were searching for the ram as Canadian officials had advised he had been exported from a Canadian flock later found to be infected.

Idaho—Canadian officials had diagnosed scrapie in a British Columbia flock and Idaho veterinarians were endeavoring to locate the exposed sheep imported from the Canadian flock when they found one of the imported Suffolk ewes showing symptoms of the disease. The second outbreak in Idaho was found when a Caribou County owner advised State officials that an imported Suffolk ewe was showing symptoms of scrapie.

Missouri—The Clay County outbreak was reported by the owner. The infected Suffolk ewe was bred and born on his farm. Both her sire and dam were from Canadian flocks found to have scrapie in Alberta in 1959.
Exposed sheep purchased by the Clay County owner had been slaughtered as were their immediate progeny. The sheep directly concerned in the Missouri outbreak involved purchases prior to the established exposure dates.

Three of the nine outbreaks reported during Fiscal Year 1961 were disclosed during tracing and inspection procedures, three were reported by veterinary practitioners, and three were reported by the owners.

In this country 105 infected flocks have been reported in 87 counties in the following 26 states: Alabama (2), California (8), Connecticut (2), Georgia (1), Illinois (21), Indiana (23), Iowa (1), Kentucky (1), Maryland (2), Michigan (2), Mississippi (1), Missouri (2), New York (3), North Carolina (2), Ohio (12), Oregon (4), Tennessee (2), Texas (1), Utah (1), Virginia (3), West Virginia (1), Wisconsin (2), Wyoming (2), Pennsylvania (2), South Dakota (1), and Idaho (3). The first report of scrapie was from Michigan in 1947. No additional outbreaks were reported until Fiscal Year 1953 when 10 were disclosed. There were three in 1954; 11 in 1955; 23 in 1956; 12 in 1957; seven in 1958; 11 in 1959; 13 in 1960; nine in 1961; and five to date in Fiscal Year 1962. Five of the infected flocks were of the Cheviot breed and the remainder Suffolk. (Report submitted by J. L. Hourrigan, D.V.M., Chief Staff Officer, Special Diseases Eradication, Agricultural Research Service, Animal Disease Eradication Division, United States Department of Agriculture.)

SCRAPIE RESEARCH DEVELOPMENTS
(A Situation Report)

There have been a number of research developments and research papers published recently. These are not only of immediate interest to your Association, but reflect favorably upon the increased interest being shown by both veterinary and medical researchers.

Noteworthy are the P. L. 480 Grants, by the United States Department of Agriculture, for expanding scrapie research in England and Scotland. Grants under P. L. 480 (Agricultural Trade Development and Assistance Act of 1954, as amended), have been extended to the Agricultural Research Council, London, England, and to the Animal Disease Research Association, Moredun Research Institute, Edinburgh, Scotland. The two grants total 300,165 U.S. dollars.

Dr. W. S. Gordon is heading the work in England which will be done at the Agricultural Research Council Field Station at Compton, Berkshire. Dr. J. T. Stamp is in charge of the work at Moredun Institute in Scotland. Both have had considerable experience in scrapie research and are considered world authorities on the disease. Many of you were in attendance when they appeared before this body in 1959, and discussed scrapie research.

The objectives of the accelerated research program will be to characterize more precisely the nature of the transmissible agent of scrapie, and to establish (a) the pathology; (b) the natural method of spread; (c) the immunology; (d) the methods of diagnosis; and (e) the methods of treat-
ment, prevention, control or eradication of the disease, and to investigate the causative factors concerned in scrapie.

Additional American contributions in the field of scrapie research include that of the National Foundation for Neuromuscular Diseases supporting studies in England by Dr. Herbert B. Parry at the University of Oxford. This reflects the interest of medical investigators who hope knowledge obtained through research on scrapie may provide clues to causes and eventual cures for some diseases of man.


Recent information developed through research, which your Committee believes will be of interest to the Association, includes the following:

*Transmission to Mice*—A degenerative disorder of the central nervous system was observed in mice inoculated with scrapie brain material passaged through goats. The disease occurred in one of three breeds of mice inoculated. Other points of similarity to scrapie were the long incubation period, the clinical symptoms seen in the mice, and the histological lesions seen in the brains of the affected mice. This work may represent an important breakthrough in scrapie research. It was done at Compton, England.

*Work on Diagnostic Tests*—Attempts were made at Compton to demonstrate antibodies in scrapie disease by using the tanned erythrocyte haemagglutination, Middlebrook Dubos, Boyden-Anderson, passive anaphylaxis (systemic), passive anaphylaxis (skin), complement-fixation, conglutinin titres, Wasserman, Kahn, and haemagglutination tests. It was concluded that there were no significant differences between scrapie and normal sera tested. Also at Compton, the haematological and biochemical tests used did not demonstrate any significant difference between the blood of chemically healthy and scrapie-affected goats. Similarly biochemical tests used showed no significant differences between cerebrospinal fluid of healthy and affected goats. Workers in Canada report that serum proteins from healthy and scrapie-infected sheep determined by chemical analysis and by paper electrophoresis show no significant differences between the groups. They also reported that scrapie occurred in experimentally affected animals irrespective of potassium or hemoglobin type.

*Work with Goats at Compton*—Further work with goats is in progress. A fifth intracerebral passage of scrapie in six goats, together with the earlier passages, produced the disease in all of 56 animals inoculated. Comparison of the intracerebral and subcutaneous routes of inoculation showed a significantly longer incubation period for the latter, in one case up to 47 months.
Cerebrospinal fluid, pituitary gland, adrenal gland, spleen, pancreas, and liver from a scrapie-affected goat is capable of producing scrapie when inoculated intracerebrally into goats. A similar thyroid gland suspension failed to produce the disease over the same period of time. A normal goat inoculated with brain tissue from a normal goat developed scrapie. Two uninoculated control goats were housed together with inoculated animals. One of the control goats developed scrapie. Twenty-eight uninoculated goats housed in a scrapie environment over periods varying from 24 to 34 months had, when the paper was published, shown no evidence of scrapie. [These animals were still negative at 47 months.] During an observation period of 29 months, scrapie did not occur in goats inoculated intracerebrally with saliva, milk, urine, or feces from a scrapie-affected goat. The author emphasized that this observation requires further examination before it can be concluded that the scrapie agent is absent from these secretions and excretions. Multiple subcutaneous inoculations in goats produced scrapie in a significantly shorter period than a single subcutaneous inoculation, but no shorter than that produced by a single intracerebral inoculation.

Scrapie Agent Possibly Becoming Altered or Adapted by Passage Through Goats—At Compton it has been found possible to obtain, by intracerebral goat passage, scrapie brain material that will produce the nervous syndrome in almost all goats inoculated, and to obtain other material that will produce the scratching syndrome in almost all goats inoculated. The authors suggest that certain "strains" of the agent will produce the nervous syndrome, while others will produce the scratching syndrome. Passage in goats may enhance the effect of the agent in this species and decrease its effect in sheep.

Transmission by Mouth Demonstrated at Compton—Both of two goats fed 100 ml. of a 10 percent emulsion of infected goat brain developed scrapie 15 and 21 months after dosing. Each of 50 sheep was drenched with 100 ml. of a 10 percent emulsion of infected sheep brain and each of another group of 46 sheep similarly received brain tissue from healthy sheep. At the time the authors wrote their paper (11 months after dosing) seven cases of scrapie had occurred in the sheep given the infected brain material. The incubation period was 5½ to 10½ months, and the infected sheep represented the Dalesbred, Derbyshire Gritstone, South Scotland Cheviot, Swaledale, and Wiltshire Horn breeds. [We understand that additional cases occurred later.] No cases had occurred in the sheep given brain tissue from normal sheep.

Age of Scrapie Manifestation—In a British study of 526 cases, the age of manifestation varied from 1¾ to 11 years in ewes and 1½ to 5½ years in rams, the mean age being three years in males and 3½ years in females; 87.6 percent of the females and 97 percent of the males manifested the disease by 4½ years of age. This work was done by Dr. H. B. Parry.

Additional Histopathological Studies—The most significant lesion in the spinal cord in scrapie was vacuolation and degeneration of neurons, whereas
vacuolated neurons were either absent or extremely rare in the spinal cords of healthy sheep. This study was made at Moredun Institute.

Heat Resistance of Scrapie—Additional recent work at Moredun Institute confirmed previous work that scrapie brain material would reproduce the disease, following either intracerebral or subcutaneous inoculation, after being boiled for 30 minutes.

When infected brain material was autoclaved at 20 lb. p.s.i. (126.5° C.) for 30 minutes it failed to reproduce the disease when inoculated intracerebrally into sheep.

Unboiled normal brain failed to reproduce scrapie; whereas unboiled scrapie brain, boiled scrapie brain, boiled scrapie brain plus boiled normal brain, and boiled scrapie brain plus unboiled normal brain did.

Additional Attempts to Characterize the Scrapie Agent—The results of inoculation with material frozen and thawed 12 times, and frozen and thawed 34 times, failed to demonstrate adverse effect on the agent.

An ether extract of goat brain was capable of reproducing the disease while an acetone extract of the same brain failed to produce scrapie in goats observed for two years.

Treatment of scrapie goat brain with ribonuclease or deoxyribonuclease did not reduce its ability to produce scrapie.

The agent was not inactivated by the presence of ether at 32° C. to 36° C. for 10½ hours or by water at 94° to 98° C. for 24 hours. The agent was also present in the whole brain, ether extract, residue from ether extraction, water extract, and residue from water extraction.

The addition of adjuvants to scrapie sheep spleen and nodes prior to injection did not appear to influence the process. The scrapie agent appeared to be self-replicating when boiled between each passage during serial transmission. This was shown to be the case through two passages and the work is being continued.

Results of research work give good reason to believe that experimental allergic encephalitis and scrapie are quite distinct entities. (Report submitted by J. L. Hourrigan, D.V.M., Chief Staff Officer, Special Diseases Eradication, Agricultural Research Service, Animal Disease Eradication Division, United States Department of Agriculture.)

FOOT ROT CONFERENCE AT LEXINGTON, KENTUCKY

May 10 and 11, 1961

At the 1959 meeting of the United States Livestock Sanitary Association the Committee of Transmissible Diseases of Sheep and Goats emphasized the need for action in attacking the problem of foot rot in sheep, and the Association passed a resolution recommending that foot rot be recognized as a reportable disease and that infected flocks be quarantined and treated under the supervision of the Livestock Sanitary Authority.
Because of the prevalence of foot rot in some of the central and eastern states and its serious effect on the sheep industry in those states, the Extension Service of the University of Kentucky, with the cooperation of the Kentucky Department of Agriculture and the Blue Grass State Sheep Association, and under the leadership of Richard C. Miller, Extension Sheep Specialist, organized a meeting to discuss the problem. This meeting was named the "First National Foot Rot Conference" and was held at Lexington, Kentucky, on May 10 and 11, 1961.

Twenty-three sheep-producing states were represented in the attendance at this meeting. The 200 people present included sheep producers, operators of livestock markets; and representatives of wool growers organizations; sheep specialists in research, teaching, and extension; research veterinarians; and representatives of disease control administration at Federal, State, and local levels.

A survey of the foot rot situation in the United States by areas was presented by Dr. E. E. Saulmon, Assistant Director of the Animal Disease Eradication Division of the U.S.D.A., and State representatives in attendance supplemented this talk by reporting on their local situations. Doctor Saulmon stated that 10 states reported that from 2 to 10 per cent of their flocks were infected, nine states reported foot rot in 10 to 20 percent of the flocks, and eight states reported flock infection above 20 percent. There were 23 states "in which foot rot was not considered to be of economic significance."

Dr. Blaine McGowan, of the University of California and Dr. H. Marsh, of the Montana Veterinary Research Laboratory, discussed in some detail the pathology, bacteriology, differential diagnosis, epizootiology, and treatment of the disease. Discussion of these talks was led by Extension Livestock Specialists, Graydon Blank of Michigan and T. R. Greathouse of Illinois.

Doctor McGowan and Dr. J. O. Heishman of West Virginia University demonstrated the proper method of preparing affected feet for treatment. This demonstration included the use of an Australian type of crate or cradle for restraining sheep for treatment.

At the final session there was a panel discussion of proposals for action towards accomplishing control or eradication of foot rot. While one State has for some years quarantined known infected flocks and treated them under State supervision, the general consensus was that the immediate need is for education of producers and veterinarians as to the true nature of the disease and the methods of control, and stimulation of the industry to start action on foot rot, before attempting to put regulatory eradication measures in effect.

In order to continue the effort initiated at this Conference, an Organizational Committee was appointed, consisting of H. Marsh, Blaine McGowan, R. C. Miller, J. O. Heishman, and L. K. Bear, who is Assistant Secretary of the Ohio Wool Growers Cooperative Association. This Committee was instructed to select additional members from the sheep industry, representing various sections of the country. (Submitted by Hadleigh Marsh, D.V.M., Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, Montana.)
OVINE VIBRIONIC ABORTION

(A Situation Report)

The Western Regional Technical Committee on vibriosis in sheep, composed of representatives of the National Wool Growers Association and research workers from the states of Arizona, California, Colorado, Idaho, Montana, Utah, Washington, Wyoming, and the U.S.D.A. has been conducting a coordinated research program on this disease since 1952. The pertinent findings of this group are:

1. Natural exposure is accomplished by ingestion of organisms from infected placentae, fetuses or fluids.

2. Abortion will occur much more readily if exposure is accomplished during the last six weeks of gestation. Abortion will rarely occur if ewes are infected during the first six weeks of gestation.

3. Natural infection and abortion confers an immunity lasting up to three years.

4. Efforts to incriminate the ram as a source of infection at the time of coitus have failed.

5. Evidence is accumulating which indicates some naturally aborting ewes become carriers by retaining infection in the gall bladder.

6. Attempts to artificially immunize ewes against this disease have met with some success. At present there is a *vibrio fetus* bacterin on the market under an experimental license. Research efforts directed towards improving this vaccine will continue. Evaluation of the efficacy of this vaccine under field conditions will take several years. (Report submitted by Blaine McGowan, Jr., D.V.M., Associate Professor of Veterinary Medicine, School of Veterinary Medicine, University of California, Davis, California.)

SHEEP AND GOAT DISEASE RESEARCH

A Summary* of Research in United States and Canada

In the summer of 1961, the experiment stations and Schools of Veterinary Medicine in the 50 States and Canada were asked to provide a list of research projects concerned with diseases of sheep and goats. With very few exceptions, this information was most courteously supplied. Also, the Committee was given a complete file of projects approved and financed by the Agricultural Research Service, United States Department of Agriculture.

A review of the projects indicated a total of 24 diseases are under investigation involving 88 projects, with the major research emphasis placed

* A complete listing may be obtained from the secretary, United States Livestock Sanitary Association. (Report submitted by Blaine McGowan, Jr., D.V.M., Associate Professor of Veterinary Medicine, University of California, Davis, California.)
on parasitic diseases (20 projects). Vibrionic abortion and bloat (nine projects each) received the next most frequent attention.

No attempt will be made to evaluate the breadth and scope of the research being conducted but, by merely noting the number of projects per disease in relation to the economic impact of the disease, some observations can be made.

It would appear that adequate research effort is being expended upon some major diseases, such as scrapie, parasitic diseases, viral and vibrionic abortion, bloat, bluetongue, white muscle disease, and even some of the minor diseases which have only one or two projects. However, there are some obvious deficiencies in the amount of research being conducted on several important diseases, such as foot rot, pneumonias, peri-natal diseases, young lamb mortality, caseous lymphadenitis, and coccidiosis. These latter diseases encompass a very large portion of the annual waste and loss to the sheep and goat industries, and some of them, for instance foot rot, could be brought more forcefully to the attention of this organization in the future.

It is recommended, therefore, that each member of this Committee, specifically the organization membership in general, solicit local, regional, and national research agencies, plus local and national industry organizations to initiate and support critical research upon these important diseases.
FURTHER STUDIES ON SALMONELLA IN HUMAN AND ANIMAL FOODS AND IN THE ENVIRONMENT OF PROCESSING PLANTS*

EMMETT B. SHOTTS, JR., M.S.; WILLIAM T. MARTIN, B.S.; MILDRED M. GALTON, Sc.M.

Atlanta, Georgia

Salmonellae have been isolated from many varied sources of public health importance (1-5). Of primary concern are certain human and animal food products, environments of the plants where these foods are prepared, animal by-products from which the food is prepared, and animal feeds.

A high percentage of fresh pork sausage samples were found to contain salmonellae by Galton, et al. (6), and Richardson, et al. (7). In the latter report, 106 sausage samples were obtained from local retail markets and salmonellae were isolated from 40 (38 percent) (7). It was noted that the product of one local producer was consistently positive. After learning of the obvious heavy contamination in their product, this firm agreed for the Veterinary Public Health Laboratory Unit of the Communicable Disease Center to study the environment of its plants and of the hogs being processed to determine the prevalence of salmonellae and to offer any suggestions to reduce the apparent high level of these organisms. Concurrently, studies were initiated in cooperation with Dr. James E. Scatterday, Florida State Board of Health, to determine the level of salmonellae contamination in six rendering plants in that State, both during the processing and in the finished products. In addition, various feed ingredients and finished poultry feeds obtained from a private laboratory were examined for the presence of salmonellae.

In effect, this report is the summary of these studies presented to further confirm the widespread distribution of salmonellae in human and animal foods.

MATERIAL AND METHODS

The slaughter and processing operation is unique in that the slaughter facility is approximately 40 miles from the processing unit. The maximum number of hogs which can be killed is 35 per day. The physical facility of the slaughter plant is crowded and offers many opportunities for recontamination of the finished carcass. The overhead rail which carries the dressed carcass to the cooler is located within ten feet of the dehairing and scraping operation. A metal shield was constructed but a four foot space between this shield and the floor still permits some splashing from the dehairing machine onto the cleaned carcasses. After overnight chilling at the slaughter plant,

* From the Department of Health, Education, and Welfare, Public Health Service, C.D.C., Epidemiology Branch, Veterinary Public Health Laboratory Unit, Atlanta, Georgia.

309
the split carcasses are stacked into an enclosed truck and transported to the processing facility. This plant produces only fresh pork sausage, frankfurters and bologna.

The hogs cultured were purchased from one supplier in Kentucky. They were primarily old sows and came from small farms throughout Kentucky, Ohio and Indiana. The animals were not placed in contact with each other until they were collected some 24 to 48 hours prior to shipment.

Through the cooperation of Dr. Joe Skaggs, Kentucky State Health Department, rectal swabs were taken just prior to loading the animals on a truck in Kentucky for transport to Atlanta. A second swab was taken upon their arrival in Atlanta, a third at slaughter and a fourth just after the carcass was dehaired. Sterile dry cotton swabs were used also to culture various sites on the carcasses and in the environments of both the slaughter and processing facilities. The use of 165°F. water to wash the outside of carcasses both before and immediately after evisceration was tried in place of the usual cold water rinse.

The procedures employed in the isolation of salmonellae by direct culture with swabs in this study are essentially those reported by Galton, et al. (8), in similar studies in Florida.

Rendering plant samples were collected at various sites in the plant during processing and from the finished products by local county health department sanitarians in Florida. These sites included the cooker, the grinder, holding bins and finished products ready for shipment. The final use of these products was for protein supplements in feeds. Samples were collected in one-half pint ice cream cartons as described by Galton (9), and mailed to the Veterinary Public Health Laboratory for examination. Approximately one week’s time elapsed between the collection of the sample and its examination in the laboratory.

The procedures used for the isolation of salmonellae from feed ingredients and feeds in this investigation parallel those of Galton, et al. (10), except that they were not done in duplicate.

Triple sugar iron agar slants (TSI) that showed reactions characteristic of Salmonella were tested with polyvalent Salmonella antiserum by slide agglutination. Cultures found to be positive were then grouped with Salmonella “O” serums (A-E₃) representing the major groups. The Spicer-Edwards pooled “H” serums were used to screen for flagella antigens before representative cultures were forwarded to the Enteric Bacteriology Unit, Communicable Disease Center, for final typing.

**RESULTS**

**Equipment and Environment of Slaughter and Processing Plants.**

Samples from the environment of the slaughter facilities were taken on 14 occasions from April 6, 1960, to June 23, 1961. The 17 sites most frequently examined are presented in Table 1. As would be expected, salmonellae were
FURTHER STUDIES ON SALMONELLA

TABLE 1
Salmonella Isolations From Equipment and Environment of Slaughter Plant
April 6, 1960—June 23, 1961

<table>
<thead>
<tr>
<th>Site</th>
<th>Swab Cultures</th>
<th>Positive Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unloading Chute</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Mud in holding lot</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Water trough</td>
<td>7</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>Ramp to kill room</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Kill room door</td>
<td>4</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Scald vat</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*D.H.M. ramp</td>
<td>11</td>
<td>5</td>
<td>46</td>
</tr>
<tr>
<td>Walkway D.H.M.</td>
<td>11</td>
<td>10</td>
<td>91</td>
</tr>
<tr>
<td>Top D.H.M.</td>
<td>11</td>
<td>6</td>
<td>55</td>
</tr>
<tr>
<td>D.H.M. Cradle</td>
<td>14</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>D.H.M. paddle</td>
<td>14</td>
<td>6</td>
<td>43</td>
</tr>
<tr>
<td>Scraping table</td>
<td>13</td>
<td>8</td>
<td>62</td>
</tr>
<tr>
<td>Chute from D.H.M.</td>
<td>12</td>
<td>7</td>
<td>58</td>
</tr>
<tr>
<td>Power saw</td>
<td>14</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>Hand saw</td>
<td>14</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td>Edible viscera pan</td>
<td>14</td>
<td>12</td>
<td>86</td>
</tr>
<tr>
<td>Knife sterilizer</td>
<td>13</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>176</td>
<td>95</td>
<td>54</td>
</tr>
</tbody>
</table>

* Dehairing machine.

not recovered from the scald vat. With this exception, the percent of swab samples positive for salmonellae ranged from 25 to 100 percent. At no time during these surveys was the environment of the plant found to be completely free of salmonellae. More than half of the 176 swab cultures (54 percent) were positive. On four occasions, samples were collected before the daily operations began and again after slaughter and dressing was completed. Fifty-six swab samples were taken before operations and 19 (34 percent) were positive. These findings indicate inadequate cleaning which allowed carry over of contamination with salmonellae from the previous day. After operations were completed but before cleanup, salmonellae were found in 28 (58 percent) of 48 swab samples taken.

During the period March 28, 1960, to June 30, 1961, 15 surveys were made in the environment of the processing plant. The findings from the 15 most frequently examined sites are presented in Table 2. The average frequency of isolation of salmonellae from a specific point was never lower than 40 percent. Salmonellae were isolated from four of five samples from the floor of the trucks used to transport carcasses from the slaughter plant to the processing plant. Surveys were made before and after the processing operations on three occasions. During one of these surveys, the recovery rate was zero at the beginning of the day's operation and 92 percent after completion of operations.
TABLE 2

Salmonella Isolations From Equipment and Environment of a Processing Plant
March 28, 1960—June 30, 1961

<table>
<thead>
<tr>
<th>Site</th>
<th>Swab Cultures Examined</th>
<th>Number Positive</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Truck floor</td>
<td>5</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>Cooler floor</td>
<td>5</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>Meat cleaver</td>
<td>15</td>
<td>13</td>
<td>87</td>
</tr>
<tr>
<td>Hand saw</td>
<td>11</td>
<td>5</td>
<td>46</td>
</tr>
<tr>
<td>Cutting knife</td>
<td>9</td>
<td>5</td>
<td>56</td>
</tr>
<tr>
<td>Cutting table surface</td>
<td>15</td>
<td>11</td>
<td>73</td>
</tr>
<tr>
<td>Skinning machine</td>
<td>9</td>
<td>7</td>
<td>78</td>
</tr>
<tr>
<td>Stainless steel cart</td>
<td>5</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>*S. grinder inlet</td>
<td>13</td>
<td>9</td>
<td>69</td>
</tr>
<tr>
<td>S. grinder auger</td>
<td>2</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>S. grinder outlet</td>
<td>15</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td>Stainless steel shovel</td>
<td>14</td>
<td>9</td>
<td>64</td>
</tr>
<tr>
<td>S. mixer</td>
<td>14</td>
<td>8</td>
<td>57</td>
</tr>
<tr>
<td>Packing table</td>
<td>5</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>Stainless steel table</td>
<td>11</td>
<td>5</td>
<td>46</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>95</td>
<td>64</td>
</tr>
</tbody>
</table>

* S = Sausage.

Rectal Swab Cultures from Swine.

Rectal swab cultures obtained from the swine at four different intervals beginning at the sale barn in Kentucky, on arrival at the slaughter plant, at slaughter and after dehairing, are shown in table 3. Positive samples from these animals increased from nine percent at the sale barn to 84 percent after dehairing. The recovery of salmonella by rectal culture from these animals appeared to be related to their length of association under crowded conditions.

Swab Cultures from Carcasses.

Swab cultures from the skin of 21 swine carcasses were taken to determine the cleansing effect of washing with 165°F. water. Salmonellae were isolated
from 20 (95 percent) of the cultures obtained after evisceration and before washing. After washing, 13 (62 percent) of the cultures taken from the same area were positive. When swab samples were taken from these carcasses again in the chill room, only six (29 percent) were positive (table 4). Another 14 carcasses were washed both before and after evisceration with similar findings.

**TABLE 4**

*The Effect of Washing Swine Carcasses With 165°F. Water on Occurrence of Contamination With Salmonellae*

<table>
<thead>
<tr>
<th>Outside Carcass</th>
<th>Single Wash</th>
<th>Double Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carcasses</td>
<td>Positive</td>
</tr>
<tr>
<td>Before wash No. 1*</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>After wash No. 1</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Before wash No. 2†</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>After wash No. 2</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>In cooler (slaughter plant)</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>In cooler (processing plant)</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>

* Wash No. 1—Before evisceration.
† Wash No. 2—After evisceration.

Aerosol spread of salmonellae was demonstrated in the slaughter plant by placing or suspending from the ceiling open 16 ounce jars containing 100 ml. of tetrathionate broth with brilliant green and placing open brilliant green agar plates with sulfadiazine added at various points throughout the slaughter room. These containers were left open for periods of from 30 minutes to three hours and yielded salmonellae consistently from the area where the finished carcasses were placed, as well as other areas of the plant.

**Rendered Animal By-products and Finished Feeds.**

Over 175 samples taken during processing and from finished products in six rendering plants were examined for the presence of salmonellae. The samples were taken from four sites in the six plants with findings as shown in table 5. It should be noted that the highest percentage of isolations (51

**TABLE 5**

*Salmonella Isolations From Materials Obtained From Six Rendering Plants*

<table>
<thead>
<tr>
<th>Sample Sites</th>
<th>Number</th>
<th>Totals</th>
<th>Positive</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>2</td>
<td>1</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Cooker</td>
<td>55</td>
<td>6</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>Press/Dryer</td>
<td>31</td>
<td>7</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Finished</td>
<td>75</td>
<td>38</td>
<td></td>
<td>51</td>
</tr>
</tbody>
</table>
percent) were made from the finished product. In at least two of these plants, S. canoga and S. cubana were cultured repeatedly from finished products for a period of at least six months. A total of 24 different serotypes were isolated from the rendering plant materials. The serotype isolated most frequently was S. thomasville.

A total of 56 finished feed samples were examined for the presence of salmonellae. Of these, nine (16 percent) were found positive, as shown in table 6. Seventeen specimens of food ingredients were examined and eight

<table>
<thead>
<tr>
<th>Type</th>
<th>Number</th>
<th>Samples Examined</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finished Feed</td>
<td>56</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Limestone</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ground Hay</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Poultry by-produce meal</td>
<td>4</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Feathers</td>
<td>2</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Feather meal</td>
<td>9</td>
<td>4</td>
<td>44</td>
</tr>
<tr>
<td>Totals</td>
<td>73</td>
<td>17*</td>
<td>23</td>
</tr>
</tbody>
</table>

*S. tennessee* and *S. montevideo* were isolated from a single feed sample.

(47 percent) of these were positive. Fourteen different serotypes were isolated from these 17 specimens. *S. worthington* was isolated three times, *S. derby* twice, and both *S. tennessee* and *S. montevideo* were isolated from a single feed specimen.

**Dried Human Foods.**

More recently, a limited number of dried cake and bread mixes have been examined. Of 22 boxes of these materials cultured, salmonellae were isolated from three (14 percent). These three products contained dried egg or egg whites. The types found included *S. tennessee, S. infantis* and *S. oranienburg*.

**Salmonellae Serotypes Encountered.**

In all, 38 Salmonella serotypes were isolated and identified as seen in table 7. Nearly one-half of these serotypes were recovered in more than one phase of these studies. In seven instances, the same serotype was found to be present in the feeds, swine, and the slaughter plant environment. Nine serotypes were isolated from swine and from sausages but not from feeds, while 21 serotypes were isolated from feeds which were not found in the animals or sausages.
## TABLE 7

*Salmonella* Serotypes Isolated

<table>
<thead>
<tr>
<th>Swine and the Environment of Slaughter and Processing Plants</th>
<th>Rendering Plants</th>
<th>Feed and Feed Ingredients</th>
<th>Human Food*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alachua</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Anatum</td>
<td>43</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Berkeley</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Blockley</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Bredeney</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>California</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>Canoga</td>
<td>—</td>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cerro</td>
<td>—</td>
<td>3</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Cubana</td>
<td>—</td>
<td>5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Derby</td>
<td>329</td>
<td>1</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Gaminara</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Gie</td>
<td>10</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>5</td>
<td>—</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Illinois</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Indiana</td>
<td>22</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Infantis</td>
<td>17</td>
<td>—</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Kentucky</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>Lexington</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Litchfield</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Livingston</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Manchester</td>
<td>3</td>
<td>—</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Manhattan</td>
<td>18</td>
<td>—</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Manila</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Meleagridis</td>
<td>—</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Minnesota</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>Montevideo</td>
<td>—</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Muenchen</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td>Muenster</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Newington</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Newport</td>
<td>21</td>
<td>—</td>
<td>—</td>
<td>22</td>
</tr>
<tr>
<td>Oranienburg</td>
<td>—</td>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Saint Paul</td>
<td>5</td>
<td>—</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Siegburg</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Senftenberg</td>
<td>—</td>
<td>4</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Tennessee</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Thomasville</td>
<td>—</td>
<td>8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>32</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Worthington</td>
<td>—</td>
<td>1</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td><strong>Total isolations</strong></td>
<td><strong>518</strong></td>
<td><strong>64</strong></td>
<td><strong>17</strong></td>
<td><strong>37</strong></td>
</tr>
<tr>
<td><strong>Total number serotypes</strong></td>
<td><strong>15</strong></td>
<td><strong>24</strong></td>
<td><strong>13</strong></td>
<td><strong>16</strong></td>
</tr>
</tbody>
</table>

* From Richardson, *et al.* (7).
Although the numbers of samples are small in these studies, they confirm earlier observations in different geographical areas and suggest possible avenues of control which warrant further investigation. The slaughter and processing of swine infected with salmonellae resulted in the production of an extensively contaminated human food product under the conditions in the plant studied. The compact design of the plant and crowding resulted in frequent cross contamination of these products. The apparent spread by aerosol could be controlled more effectively in a building where the various processing operations were in separate rooms.

Several points must be considered in an attempt to interpret the findings on rectal swab samples collected on swine from their collection point through the slaughter process. A single rectal swab to determine the status of an animal as a salmonellae carrier, is meaningful only if positive. Althought the number of organisms present in the intestinal tract may be small, they still serve as a source of infection for other animals.

The washing of carcasses in 165°F. water reduced the number of recoverable Salmonellae but this reduction was small and not constant under the conditions existing in this plant. The greatest reduction (35 percent) was seen following the wash prior to evisceration. During the evisceration process, the contamination of the outside of carcasses increased from 29 percent to 50 percent. After the second wash, the number of contaminated carcasses was reduced to 35 percent. This reduction was similar when a single wash was used. According to plant personnel, the hot water washing resulted in a more desirable carcass for sausage. A 50 percent reduction in positive findings immediately following the final wash and in the first cooler was observed. A 30-minute to one-hour period lapsed between collection of the cultures in these two areas. This decline suggests the hypothesis that chilling the carcass may fix or coat the salmonella in fatty material and make it more difficult to remove from the carcass by swab culture technique.

During transportation from the slaughter plant cooler to the processing plant cooler, contamination of the carcasses increased from 14 percent to 50 percent.

In the studies of rendering plants, a high percentage of rendered materials and ingredients of poultry origin being used as protein supplement in animal feeds were found to contain Salmonella. Galton, et al. (8) (10), have shown the presence of salmonellae in animal feeds, especially dog foods, where 26 percent of meals yielded salmonellae. Information obtained from rendering plant personnel indicate that an average temperature of 325°F. was held for two to four hours in the cookers. This should be sufficient to eliminate viable salmonellae. However, without exception, salmonellae were isolated more frequently from the finished materials than from materials obtained along the processing lines.

With the exception of one plant, all had open conveyor systems designed so that uncooked material could easily become mixed with the cooked materials. The interchange of personnel and tools, without proper cleaning,
also presented a source of contamination of cooked materials. It has been shown that dust particles carrying salmonellae can contaminate materials which may support the growth or survival of the organism. The settling of this dust under favorable conditions of temperature and humidity could offer a source of continuing contamination. This could account for the persistence of *S. cubana* and *S. canoga* in finished products over a six months period from two of the plants studied.

From these studies, it is obvious that many animal protein supplements are contaminated with Salmonellae and offer a source of animal infection. These infected animals may eventually find their way to slaughter. During the slaughter and processing, the carcasses frequently become contaminated with Salmonella. The foods prepared from these carcasses are potential sources of human infection.

The distribution and spread of Salmonella serotypes encountered in this study closely parallel those found by Galton, *et al.* (8), in similar studies in Florida. It is of interest that *S. derby*, *S. anatum*, and *S. infantis* were the most prevalent types encountered and were isolated from all sources sampled.

**SUMMARY**

It has again been demonstrated that swine carcasses and fresh pork products are often contaminated with salmonellae during processing for human consumption. A hot water rinse used before and after evisceration appeared to reduce the numbers of salmonellae recovered from the skin of carcasses.

Salmonella were recovered from 23 percent of the animal feeds and from approximately 50 percent of animal feed ingredients and rendered material.

**REFERENCES**


PROBLEMS ENCOUNTERED IN THE INTERSTATE MOVEMENT OF FEEDER PIGS

K. Myers

Grundy Center, Iowa

It is a real honor for me to be invited to speak on the subject which you have assigned. It is one with which the organization I represent, the Wisconsin Feeder Pig Marketing Cooperative has had a little experience, for this year we will ship almost one-third of a million feeder pigs and these pigs will go into 20 or perhaps more, States.

The problems encountered in the interstate movement of feeder pigs can be easily divided into two parts.

Number 1, the problems with pigs, and number 2, the problems with people. From our experience and standpoint, the problems with pigs are to be preferred to the problems with people.

Because of this, I would like to devote the first part of my remarks to some of the problems with people. This part of the presentation can also be divided rather easily into several sections or parts. Perhaps a logical division would be to divide the problems dealing with people into the following groups:

1. Producers of feeder pigs.
2. Buyers of feeder pigs.
3. Dealers in feeder pigs.
4. Miscellaneous interested parties in feeder pigs, including veterinarians, feed dealers, druggists, patent medicine peddlers, etc., and
5. Regulatory officials.

I shall have a few comments on each as I go along.

Before I go further, I'd like to make a point that I feel should be kept in mind in any discussion involving the interstate movement of feeder pigs. The interstate movement of feeder pigs in itself creates no more problems as far as the pigs are concerned than does the intrastate movement of feeder pigs. A State line just doesn't make this difference, and if you think it does, you'll have a pretty tough time convincing me that moving a pig 200 miles within a State is easier on a pig or creates any less stress on the pig than moving a pig 200 miles across a State line. There isn't one single thing at the State line that is one bit different than a line one, five, or ten miles on either side of the State line.

In saying this I am in no way overlooking the need and importance for a State line in so far as regulatory matters and enforcement are concerned. I have pointed this out to illustrate that the mere moving of a pig across a
State line just doesn't cause the pig to get Cholera, Rhinitis, T.G.E. or whatever other swine disease you might name.

I am sure that the feeder pig industry deserves much of the bad reputation it has earned over the years, but it isn't due to the State line. The problems encountered came about by someone, producer, dealer, purchaser, yes, even occasionally a veterinarian, doing something wrong.

I shall start with the producer. Far too often in the past the producer has had little if any concern over what happens to his pigs once they leave his front gate. Unfortunately this still exists. However, this attitude is on a rapid decline, for the growth of quality feeder pig production programs has grown rapidly during the past five years. A name is just as important in the feeder pig business as it is in the automobile, drug or any other business. The Missouri Cooperative Feeder Pig Sales, and others, yes, the organization I represent, are building a reputation for quality pigs. Producers who deliver an inferior or diseased pig might get by once, but you can bet he doesn't get by the second time. Placing a name and an eartag with a number that can trace the ownership of the pig is to my way of thinking one of the best things that has happened in the swine industry. Believe you me, as a producer you'll think twice before sending someone else your problems.

The handler or dealer plays a big part in the problem, for I don't care how healthy a pig you start with and how well he's handled after the customer receives him, if the pig is improperly handled between producer and customer, you're in for problems. Dirty, improperly cleaned and disinfected premises and trucks can ruin the best of pigs. Overcrowding and too long a stay on trucks is still far too prevalent in the industry. These are not problems of pigs, but problems of people.

The buyer is not without some faults. Too often he has a touch of larceny in his heart and he thinks he's pulling a fast one when he buys a bunch of pigs from a stranger for two bits less than the peddler asked, when the pigs were overpriced a dollar to begin with. If I were to place the blame on anyone for keeping the peddler, the jockey, or whatever appropriate name you might give him, in the feeder big business, I'd have to place it solely on the buyer of feeder pigs. The buyer can place a stop on all the abuses I know of overnight by his refusal to buy; but then as I mentioned, many of them have a "touch of larceny" in their hearts and so I suppose we'll always have them.

Buyers of pigs vary considerably in their ability. This variation in ability causes us more concern than anything else I know of. We haul most of our pigs in semi-loads of 600 head. It isn't too often that one buyer takes an entire load and most loads are split between four to six customers.

The difference in ability between these four to six people who receive pigs by gate cut when loaded onto the truck, hauled on the same truck and delivered within hours of each other is the difference between night and day.

If trouble is reported to us by a customer, we immediately check the other pigs on the same load. I could relate some very interesting experiences and observations as a result of this if time permitted.
The group of people I classified as Miscellaneous also create a few problems. These problems range all of the way from the druggist who diagnoses hog diseases from back of a drug counter without ever seeing the hogs, to the feed dealer whose Uncle Joe's Super Snort will take care of everything from Mange to Rhinitis, to the veterinarian who diagnoses Hog Cholera everytime a hog comes into his territory that has been vaccinated by another veterinarian. All these, and a number of others I could name, contribute to the problem. These are all problems with people.

And the last group, but not the least, are the regulatory officials. I bring them into this for it is my understanding that they have been trying, and I place a question mark after trying, to come up with some uniform regulations for 20 years. From our experience in shipping pigs into 20 or more States, it doesn't appear as if too much progress has been made.

I don't believe any two States have identical laws and regulations. Some are similar. Some States require permits. Some States will wire permits, others will send them only by mail. Some require the name of the local veterinarian. Some require the location of the farm. Some States require ear tags, some require vaccination prior to shipment, some prohibit vaccination prior to shipment. Some States require a license and a bond. Where forms are required, there is no similarity.

Far too often the laws and regulations are written for an economic advantage rather than out of concern for the health of the pigs.

Personally, I feel that it's a disgrace to the swine industry that the regulatory officials haven't been able to get together and come up with some workable, uniform rules and regulations governing the interstate movement of swine.

So far I haven't mentioned a single problem of the pig. The problems I have discussed so far are problems with people. Now the pig does have some problems and anyone who tells you that they've never had any trouble has either never handled pigs or is the biggest liar you've ever met.

Earlier in my talk I mentioned this thing called stress. You just can't move pigs, be it across a State line, within a State, from one farm to the next or even from one end of the same farm to the other, without creating some stress conditions. I am sure that the diseases brought on by this stress are our biggest problems. The two which are of the most concern to us are—and in a meeting of veterinarians I hesitate to be so general—scours and respiratory diseases. I know full well that there are all kinds of scour and all kinds of respiratory diseases.

The scours I refer to are the kind brought on by moving, and a complete change of feed and water. Often times they are helped along by too rich a feed to start with.

The respiratory problems I refer to are the cough and the occasional pig that sometimes develops pneumonia.

Fortunately both of these simple diseases—and please forgive me for not being more specific and for calling them simple diseases—respond readily to sulphathiazole and electrolytes and if used immediately upon delivery reduces
the chances of losses from these and other more serious diseases or forms of the two I referred to to a very low level.

Now there is a whole list of diseases that are a constant problem, as you know far better than I. There is Rhinitis, T.G.E., Virus Pneumonia, P.P.L.O., Salmonella Infections, Erysipelas, Mange, Worms, Lice, but to name a few. In a discussion of this type I do not feel it necessary to go into any specific disease in detail. I doubt if the time will ever come when we'll be free of the problems of disease in hogs. I would hope that we can eradicate cholera as well as some of the other diseases. Chances are as we do we'll have some new ones to contend with. Ten years ago or so when I first got into the business, Rhinitis was unheard of in northern Wisconsin. It isn't unheard of today. At the same time where Erysipelas was once a major problem, we encounter little difficulty with it today.

In summary, the biggest problem we have encountered in the interstate movement of feeder pigs is the problems of people. There isn't a great deal we can do here today as far as producers, dealers, customers and some of the others who are interested in the industry are concerned, but there is something that this group can do and that is to come up with some uniform laws and regulations governing the movement of feeder pigs and all swine across State lines.

In my opinion number one consideration should be given to the health of the animals, and economic advantages, either to local producers of feeder pigs or to the veterinarian, should have no part in them.

This thing of uniform rules and regulations has also been given much consideration by the National Hog Cholera Committee. Having served as Chairman of this Committee for three years and listening to the discussion on the problems brought on by the lack of uniform rules and regulations, I was constantly asking "Why not?"

I ask the same question today, "Why not?"
INVESTIGATIONS INTO "SERUM BLOCK"

H. W. Dunne,* D.V.M., Ph.D., and D. C. Kradel,† D.V.M.

University Park, Pa.

Previously (1) it was reported that the administration of anti-hog cholera serum (hereafter referred to simply as serum), given alone to pigs at sales barns was responsible for the failure of those pigs to develop active immunity when subsequently vaccinated with hog cholera vaccine. Experiments showed that pigs which received two doses (one dose being 1.0 cc. per pound body weight) of serum, which frequently occurs when they pass through two sales barns, could not be actively immunized with an attenuated vaccine given three to four days after the last administration of serum. This inhibitory effect upon the development of active immunity has been given the term "serum block." This was true even if as many as 25 doses of vaccine were given four days after the administration of serum. The increased vaccine dosage, however, did overcome in part the effect of "serum block." Two doses of serum effectively neutralized or blocked the production of active immunity by one dose of vaccine given without serum on the third, tenth or twentieth day following the last administration of serum. Even one injection of serum provided an effective block to the production of active immunity when one dose of vaccine alone was given four days after the serum. It was shown, however, that the immunizing response to vaccine given at the time the first serum was administered, was not impaired by repeated vaccinations with serum and vaccine.

Certain questions were raised in respect to the vaccination of the non-infected herd which received serum alone. They concerned the effect of giving two doses of vaccine, one at 10 and the other at 20 days after the serum had been injected.

What would happen if the herd was quarantined 30 days and then vaccinated with vaccine and serum? What protection would virulent virus provide under the conditions of "serum block?"

Other questions directly or indirectly associated with "serum block" were raised. One concerned the treatment of "breaks," i.e. the subsequent occurrence of hog cholera in such herds. It was proposed by some that virulent virus was necessary in treatment—that neither serum alone nor serum and vaccine as treatment were effective.

The following experiments are designed to provide some answers to the above questions. The first set of experiments involves additional evidence of

* Department of Veterinary Science, Pennsylvania State University.
† Pennsylvania Bureau of Animal Industry.

Authorized for publication as journal series No. 2618 of the Pennsylvania Agricultural Experiment Station, University Park.

323
"serum block." The second set is a titration of the immunity potential of the product in an attempt to explain differences between attenuated vaccines and virulent virus. The third set is concerned with treatment of the infected herd following "serum block."

Throughout these experiments "serum" refers to commercially produced anti-hog cholera serum. "Vaccine" refers to a rabbit origin vaccine commercially produced. Experiments previously reported (1) and those reported here were conducted on two lots of vaccine. One had an expiration date of March, 1962 and the other May, 1962. These were 24 month expiration dates. All experiments were conducted within the first 12 months after release of the vaccine. Challenge and vaccination with virulent virus were made with a Pennsylvania strain of hog cholera virus of blood origin which contained at least $1 \times 10^6$ M.L.D. but less than $1 \times 10^7$ M.L.D. Challenge by injection was made by introducing one cc. of undiluted blood virus subcutaneously. Challenge by contact was made by inoculating the contact pig with the above virus not less than three days prior to contact with animals to be challenged. An animal was judged sick when it showed visible signs of illness and a temperature rise to $104^\circ$ F. or more. All pigs used in these experiments were from hog cholera susceptible sows and were not previously treated with any biologic product unless specified in the experiment.

Additional Evidence of "Serum Block."

One of the proposals made last year was that the blocking effect of serum given prior to vaccine administration could be countered by giving two doses of vaccine without serum at 10-day intervals after injection of the serum. Therefore, an experiment was initiated (Table 1) in which 30 pigs, weighing 30 to 35 pounds, were divided into two groups A and B. Both groups were given 30 cc. of serum. Both groups were given vaccine nine days later. Group B also received a second dose of vaccine 19 days after administration of serum. Pigs were challenged by injection of virulent virus 35 days after the last vaccination. Of group A (vaccinated at nine days), 15 of 15 became ill and 14 died. Of group B (two vaccinations, nine and 19 days), 14 of 15 became ill and nine died.

Another proposal for handling pigs during "serum block" was to quarantine the pigs for 30 days after receiving serum and then vaccinate with vaccine and a minimal dose of serum. An experiment to simulate this condition was devised (Table 2). Following the administration of 30 cc. of serum, 20 pigs weighing approximately 30 pounds each were isolated for 30 days. At the end of this period, the pigs were divided into four groups of five pigs each. Each of five pigs of group A was given one dose of vaccine and 10 cc. of serum, each of five others (group B) was given one dose of vaccine and no serum. A third group of five (group C) was given 20 doses of vaccine and 10 cc. of serum each. The remaining five (group D) were controls, each of which received 10 cc. of serum only. Thirty-five days later all pigs were challenged by injection of virulent virus. In group A (one dose vaccine and 10 cc. serum), four of five became ill and two died.
INVESTIGATIONS INTO "SERUM BLOCK"

Of the five pigs in group B (one dose vaccine and no serum), three pigs became ill and one died. Of the five pigs in group C (20 doses of vaccine and 10 cc. of serum), three pigs became ill and one died. All controls (group D) died.

Obviously neither the first suggestion nor the second proved to be an adequate solution to problem of "serum block." The only workable method of alleviating the effects of "serum block" lies in prevention. Elimination of the practice of using serum alone at community sales is the only logical method of curbing the problem.

The Titration of Immunizing Potential of a Vaccine.

Although the dangers of spread of hog cholera by "breaks" from the use of virulent virus are well recognized, most of us cannot help but recall the immunizing potential of that product at its best. Early users of virulent virus are quick to point out that they didn't have "serum block" problems—and they are right. At the risk of creating even more problems, we set up one experiment with two doses of serum, a four day interval, and 2 cc. of virulent virus and 30 cc. of serum to 20 pigs weighing 30 pounds. Four additional pigs were controls. Challenge was made at 90 days by the subcutaneous injection of virulent virus. Although several pigs had a temperature rise, only the controls died. This was not done to suggest that virulent virus should be reinstated. On the contrary, it was done to determine if a real difference exists and, if so, to determine the cause.

It is obvious that a difference does exist between virulent virus and attenuated vaccines in establishing active immunity in the presence of passively acquired antibodies. Is then the nature of this difference in the character of the virus or is it a matter of virus quantity—of numbers of virus particles?

It has long been our contention that the difference was primarily a matter of the number of virus particles present. In an attempt to prove this point, some in vivo immunity titrations were conducted using vaccine virus diluted in tenfold (one log) dilutions from $1 \times 10^0$ (undilute) to $1 \times 10^{-4}$ ($1:10,000$) in buffered salt solution (Table 3). Twenty pigs were divided into five groups to correspond with the number of vaccine dilutions. Each of the four pigs in a group was inoculated with 1 cc. of the dilution designated for that group and with 15 cc. of serum. Immunity challenge was made by contact exposure to infected control pigs 90 days after inoculation. Only those pigs receiving 1 cc. of undiluted vaccine and 15 cc. of serum lived. One pig in this group became sick but recovered. In the group receiving the $1:10$ ($1 \times 10^{-1}$) dilution of vaccine virus and 15 cc. of serum, all four pigs became ill and one died. Pigs receiving $1:100$ and higher dilutions of vaccine virus were not protected and died of hog cholera.

The same experiment was repeated six months later with the same vaccine using five pigs per group in dilution from $1 \times 10^0$ (undilute) to $1 \times 10^{-3}$ ($1:1,000$) five control pigs received 15 cc. of serum only (Table 4). Challenge this time was made by inoculation of one cc. virulent virus 90 days after
vaccination. Of five pigs receiving one cc. of the undiluted vaccine and 15 cc. of serum, one became ill and died. In the group receiving 1:10 dilution of virus, four became ill and all four died. None of the pigs receiving the higher dilutions of vaccine were protected.

In a companion experiment to the first vaccine immunity titration experiment and using 15 pigs from the same source, five groups of three pigs each were inoculated in a similar manner with one cc. of dilutions of virulent virus and 15 cc. of serum (Table 5). Dilutions again ranged from undilute to $1 \times 10^{-4}$ ($1:10,000$). There were no indications of infection or "break" following vaccination. Challenge of immunity was made by contact with five infected control pigs. None of the pigs from any dilution died although seven of the 15 pigs showed temperature reactions but recovered.

Two attempts to duplicate the virulent virus dilution experiment failed because 15 cc. of serum was not adequate to neutralize one cc. of any of the dilutions including the $10^{-6}$ dilution of the virus in simultaneous vaccination. Pigs in all dilution groups showed illness and some died during this initial phase of the experiment. A third attempt was partially successful in that hog cholera infection from vaccination was controlled in the four dilution groups above the $1 \times 10^{-2}$ dilution. Surviving animals showed no illness upon subsequent challenge with virulent virus. The first "break" with virulent virus and serum was associated with severe sunburn of the pigs four days prior to vaccination. Pigs from the same herd, however, at the same time, with the same exposure to sunlight, and the same degree of sunburn were in the companion experiment—the second vaccine titration group (Table 4) and did not react adversely to the vaccination. The second and third attempts at virulent virus-immunity titration were complicated by pre-vaccination diarrhea. The pigs used in the third attempt were treated with antibiotics prior to vaccination but apparently were not completely recovered at the time of vaccination. Control pigs not exposed to vaccination recovered uneventfully, subsequently were challenged with hog cholera virus and were completely susceptible. These pigs would not have been used if the need for data had not been urgent for this presentation and no other animals were immediately available.

While one cannot treat lightly the possibility of a difference in the invasiveness of vaccine virus and virulent virus, the big difference appears to be in the titer of the virus. It is known that rabbit origin virus tissues are suspended in a diluent in the production of the vaccine. What this dilution factor is may vary but it is not likely to be less than one log (1:10). It is generally recognized that freeze drying can cause a drop of one to two logs ($1:10$ to $1:100$) in virus titer. Thus if the original virus in the rabbit would have had $1 \times 10^5$ immunizing doses, it is quite possible for the product at the time of release to have not more than $1 \times 10^2$ (100) minimum infective doses per cc. Anything which might happen in handling the vaccine from that time to the time of injection into the pig might possibly cause a drop of another log in virus titer.
The evidence presented in the immunity titrations above and the earlier information (1) which showed that increased vaccine dosage did decrease the losses due to "serum block," strongly point to the great variation between virus particle numbers in attenuated vaccines and virulent virus as being the major contributing factor in the differences shown. If the present standards of vaccine titration were in force during the days of virulent virus usage, it would have been possible to dilute 100 cc. of virulent virus and use it to immunize one million pigs. This, of course, is an unfair comparison but it points out some of the problems arising from the decrease in numbers of virus particles.

Another possible factor is raised in determining the degree of attenuation that has been achieved for any vaccine. In the titration of virulent virus, sometimes endpoint dilutions result in prolonged incubation periods and a disease which runs a chronic course. If vaccines in which adequate attenuation is questionable are produced and tested near the endpoint of their titration, the question is posed, "Is this test a true measure of their attenuation?" Or is it affected by dilution? Would the testing of a more concentrated product reveal a more realistic evaluation of the degree of attenuation?

Treatment of the Infected Herd with Serum and Vaccine Following "Serum Block."

Since virulent virus has been outlawed in most States it may be anticiplastic to provide evidence that it is not needed to protect swine when active infection occurs in a herd following "serum block." It is a common belief that a "break" cannot be curbed without the use of virulent virus in conjunction with anti-hog cholera serum. It is believed by many that, as passive immunity is dissipated, infection will take place and death result. It has already been established (2) that swine infected with hog cholera cannot be protected by giving them serum if the animals have been infected for more than four days. This is true only if the pigs have had no previous exposure to passive antibodies or other substances which might cause some increase in resistance.

Two experiments were conducted to show the effect of revaccination on a herd following "serum block" in which a pig was observed sick with hog cholera. The first was the treatment of a herd infected five weeks after "serum block" and vaccination. The second was the treatment of a herd with active infection three weeks after "serum block" and vaccination.

In the first experiment (Table 6) 36 pigs weighing 30 to 35 pounds each were given two 30 cc. doses of serum four days apart. Three days later (day seven) they were given one dose of vaccine and 15 cc. of serum. After five weeks (day 42) they were placed in contact with a pig infected with hog cholera. On the sixth day of contact, temperatures were taken and all animals treated with 30 cc. of serum and one dose of vaccine.
At the time of revaccination, 18 of 36 had temperatures of 104° F. or higher. Sixteen additional animals showed a temperature rise to 104° F. or more after revaccination, bringing the total of sick animals to 34. Two pigs remained well throughout the experiment. Eleven of the 18 pigs (61 percent) showing a temperature rise to 104° F. or more at the time of revaccination died. All of the pigs with temperatures lower than 104° F. at the time of revaccination lived.

The second experiment on serum treatment (Table 7) was designed to show the effect of a higher concentration of residual passive antibodies in pigs in an infected herd, three weeks after the last administration of serum alone.

Thirty-two pigs were given two doses of serum three days apart. On day seven they were vaccinated with one dose of vaccine and 15 cc. of serum. On day 28 (three weeks after the last injection of serum), they were placed in contact with control pigs infected with hog cholera. Temperatures were taken on day 34 (six days after contact exposure) and 30 cc. of serum and one dose of vaccine were given.

Nineteen of the 32 pigs had temperature reactions of 104° F. or higher at the time of revaccination. Only six of the remaining 13 showed a temperature elevation after revaccination. Seven of 32 were completely resistant throughout. Only one pig died following treatment. All animals resisted challenge five weeks after revaccination.

Nothing has been said about the role of vaccines in these treatments. However, we have already shown that active immunity is difficult to establish with vaccines following “serum block.” In a situation requiring treatment they cannot be expected to be more effective. Is it possible that the virulent virus being shed by infected animals in an “outbreak” of hog cholera would be capable of establishing active immunity in animals retaining passive protection?

To establish the point made above 12 pigs (Table 8) were given 30 cc. of serum and seven days later exposed by continuous exposure to a hog cholera-infected pig (to simulate the “break” of a single pig in a herd purchased at a community sale). All of the pigs showed some temperature reaction but all lived. Three control pigs with no treatment died. Three other pigs which had received vaccine and serum showed no ill effects and lived. This proves that animals having only passive protection can survive an “outbreak” and develop active immunity if the amount of serum administered is adequate.

Certain conclusions can be drawn from these observations. Practically all animals with temperatures less than 104° F. can be saved. Bear in mind that a hot holding pen can cause a normal animal’s temperature to rise to 105° F. or above. Therefore, it would be helpful in arriving at a prognosis to know what portion of the group has a temperature rise, using 104° F. as the top of the normal temperature range providing that environmental conditions are moderate.

The number of pigs saved with temperatures over 104° F. is largely dependent upon two factors:
1. The resistance of the animal. This usually is directly related to the period between the last administration (and amount) of serum and onset of infection which determines the number of residual antibodies.

2. The period that infection has existed in the herd. From past experience and from these experiments the critical period (when pigs are in fairly close contact with each other) appears to be about six days after exposure by contact to a single infected pig. At this time approximately 50 percent of the exposed pigs will have a temperature rise. Minimum loss (as little as three percent of sick animals) will be observed in infected herds that are treated when the total number of animals having a temperature above 104° F. does not exceed 50 percent of the herd and treatment of the infected herd with serum (and vaccine) is administered within four weeks of the last injection of serum. Losses of 60 percent or more can be expected among pigs already sick if they are treated with serum (and vaccine) when 50 percent or more of the pigs have a temperature of 104° F. or higher and the interval between treatment of the infected herd and the last previous administration of serum is six weeks or longer. In other words at four weeks after serum injection there is still likely to be a residual antibody effect. At six weeks postserum inoculation the residual antibody appears to have a negligible role.

Neither the administration of vaccine nor virulent virus is necessary to establish active immunity in infected herds in which pigs have reasonably close contact during the period of infection.

SUMMARY

1. Satisfactory immunization was not obtained with either two vaccinations with vaccine alone, nine and 19 days after "serum block," or with vaccination with vaccine and serum after a 30-day quarantine following "serum block."

2. The difference between the degree of immunity established by virulent virus and that produced by vaccine virus in "serum blocked" animals appears to be primarily a matter of numbers of virus particles in the inoculum rather than variation in the character of the virus.

3. Treatment of the infected herd following vaccination or serum alone treatment is dependent upon the residual antibody level (directly proportional to the time and amount of serum administered and the time of exposure to the virus) and the period of time over which the pigs were exposed to infection. Fifty percent of the animals can be expected to have a temperature raise after six days of exposure to the virus.

4. Neither virulent virus nor vaccine must be administered to the exposed pigs in an infected herd to establish an active immunity. Even in the presence of previously administered serum such pigs will survive and be actively immune if the antibody level was high enough at the time of exposure and if
exposure to infected pigs was relatively constant over the period during which the passively acquired antibodies were being depleted.

REFERENCES


**TABLE 1**

*Delayed Vaccine Alone*

<table>
<thead>
<tr>
<th>Day</th>
<th>Reaction to Challenge</th>
<th>Died</th>
<th>Lived</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>Challenge 1 cc. virulent virus subcutaneously</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**

*Thirty-Day Quarantine of “Serum Blocked” Pigs*

<table>
<thead>
<tr>
<th>Day</th>
<th>Vaccination as indicated below</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30 cc. serum alone</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Vaccinate as indicated below</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>Challenge 1 cc. virus subcutaneously</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 3**

*Vaccine Immunity Titration 1*

<table>
<thead>
<tr>
<th>Vaccination Dilution</th>
<th>Vaccination Serum</th>
<th>Challenge by Contact at 90 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sick</td>
</tr>
<tr>
<td>1 cc. 1 x 10^0</td>
<td>15 cc.</td>
<td>1</td>
</tr>
<tr>
<td>1 cc. 1 x 10^-1</td>
<td>15 cc.</td>
<td>4</td>
</tr>
<tr>
<td>1 cc. 1 x 10^-2</td>
<td>15 cc.</td>
<td>4</td>
</tr>
<tr>
<td>1 cc. 1 x 10^-3</td>
<td>15 cc.</td>
<td>4</td>
</tr>
<tr>
<td>1 cc. 1 x 10^-4</td>
<td>15 cc.</td>
<td>4</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>5</td>
</tr>
</tbody>
</table>
TABLE 4

Vaccine Immunity Titration 2

<table>
<thead>
<tr>
<th>Pigs</th>
<th>Vaccine Dilution</th>
<th>Serum</th>
<th>Challenge With 1 cc. Virulent Virus Subcutaneous Inoculation at 90 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1 cc. 1 $\times$ 10$^6$</td>
<td>15 cc.</td>
<td>Sick 1 Died 1 Lived 4</td>
</tr>
<tr>
<td>5</td>
<td>1 cc. 1 $\times$ 10$^{-1}$</td>
<td>15 cc.</td>
<td>Sick 4 Died 4 Lived 1</td>
</tr>
<tr>
<td>5</td>
<td>1 cc. 1 $\times$ 10$^{-2}$</td>
<td>15 cc.</td>
<td>Sick 5 Died 5 Lived 0</td>
</tr>
<tr>
<td>5</td>
<td>1 cc. 1 $\times$ 10$^{-3}$</td>
<td>15 cc.</td>
<td>Sick 5 Died 5 Lived 0</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>15 cc.</td>
<td>Sick 5 Died 5 Lived 0</td>
</tr>
</tbody>
</table>

TABLE 5

Virulent Virus Immunity Titration

<table>
<thead>
<tr>
<th>Pigs</th>
<th>Virus* Dilution</th>
<th>Serum</th>
<th>Challenge by Contact at 90 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1 cc. 1 $\times$ 10$^6$</td>
<td>15 cc.</td>
<td>Sick 2 Died 0 Lived 3</td>
</tr>
<tr>
<td>3</td>
<td>1 cc. 1 $\times$ 10$^{-1}$</td>
<td>15 cc.</td>
<td>Sick 0 Died 0 Lived 3</td>
</tr>
<tr>
<td>3</td>
<td>1 cc. 1 $\times$ 10$^{-2}$</td>
<td>15 cc.</td>
<td>Sick 1 Died 0 Lived 2</td>
</tr>
<tr>
<td>3</td>
<td>1 cc. 1 $\times$ 10$^{-3}$</td>
<td>15 cc.</td>
<td>Sick 3 Died 0 Lived 3</td>
</tr>
<tr>
<td>3</td>
<td>1 cc. 1 $\times$ 10$^{-4}$</td>
<td>15 cc.</td>
<td>Sick 1 Died 0 Lived 3</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>None</td>
<td>Sick 5 Died 5 Lived 0</td>
</tr>
</tbody>
</table>

* Virus titer 1 $\times$ 10$^6$.
† One animal died before challenge from causes other than hog cholera.

TABLE 6

Treatment of the Infected Herd Following Serum Break 1

Number of pigs — 36
Day 0  30 cc. serum
Day 4  30 cc. serum
Day 7  2 cc. (1 dose) vaccine, 15 cc. serum
Day 42 Contact with hog cholera infected pig†
Day 48 Temperatures taken and revaccinated 30 cc. serum and 2 cc. (1 dose) vaccine
Initial temperature reaction at revaccination (at 6 days post contact) 18 of 36
Initial temperature reaction after revaccination 16 of 18
Total showing temperature reaction 34 of 36
Total dying* 11 of 36

* All dying had a temperature rise of 104°F or higher at the time of revaccination.
† Hog cholera susceptibility control from same herd.
DUNNE AND KRADEL

TABLE 7

Treatment of the Herd Infected Following Serum Block 2

<table>
<thead>
<tr>
<th>Day</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30 cc. serum</td>
</tr>
<tr>
<td>3</td>
<td>30 cc. serum</td>
</tr>
<tr>
<td>7</td>
<td>2 cc. (1 dose) vaccine, 15 cc. serum</td>
</tr>
<tr>
<td>28</td>
<td>Contact expose to hog cholera-infected control</td>
</tr>
<tr>
<td>34</td>
<td>(6 days post contact) Take temperatures and treat with 30 cc. serum and</td>
</tr>
<tr>
<td></td>
<td>2 cc. (1 dose) vaccine</td>
</tr>
</tbody>
</table>

Initial temperature of 104°F on day of revaccination

Initial temperature of 104°F after revaccination

Died

Completely resistant throughout

<table>
<thead>
<tr>
<th></th>
<th>19 of 32</th>
<th>6 of 13</th>
<th>1 of 32</th>
<th>7 of 32</th>
</tr>
</thead>
</table>

|               |          |         |         |

TABLE 8

Development of Active Immunity in Passively Immunized Swine

Immunization on day 0
Contact with infected pig on day 7

<table>
<thead>
<tr>
<th>No. Pigs</th>
<th>Vaccination Serum</th>
<th>Vaccine</th>
<th>Reaction to Contact Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 cc.</td>
<td>0</td>
<td>Sick Died Lived</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>12 0 12</td>
</tr>
<tr>
<td>3</td>
<td>(control) 30 cc.</td>
<td>1 dose</td>
<td>0 0 3</td>
</tr>
<tr>
<td>3</td>
<td>(control) 0</td>
<td>0</td>
<td>3 3 0</td>
</tr>
</tbody>
</table>
A SEROLOGICAL (NEUTRALIZATION) TEST FOR HOG CHOLERA*

LEROY COGGINS AND BEN E. SHEFFY

Ithaca, New York

Neutralization tests have become very important in identification of viruses, assessment of colostral protection, evaluation of vaccine efficacy and in studies of antibody production. After the finding of a cytopathogenic strain of hog cholera virus (1, 2) a neutralization test for this virus became possible. This study was planned in order to develop an accurate test for measurement of antibodies against hog cholera virus, as well as to explore applications of such a test in various hog cholera problems related to immunity.

MATERIALS AND METHODS

Tissue culture. Porcine kidney tissue cultures were prepared from disease-free pigs maintained by the Institute. Kidneys from pigs of various ages (one to 10 weeks) were used. After removal from the pig, the kidneys were minced into small pieces and trypsinized over night at 4 C. The cells then were washed and diluted 1:250 with medium at pH 7.3 that consisted of Hank's balanced salt solution (80 percent), 0.5 percent lactalbumin (10 percent), bovine serum (10 percent) and antibiotics. After suspension, one ml. was distributed into each tube and the tubes were placed into stationary racks. The cells were incubated at 37 C until full sheets were seen usually after four to five days. At this time, fluid was removed and replaced with medium at pH 7.6 that contained Earle’s solution (80 percent), 0.5 percent lactalbumin (10 percent), bovine serum (10 percent) and antibiotics. The cells were kept on this medium with a fluid change twice each week until used. Inoculation medium at pH 7.8 consisted of 1.5 cc. of Parker 199 (86 percent), 0.5 percent lactalbumin (10 percent), lamb serum (four percent) and antibiotics. Not all lamb serums supported cytopathogenicity and, therefore, each was tested before use. The antibiotics in all media were 500 units penicillin, 100 mg. streptomycin and 100 units mycostatin per ml.

Virus. Tissue-cultured persistent strain A hog cholera virus that had been serially transferred between 25 and 30 passages was used. Growth curve studies of virus were made to determine optimal time for harvesting virus for neutralization tests and to avoid accumulation of dead virus (figure 1). Virus appeared at a titer of CPID$_{60}$ $10^{1.7}$ per ml. after 24 hours of incubation and reached a maximum titer of CPID$_{50}$ $10^{2.5}$ per ml. in 72 hours. Frequently, virus titers of CPID$_{50}$ $10^{3.5}$ per ml. were obtained; stock virus, therefore, routinely was harvested after 96 hours of incubation. Because of low titer of virus it was necessary to prepare virus continuously for neutralization tests. Virus was stored at —70 C until used.

* The authors acknowledge with appreciation support from the Office of Naval Research.

Veterinary Virus Research Institute, New York State Veterinary College, Cornell University, Ithaca, N. Y.
Varying the pH of the inoculation media from seven to eight did not inhibit the cytopathic effect of the virus but the normal cell controls degenerated in four days when the pH was near seven.

Serums. Serums were taken at zero, four, seven, 10, 14 and 21 days after inoculation in the study of hog cholera antibody production. In the study of maternal fall-off of antibodies, serums were taken at intervals of two weeks. Serums were stored at —20 C.

Test procedure. Either five- or tenfold dilutions of serum were made in Parker 199. An equal amount of a serum dilution was mixed with undiluted tissue-cultured virus. This mixture was placed at 4 C for two hours. Each of five tissue-cultured tubes was inoculated with 0.2 ml. of a virus-serum mixture. Virus titration and cell controls were included in all tests. Tubes were incubated at 36 C until a final reading was made on the sixth day. Degeneration of the cell sheet infected with virus began on the second or third day after inoculation and maximum degeneration was seen by the fourth or fifth day. Uninoculated cells did not show degeneration until seven to 10 days later.

Viral degeneration consisted of rounding of cells which became smaller and more refractile. Holes developed in cell sheets with long thin strands of cytoplasm between cells; many small refractile cells appeared to cling to these strands. If the virus inoculum was heavy, CPE progressed to complete destruction of the cell sheet. It was necessary to have confluent cell sheets at the time of inoculation and to read results at the proper time because hog cholera virus effects were similar to those seen later in uninoculated cells.

Figure 1. Growth of hog cholera (HC) virus in tissue culture.
An attempt was made to establish the relationship between an amount of virus and serum titer (figure 2). The greatest change in serum titer occurred with the first half log dilution of virus. A change of $10^{1.0}$ in virus titer resulted in a change of $10^{0.8}$ in serum titer.

![Figure 2. Relationship between amount of virus and titer of serum in the neutralization test.](image)

To measure the error of the neutralization test a serum was diluted 0, 1:3, 1:9, and 1:27. Then each dilution was treated as a separate serum. A titer was determined for each, using twofold dilutions. The titer as determined by the neutralization test had a range of $10^{0.29}$ (Table I).

**TABLE I**

*The Accuracy of the Neutralization Test as a Measure of Antibodies*

<table>
<thead>
<tr>
<th>Serum Dilution</th>
<th>Serum Titer ($-\log_{10}$)</th>
<th>Calculated Serum Titer ($-\log_{10}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.92</td>
<td>1.92</td>
</tr>
<tr>
<td>1/3</td>
<td>1.56</td>
<td>2.03</td>
</tr>
<tr>
<td>1/9</td>
<td>1.26</td>
<td>2.21</td>
</tr>
<tr>
<td>1/27</td>
<td>0.65</td>
<td>2.08</td>
</tr>
</tbody>
</table>

Range 0.29 Log
Applications of the neutralization test. The neutralization test was used to follow the development of hog cholera antibodies (figure 3). The curve represents an average antibody production of three pigs following hog cholera vaccination. Antibodies appeared between the seventh and eleventh day in two of the three pigs and rose rapidly. The other pig developed antibodies between the fourteenth and twenty-first day.

![Figure 3. Development of hog cholera (HC) virus in vaccinated pigs.](image)

Fate of maternal hog cholera antibodies in piglets. The average fall-off of maternal hog cholera antibodies in 18 pigs expressed as log percent of sow titer is shown in figure 4. When a straight line was fitted to these data, pigs with titers of $10^{-3}$ did not completely lose their titers by 14 weeks of age and were resistant to hog cholera challenge. Titers of $10^{-0.5}$ to $10^{-2}$ dropped to zero between two to eight weeks. The half-life of maternal antibodies was 13 days.

DISCUSSION

Although one of the major difficulties with the neutralization test has been the low virus titer, 30 to 300 CPID$_{50}$ can be obtained in the test. Uniform, confluent cell sheets are necessary for the endpoint to be distinct. The absence of a clearly specific type of degeneration no doubt results in inaccuracies at the endpoint and accounts for much of the error in the test. Nevertheless, a neutralization test has been developed which is satisfactory enough to allow measurement of development and fall-off of high cholera antibodies. The potential of the neutralization test in the evaluation of hog cholera vaccines or in the development of a nomograph is clear. Work along these lines is
now in progress. Studies to show a correlation between antibody titer and immunity are also underway (3).

The fact that pigs can retain their maternal antibody titer for as long as 14 weeks raises a question which must be resolved. What titer can a pig have and still be actively immunized with a live-virus vaccine or inactivated product? Experiments now in progress indicate that pigs with a titer of 32 were immunized with virulent virus but not with attenuated live-virus vaccine. This work will be necessary if pigs are to be immunized properly.

SUMMARY

A neutralization test has been developed for hog cholera virus. Although the test needs further improvement, development and fall-off of hog cholera antibodies can be measured. Studies on the virus growth curve, virus titer versus serum titer and the reproducibility of the test are presented. Neutralization tests show that hog cholera antibodies develop between the seventh and eleventh day after vaccination and that maternal antibodies may last for as long as 14 weeks.

REFERENCES

Serological tests for specific antibody provide an invaluable aid to the understanding and control of animal disease, and with the discovery of a strain of cytopathogenic hog cholera virus (1) a long-existing and urgent need has been fulfilled. The development of a serum neutralization test for hog cholera immunity will serve to remove a large amount of the uncertainty which in the past necessarily has attached to any hog cholera control program. This uncertainty now can be replaced by a definitive picture of the immunity status of the animals involved in the program, both before and after the control measures are instituted. The ultimate goal of hog cholera eradication has, by the same token, been brought potentially closer to realization.

One of the immediate benefits to be derived from the serum neutralization test as an indicator of immunity is its application to vaccine evaluation. The effectiveness of a vaccine can be ascertained only by vaccination tests in susceptible animals, which now can be identified serologically, and the immunity induced by vaccination can now likewise be assessed by serological test, replacing the more costly and less certain method of postvaccination challenge of vaccinates and control animals of unknown immunity status.

A prerequisite to the use of the neutralization test as a substitute for the challenge test in the evaluation of a vaccine or other control measure is the sound demonstration of a high level of accuracy in the neutralization test as an indicator of immunity. It must be shown by a convincingly large number of tests that animals which are negative serologically do in fact develop typical hog cholera when challenged and that serologically positive animals are unaffected by challenge.

In reality, of course, the problem is not quite so simple as outlined here; aside from the purely statistical problem of determining what constitutes a convincingly large number of tests there are several complications arising from biological factors—all of which, it is pertinent to remark, have been revealed by serological methods.

First among these complicating factors is the passive immunity produced by maternally transferred antibody. As reported (2), the amount of maternal antibody in a young pig steadily declines with age until eventually it reaches an undetectable level and presumably finally disappears entirely. If the immunity status of the pig during this period of antibody decline follows in

* The authors acknowledge with appreciation support from the Office of Naval Research. Veterinary Virus Research Institute, New York State Veterinary College, and Biometrics Unit, Department of Plant Breeding, Cornell University, Ithaca, N. Y.
THE SERUM NEUTRALIZATION TEST

principle the same course as that of a puppy in relation to distemper immunity (3) then we cannot expect an abrupt transition from a pure immune state to a pure susceptible state as the antibody level approaches zero. Rather, at low antibody levels the animal may be expected to go through a gradual transition from immunity to susceptibility with a corresponding increase in severity of response were it challenged with the infectious agent. During this transitory period at low antibody level, the response to challenge cannot be predicted with any certainty by means of a serological test—or, to put it another way, the serological test would predict that the response to challenge is uncertain. In the final stages before the disappearance of maternally transferred antibody there is no typical response to challenge; what is typical is that the response varies from no signs of illness through moderate signs of illness to the most severe form of illness. In these circumstances the neutralization test might be said to fail as an indicator of immunity; the circumstances themselves are easily recognizable, however, and may therefore be avoided if this is not a point at issue. For the purposes of vaccine evaluation this does, in fact, become an advantage of the serological test over the challenge test, for if the vaccine is poor and produces only low antibody titers the serological test will always detect this fact while the challenge test would do so only part of the time.

A second factor which might invalidate the serological test as an indicator of immunity is the time factor as it relates to the development of active immunity following vaccination. Antibody development has been charted and it was found that there may be a lag of as much as two weeks before detectible antibody appears (2). The antibody level is then rapidly rising, however, and at this stage just before the antibody reaches a serologically detectible level the animal may well be in an already immune state because of its potential for immediate rapid antibody production. Thus, under these circumstances, the serological test would again be an apparent failure, giving a negative result for an immune animal. This, of course, is a relatively trivial point, since the circumstance is readily avoided in practice by waiting until at least three weeks after vaccination to take the serum sample.

The third and most striking error-producing factor is virus diarrhea virus and the role it plays in protecting pigs against hog cholera (4). It has been found that an animal with VD antibody but no hog cholera antibody would be classified negative by the hog cholera serum neutralization test, yet might resist a challenge dose of virulent hog cholera virus. Consequently, hog cholera immunity can be ascertained only by running two serological tests, one for VD antibody and one for hog cholera antibody. Two tests would not be necessary, of course, for the purpose of a hog cholera vaccine evaluation, for VD antibody, if present, does not inhibit the information of hog cholera antibody, and the only concern in this case would be whether hog cholera antibodies were produced following vaccination. This, again, actually represents an advantage of the serological test over the challenge test as a method of vaccine evaluation, for an ineffective vaccine administered to a pig with VD antibody would produce an apparent immunity according
to the challenge test, but would be revealed as a vaccination failure by serological test.

Bearing these several complicating factors in mind we can now turn to an examination of the experimental results obtained in a study of the correlation between antibody titer as measured by the neutralization test and immunity status as measured by challenge. The pigs used in this analysis were taken from several different experiments, including vaccine and serum tests, material antibody transfer studies, and VD protection experiments. In the latter case the VD treated pigs were excluded and only the control pigs are used here, thereby eliminating one of the aforementioned complicating factors. Pigs from the maternal transfer study might be expected to raise problems if they are nearing the expiration date of their maternal protection, and pigs from the vaccine tests were, unfortunately, bled and challenged just two weeks after vaccination, when the pigs are in an immune state with rapidly rising but low antibody levels.

A total of 68 pigs have been accumulated from these experiments and are classified here according to their antibody titer at the time of challenge and according to their response to challenge. In most cases, the class to which a pig belonged was readily apparent; the 38 pigs which were serologically negative and responded to challenge did, in fact, show typical signs of hog cholera, and all but one of them died. At the other extreme, the 21 pigs with antibody titers in excess of 10 exhibited no signs of illness whatsoever when challenged. In the central column of the table the situation was not always so clear; two pigs did exhibit no signs of illness whatsoever, and these were two pigs that had been vaccinated and then bled and challenged two weeks later when their titers were just beginning to rise and had become detectable. They were truly immune. The remaining five pigs with low titers came from the maternal transfer study and were approaching the susceptible state; three of these five did, in fact, develop typical signs of hog cholera and two of the three died. The other two, which were littermates, developed only moderate signs of illness—a three-day fever—and were here somewhat arbitrarily classified as nonrespondents. A third member of this litter had a still lower antibody level which was serologically undetectable, and it also developed only moderate signs of illness; this pig was therefore classified, again somewhat arbitrarily, as a negative nonrespondent. The other negative nonrespondent came from a vaccine test; at two weeks after vaccination this pig had no detectable antibody and yet showed no signs of illness following challenge. Later, this interesting pig was again bled and still showed no antibody titer. Thus, in this one pig and, in fact, only in this one pig, do we find absolutely no indication of a correlation between antibody titer and immunity status.

If we now ask what conclusions can be drawn from this preliminary study we are brought back to the statistical question of whether the present data are extensive enough to justify any firm decisions. The chart shown in figure 1 is designed to answer this question and was constructed on the basis of statistical formulas, to test the hypothesis that the serological test is at
least 95 percent accurate as an indicator of immunity. Information is accumulated on this chart until a decision to accept or reject the hypothesis is reached. Using all 68 pigs, we see that the plot falls completely off the chart in the rejection region; excluding all low titer pigs from the maternal transfer study, however, we find that the remaining single error plot falls in the region for continued testing.

SUMMARY

Preliminary tests of the accuracy of the serological test as an indicator of immunity against hog cholera show that the neutralization test appears to be a reliable predictor of immunity except when (a) young pigs are nearing the expiration of their maternal protection (b) vaccinated pigs are just beginning their antibody production in the two-week period immediately following vaccination (c) pigs having no hog cholera antibody are protected by a previous infection with virus diarrhea. A sequential challenge test is under way to demonstrate that when these factors are excluded, the serum neutralization test in tissue culture is at least 95 percent accurate as an indicator of immunity.

REFERENCES

TABLE I

Relationship Between Serum Neutralizing Titer and Response to Challenge

<table>
<thead>
<tr>
<th>Response</th>
<th>Titer at Time of Challenge</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$&gt;10$</td>
<td>$&lt;10$</td>
<td>Negative</td>
</tr>
<tr>
<td>Hog Cholera</td>
<td>0</td>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td>No Illness</td>
<td>21</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>7</td>
<td>40</td>
</tr>
</tbody>
</table>

REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE


Mindful of the serious economic loss to the swine industry because of the widespread prevalence and high incidence of several contagious diseases of swine, this Committee did, in its report for 1960, direct attention to several factors believed to be most responsible. It is our belief that little has changed within the year except for the growing interest in a program for the eradication of hog cholera. Since most of its recommendations await acceptance and positive action, this Committee reaffirms the 1960 report as approved by this Association.

Mindful that the swine industry has requested and that it has a right to expect more uniform and effective requirements at least for movement of swine between States, this report confines its recommendations to the area of movement rather than to programs for specific diseases.

Minimum requirements for both inter- and intra-state movement of breeding and feeding swine are proposed as follows:

I. All diagnostic tests to be made in State-Federal Cooperative or State Approved Laboratories.

II. That movements to and from concentration points be restricted to:
   a. Public Stockyards under Federal Supervision; and,
   b. Approved or licensed markets, dealers and order buyers.

III. Inspected by a licensed accredited veterinarian within eight (8) hours preceding shipment or release.

IV. Identified by registration number, breed tattoo or an identification eartag firmly affixed at the juncture of the medial and middle one-third of the upper margin of the ear and showing the state of origin. An ear punch would be considered acceptable for movement of swine within a State.

V. That neither raw or cooked garbage has been fed at any time within the lifetime of the swine in the shipment.

VI. Feeder swine permitted to move only under permit from State of Destination.
VII. Must arrive at destination within 72 hours from time of consignment from originating premises, or from time of arrival at a market, dealer's premise or concentration point.

VIII. Must be quarantined upon arrival at destination; maintained in strict isolation for a period of twenty-one (21) days from time of inspection and/or vaccination, except that breeding swine moving directly from the farm premises of the breeder of origin to the farm premises of a breeder may be exempted at the option of the State of Destination when vaccinated for twenty-one (21) days or longer.

IX. Consignment to an intermediate destination (Approved markets, Dealer's premises, Concentration points and Public Stockyard markets under Federal Supervision) permitted one time only with resale and delivery to final destination to be completed within 72 hours from time of inspection and/or vaccination at point of origin.

X. Breeding Swine to be:
   a. From "Validated" Brucellosis Free herds without test; or,
   b. From "Qualified" negative herds without test when the herd of origin has passed a negative test for Brucellosis within six months preceding movement; or
   c. Tested and negative for brucellosis within 30 days preceding movement; quarantined, isolated and subject to a negative retest at destination in not less than 30 nor more than 60 days.

XI. Vaccinated against hog cholera by one of the following:
   a. Modified Live Virus Vaccine when administered for not less than 21 days preceding shipment; or,
   b. Modified Live Virus Vaccine with a minimum dosage of 20 cc. hog cholera antiserum administered within 72 hours preceding arrival at destination only if quarantined and isolated at destination as required under VIII.
   c. Killed or inactivated vaccine administered for not less than 21 days nor more than 10 months preceding shipment; or,
   d. Under special permit from State of Destination in the case of breeding swine moving directly from breeder to breeder.

XII. Swine to be sprayed in approved cresylic disinfectant in approved strength solution preceding release and loading from a market, dealer's premise or concentration point.

XIII. Accompanied by a health certificate (State or Federal) showing:
   a. Name and address of consignor and point of origin.
   b. Name and address of consignee and destination.
c. Identification of swine in shipment and general description as to breed, approximate age, size or weight, except that in the case of feeder pigs graded after identification, recording of numbers waived with statement that "tag numbers are on record at market of origin."

d. Date and method of vaccination and by whom vaccinated.

e. Dates and results of any tests as required.

f. Permit number when permit is required.

g. Name and address of carrier, car or license number of railroad car, truck or conveyance.

h. A statement as to health status of animals in shipment and in the case of breeding swine, a specific statement as to herd health status especially with regard to chronic diseases as atrophic rhinitis, virus pig pneumonia, Transmissible Gastro- enteritis (TGE) etc.

XIV. Any extension of time beyond the 72 hour limit on movement from point of origin to point of final destination should be considered only when distance is greater than 1,000 miles and then ONLY by permission from the Chief Livestock Disease Control official for the State of Destination. A 24 hour limit on movements within a State appears adequate.

XV. "Approved" markets, dealer's premises, concentration points and order buying stations as used herein shall mean those which:—

a. Maintain acceptable standards of sanitation; and,

b. Are inspected and under the supervision of a licensed, approved veterinarian; and,

c. Are operating under the supervision of and cooperating with the State Disease Control Agency; and,

d. Maintain complete records to show: (1) name and address of consignor; (2) origin of each shipment of swine; (3) number and general description of all swine in each consignment received or consigned or released; (4) date received and released; (5) name and address of purchaser and/or his agent and destination; (6) name and address of carrier including car or license number; (7) retain all in-and-out waybills, conductors manifests, permits, and/or certificates for a period of one year—these to be available on demand to a representative or agent of the State Disease Control Agency or of the United States Animal Disease Eradication (ADE) Division.

XVI. Public Stockyards Markets as used herein shall mean those markets under Federal supervision and meeting the requirements of XV.
XVII. "Acceptable Standards" of sanitation as used herein shall mean:—
a. That all pens, alleyways and chutes used for the handling and
penning or holding of swine be constructed of smooth con-
crete or other approved impervious material properly
graded and curbed for maximum control of liquid wastes
and drainage; and,
b. That all pens are thoroughly cleaned and disinfected with
an approved disinfectant in proper or designated strength
prior to each use.

XVIII. State Disease Control Agency, State of destination or origin, as
used herein shall mean the Chief Livestock Disease Control
official for the State of origin and/or destination.

This Committee further recommends:

1. That Federal Inter-state regulations be adopted at the earliest possible
date and in accordance with the requirements as outlined herein
and that the various States develop laws or regulations in accord-
ance with these recommendations.

2. That an improved system for the reporting and statistical evaluation
of contagious and infectious diseases of swine be developed and
initiated at an early date. This is particularly needed for more
accurate estimation of economic loss; regional prevalence and
incidence and for research project and program emphasis.

3. That the various States give serious consideration to the develop-
ment of programs for the control and eradication of Swine Bru-
cellosis. Breeders, particularly breeders of registered breeding
swine, should be encouraged to qualify their herds under the
"Validated" Brucellosis Free Herd Plan.

4. That all breeding swine sold or moved be tested and negative for
Brucellosis.

5. That Market and/or Slaughter House Testing programs be developed
for the purpose of screening swine herds. These should be initiated
on a "pilot" basis until workable procedures are developed.

Signed by a majority of the members of the Committee on Transmissible
Diseases of Swine.
PROTECTION OF PIGS AGAINST HOG CHOLERA WITH VIRUS DIARRHEA VIRUS OF CATTLE

Ben E. Sheffy, Leroy Coggins and James A. Baker*

Ithaca, New York

Recent studies have indicated that animals can be protected against certain viruses by others that are immunologically different, not through production of common antibodies but by inducing a state of secondary response so that specific antibodies are formed more quickly (1, 2). The secondary response relationship between hog cholera (HC) virus and virus diarrhea (VD) virus is presented here.

CROSS-PROTECTION STUDIES IN PIGS AND CALVES

SPF (specific pathogen free) pigs. Three litters of pigs were obtained from the SPF herd maintained by the Institute and each litter was placed in an isolation unit. In the tests, part of each litter was left uninoculated for controls. One-half of the remaining pigs in two litters were given VD virus strain N.Y. 1, maintained as infected spleen from a calf, while the others were given VD virus Oregon strain C24V after nine serial passages in tissue culture. In another litter, only strain N.Y. 1 was given. Inoculations were given intramuscularly; they consisted of either one ml. of a 10 percent spleen emulsion for strain N.Y. 1 or of undiluted tissue culture fluid for strain Oregon C24V. After four weeks, each pig was given intramuscularly one ml. of a 10 percent spleen emulsion from a pig infected with virulent strain A HC virus. In another test, a litter of pigs was given strain N.Y. 1 in a similar manner. Then, one day, 10 days, one, two, three, four, five and six months after inoculation a pig was removed, placed in another isolation unit and given an inoculation of virulent HC virus.

No signs of illness were seen in any SPF pigs, whether they were inoculated with VD virus or exposed by contact. After inoculation with HC virus, all pigs showed a temperature elevation two to four days later. In the pigs that had not been inoculated with VD virus, temperatures remained elevated and the pigs died eight to 15 days after inoculation. In contrast, with the exception of the pig given HC virus on the day after VD virus and which subsequently died, pigs initially given VD virus showed temperature elevations of lesser degree that lasted for only one to two days. These pigs continued to eat and showed no other signs of illness (figure 1).

Veterinary Virus Research Institute, New York State Veterinary College, Cornell University, Ithaca, N. Y.

* The authors acknowledge with appreciation support from the Office of Naval Research.
Field test pigs. In addition to tests on SPF pigs, pigs that came from a farm near Ithaca, New York, and from dams that had been immunized to hog cholera (titers ranging 0 to 760) were tested also. In these tests, Oregon C24V was used after 11, after 21 and after 32 serial passages in tissue culture. As before, one-half to two-thirds of each litter were inoculated with VD virus while the others were left uninoculated.

From two to four days after inoculation with virulent HC virus, pigs showed temperature elevations that lasted approximately 10 days. All pigs
TABLE I
Protection of Pigs Against Hog Cholera (HC) by Virus Diarrhea (VD) Virus

<table>
<thead>
<tr>
<th>Virus Used</th>
<th>HC Status of Dam</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. Y. 1 (Calf spleen)</td>
<td></td>
<td>14/14</td>
<td>100</td>
</tr>
<tr>
<td>C24V 9th TC</td>
<td>Disease-free</td>
<td>4/4</td>
<td>100</td>
</tr>
<tr>
<td>Uninoculated</td>
<td></td>
<td>0/8</td>
<td>0</td>
</tr>
<tr>
<td>C24V 11th TC</td>
<td></td>
<td>14/24</td>
<td>58</td>
</tr>
<tr>
<td>C24V 21st TC</td>
<td>Immunized</td>
<td>6/12</td>
<td>50</td>
</tr>
<tr>
<td>C24V 31st TC</td>
<td></td>
<td>5/12</td>
<td>42</td>
</tr>
<tr>
<td>Uninoculated</td>
<td></td>
<td>7/37</td>
<td>19</td>
</tr>
</tbody>
</table>

* Denominator indicates number tested. Numerator—number protected.

...went off feed and showed other signs of illness usually associated with hog cholera. Some pigs survived. Neutralization tests on control pigs that survived showed persistence of HC antibody that had been maternally transferred. Since the number of pigs that had maternally transferred HC antibody was equally distributed among the various groups, the relative efficacy of passage levels still could be evaluated even though protection given some pigs by VD virus was masked by maternal protection against HC virus. It appears, therefore, that strain Oregon C24V virus, while having some protective capacity at these passage levels, was not completely effective. Protection appeared to be related to the number of passages and, with increase in passage level, effectiveness of protection decreased.

Calves. In reciprocal tests, each of six calves was given intravenously one ml. of spleen emulsion from a pig infected with virulent HC virus. In four of six tests, a calf was exposed by contact. One month after initial inoculation, or exposure, each calf was given intravenously one ml. of spleen emulsion from a calf infected with strain N.Y. 1 VD virus. Daily temperatures were recorded and leukocyte counts were made.

Neither calves inoculated with HC virus nor those exposed to inoculated calves showed an elevation of temperature or changes in leukocyte count. After inoculation with VD virus, calves that had been given HC virus and control calves developed a typical diphasic temperature response (figure 2) and a leukopenia.
FIGURE 2. Response of calves inoculated with hog cholera (HC) virus followed by virus diarrhea (VD) virus.

SEROLOGICAL STUDIES

By use of the gel-diffusion technique a serological relationship between mucosal disease virus and swine fever virus has been reported (3). This work strongly suggested that the two viruses share a common antibody. In order to clarify the protection that was found and to increase understanding of the relationship suggested by gel-diffusion studies, further studies were made.

Serum transfer of protection. A litter of six SPF pigs was placed in an isolation unit. Each of two pigs was given 40 ml. intraperitoneally of serum taken from a pig 28 days after VD virus inoculation. To each of two other pigs there was given intraperitoneally 225 ml. of colostrum from a cow that had a titer of 181 for VD antibody. The other two pigs were left uninoculated. Then, 24 hours later, each pig was given intramuscularly one ml. of spleen emulsion from a pig infected with virulent HC virus. All died.
In another test, six SPF pigs were placed in an isolation pen. One pig was given 6250 HC antibody units per pound of body wt. A second pig was given five times this number of VD antibody units per pound of body wt. and a third was given 25 times this amount. These VD antibodies were produced in pigs. In a similar test, two pigs were given VD antibodies that had been produced in calves. One pig was left uninculated. Then, after 24 hours, all pigs were tested for immunity with the usual inoculation of virulent HC virus. All died except the pig given HC antibodies.

Reciprocal neutralization tests. After the neutralization test for VD virus (4) and for HC virus (5) had been developed, reciprocal neutralization tests were done and, as can be seen in Table II, they appeared different immunologically.

<table>
<thead>
<tr>
<th>Virus Used</th>
<th>Serum Neutralization Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>VD</td>
<td>10/10</td>
</tr>
<tr>
<td>HC</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* Denominator indicates number of serums tested; numerator those that neutralized 50 to 100 TCID₅₀ of virus. Homologous titers for VD ranged from 70 to 3160 and for HC from 100 to 2800.

Serological findings on SPF pigs and on calves that had been used in cross-protection tests. Each pig was bled when VD virus was inoculated or exposure began, again at the time HC virus was inoculated and on the fourth, seventh, tenth, fourteenth, and twenty-first day afterwards. Serums were assayed for VD antibodies and for HC antibody titers by neutralization tests. These results are recorded in figure 1 and clinical features are included for comparison.

After inoculation of VD virus, pigs developed an average antibody titer of 27 against VD virus and no titer against HC virus. Littermate pigs that had been exposed by contact showed no antibodies to either virus. After inoculation with HC virus, pigs that had been given VD virus initially showed HC antibodies four to seven days after inoculation, and 21 days afterward the average titer was 515. Also the titer against VD virus increased from 27 to 729 and paralleled the development of titer against HC virus. In the control pigs no antibodies developed against either VD or HC virus before they died. Pigs from the field test that survived showed antibody responses similar to the SPF pigs.

In a similar manner, all calves were bled when the study began, again at the time VD virus was inoculated and on the fourth, seventh, tenth, fourteenth and twenty-first day afterward. Serum samples were assayed for HC and for VD antibodies. These results are recorded in figure 2.

Calves showed no antibody against either HC or VD virus at the time of HC virus inoculation. After HC virus inoculation they developed an average
titer of 14 for HC virus and showed no titer for VD virus. After VD virus inoculation, however, these calves developed VD antibodies after four days, and 21 days after inoculation VD titers rose to 988; HC antibody titers rose from 14 to 1,080. The contact calves showed VD antibodies 11 days after inoculation which rose to 720 after 21 days, and no antibodies to HC virus developed in these calves.

**DISCUSSION**

From the data presented it has been concluded that VD virus can protect pigs against lethal doses of HC virus. Because the two viruses do not share a common neutralizing antibody, an explanation other than immunity appears necessary. Indeed, tests for the appearance of HC antibodies in pigs, after inoculation with VD virus, indicate the manner of this protection. Pigs given hog cholera vaccine virus show antibodies approximately 14 days afterwards, whereas pigs given VD virus will show antibodies within seven days after HC virus inoculation. It appears, therefore, the VD virus induces a state of secondary response to hog cholera virus. In this way it resembled the effect produced by a single inoculation of homologous inactivated virus vaccine (6). This state of secondary response still was present six months later. HC virus provided a similar secondary response in calves against the VD virus.

Textbooks usually do not differentiate resistance and immunity completely, but include such indefinite factors as nutrition, heredity and environment as nonspecific factors of resistance. While nonspecific factors may be important, it would appear now that in some instances resistance to pathogenic agents may be due to previous infection by an organism that is distinct immunologically but capable of inducing a state of accelerated antibody response. Perhaps this could be indicated by complement-fixation or by gel-diffusion, as was found for adeno-ICH (7) and reported for VD-HC (3). Heterotypic responses also have been noted within the human adenovirus group following vaccination (8, 9) and complement-fixing antibodies have been observed in individuals to polio virus following ECHO 9 infection (10). While serological studies have not been made, cross-protection among certain of the group A arboviruses has been found (11, 12, 13, 14).

Application of this resistance concept introduces an entirely new means of controlling diseases, although field test application of laboratory findings for VD virus to protect against HC virus indicates that additional studies need to be made before its use can be recommended. Obviously, a strain of VD virus must be found that is considered both safe and efficacious.

**SUMMARY**

Pigs inoculated with VD (virus diarrhea) virus survived inoculations of virulent HC (hog cholera) virus which killed littermates that had not received VD virus. Survival was signaled by accelerated antibody production both to HC and VD viruses. In reciprocal tests, HC virus produced secondary response in calves. Reciprocal neutralization tests between VD virus and HC
virus showed they did not share a common neutralizing antibody. Protection appeared related to accelerated antibody production that resembled the secondary response induced by inactivated viral antigen.

REFERENCES


IMPLICATIONS OF NEWER KNOWLEDGE IN THE ERADICATION OF HOG CHOLERA

JAMES A. BAKER

Ithaca, New York

A primary purpose of all those concerned with control of swine diseases is to eradicate hog cholera. Towards this end Congress already has given approval, and the Animal Disease Eradication Branch of the Agricultural Research Service is expected to proceed immediately.

For total eradication of hog cholera, all of the live hog cholera virus in the United States must be eliminated, with the exception of the carefully handled virus permitted only in those laboratories whose facilities can guarantee that no accidental escape of virus can take place.

Total eradication does not mean partial control in the sense that, within a stated interval of time, no losses will occur from hog cholera. Rather, total eradication must mean just what the name implies, the total obliteration of hog cholera virus except in research laboratories as above stated; it must mean an end to vaccination with any live hog cholera virus; and it must mean that eventually, the pig population will become totally non-immune. Although at first thought, this may seem contrary to the main objective of total eradication, the technical meaning of "non-immune" does not necessarily imply "non-resistance" to hog cholera. For instance, after vaccination with cowpox virus, an individual is considered immune to smallpox virus; it might be more correctly stated that an individual is immune to cowpox virus and resistant to smallpox virus since they are different viruses. In a similar manner, resistance may be possible against hog cholera by use of a virus other than hog cholera (1), thereby permitting a pig population that is non-immune yet resistant to hog cholera.

In earlier days, when the etiology of hog cholera was being discovered (2), hog cholera caused devastating losses in swine. The introduction of a protective vaccine (3), even though it meant using virulent hog cholera virus simultaneously with serum, was hailed as salvation by swine producers. With such vaccination the swine industry grew; no longer did hog cholera create the panic it did before vaccination. Therefore, it cannot be true that all of the hog cholera found today is the result of virulent vaccine virus, although few would doubt that virulent vaccine virus can spread hog cholera and that it must no longer be used. Virulent vaccine virus must be eliminated as a preventive, and has been to a large extent as the newer modified virus vaccines (4) have replaced the older, more dangerous method. But, even modified vaccine viruses eventually must go if total eradication is to be an accomplished fact. This is not to imply that eradication cannot proceed with

Veterinary Virus Research Institute, Cornell University, Ithaca, N. Y.

354
modified viruses, but it can succeed only if the vaccine viruses meet all of the rigid requirements of efficacy and cannot spread the disease they are designed to prevent.

While it is conceivable that hog cholera could be eradicated simply by quarantine and slaughter of all infected animals, without the use of any preventive products, this procedure would be most unwise unless we are fully prepared to accept losses such as occurred before any vaccine whatsoever was used.

Because preventive products appear to be necessary, careful consideration should be given to those available or suggested. These include:

I. Antiserum.

II. Virulent hog cholera virus.

III. Modified hog cholera virus.
   A. Does not require serum:
      1. Spreads.
      2. Does not spread.
   B. Does require serum:
      1. Spreads.
      2. Does not spread.

IV. Inactivated hog cholera virus.

V. Virus of virus diarrhea (VD) of cattle.

In these proceedings of the United States Livestock Sanitary Association, reports have been made of three potential products not in general use. These products are designed either to immunize or to prevent losses from hog cholera. One of these is an inactivated vaccine virus (5) that is effective when given in two doses and which does not spread hog cholera, therefore, it would be safe and efficacious. Unless this vaccine differs from others of this type, duration of actual immunity will be short although resistance characterized by secondary response should last for a longer interval of time. Preparation of this particular vaccine requires the preliminary use of considerable amounts of virulent hog cholera virus, and, under present government regulations, inoculation of susceptible pigs with fully virulent virus is required in the necessary potency tests.

The tissue-cultured cytopathogenic hog cholera virus (6) has the more desirable attribute of permitting assay of virus content of vaccine without actual use of swine. If field tests bear out present laboratory findings that it is safe and does not spread, then an ideal live virus has been found. Furthermore, if the neutralization test (7) made possible by this cytopathogenic strain of hog cholera virus continues to prove an indicator of immunity (8) (9), then tests using virulent virus for efficacy evaluation would be unnecessary.
The newest method of all should receive attention also, because it may prove to be the best method, if it can be made safe and efficacious. This is the use of virus of virus diarrhea (VD) of cattle for the preparation of a vaccine designed to give resistance to hog cholera and thus prevent losses from this disease (10). A finding of this order should stimulate and greatly shorten the whole procedure of eradication in that vaccines made from hog cholera virus of any sort could be terminated.

Important as immunizing products may seem to be, only by proper and extensive use can they help eradicate hog cholera. They should function to prevent enough natural transmission of virulent hog cholera virus in order to bring about a biological die-off of the virulent virus. To attain this goal, a program more comprehensive than mere vaccination must be initiated. In general, there should be:

1. A genuine desire to eradicate hog cholera by those concerned in swine production.
2. A program based upon practical regulatory methods.
   a. Funds to make possible execution of this program.
   b. Education to obtain and coordinate cooperation.
   c. Products that are efficacious, yet which will not require consistently virulent virus, either for their production or their evaluation. If virulent virus ever is necessary, then it must be carefully controlled.
   d. Control over movements of any infected swine or material contaminated with virulent virus, in order to prevent further spread.
   e. An enforcement procedure under Federal supervision.
   f. Further research to develop still better methods of prevention and diagnosis for the future.

The signal to begin a program of eradication of hog cholera has been given. All of the factors that make for success seem to be present. Communication for understanding directives and regulatory procedures between authorities and livestock owners is possible through the press, radio and television. Preventive products are ample and can be supplied in efficacious form wherever and whenever they are needed. Experienced and capable people in the Animal Disease Eradication Branch, working under expert direction in the new government laboratory at Ames that is the most modern and best equipped animal disease laboratory in the world, will execute the program. Although unforeseen problems, of course, will arise, there could be criticism by the uninformed, and personal ambition may intervene to make the task more difficult than it should be, success can be expected.

DISCUSSION

DR. VAN ROEKEL: I should like to ask Dr. Baker. Do you propose live VD virus for hog cholera?
DR. BAKER: One of the intriguing points about live virus vaccines is the long period of protection that they provide. If live VD virus is shown to be safe and efficacious, then I certainly would propose its use as better than either live hog cholera virus vaccine or inactivated hog cholera virus vaccine.

DR. SUTTON: I would like to ask Dr. Sheffy, what he attributes the lack of resistance in the natural pigs due to VDV?

DR. SHEFFY: At this time, we are unable to draw final conclusions. It could be that maternally transferred hog cholera antibodies interfere with VD virus protection against hog cholera; it could be the strain of virus at the high passage levels used that is causing this lack of effectiveness. Tests are underway to decide these points. Of course, if it is interference from hog cholera antibodies, then VD virus vaccine would be used as hog cholera virus vaccines are used. If it is the strain of virus, then search for a better strain would solve this problem. Already, we have strain N.Y. 1 VD virus under test in this search. We hope the near future will resolve your question, Dr. Sutton.

REFERENCES


REPORT OF THE COMMITTEE ON THE NATIONWIDE ERADICATION OF HOG CHOLERA


A decade has passed since the inception of this Association's Committee on the Nationwide Eradication of Hog Cholera. During this period of time much information has been presented, evaluated and disseminated relative to the eradication of this most costly disease affecting the United States swine population. Over these years many hours of toil have gone into the development and coordination of plans for elimination of this insidious problem. We now stand on the threshold of launching this effort for which we have been preparing during these years. At least a dozen States are in process of either establishing Statewide hog cholera eradication committees or are preparing immediately to put into operation an all-out eradication effort.

Since our Committee report of last year, an additional eight States have adopted laws or regulations to prohibit the use of virulent hog cholera virus, thus bringing the present total to 40 States.

Your Committee wishes to commend the College of Veterinary Medicine of the University of Minnesota in cooperation with the Animal Disease Eradication Division of the United States Department of Agriculture for their efforts in organizing and presenting the excellent Symposium on Hog Cholera held in conjunction with this year's meeting of the Association. This was one of the most informative sessions ever held dealing with this disease.

The Committee wishes to take note of a cooperative effort on the part of the serum-virus producing industry and the Biologics Inspection Section of the United States Department of Agriculture with reference to the recommendation of this Committee in our report last year—that of giving consideration to the shortening of expiration dates of modified live virus vaccines so as to increase the degree of immunity conferred to swine vaccinated with these products. Information developed within the Suwannee County, Florida, pilot test program indicates that within the past nine months, as a result of effecting these recommended changes on products used within that area, the degree of immunity conferred in animals challenged during this time has increased from a low of 57.5 percent to 76.5 percent, and is continuing to rise.

It is also noteworthy that a year has elapsed since the confirmation of the last case of hog cholera in Suwannee County.

Your Committee has been advised that the United States Department of Agriculture, cooperating with the State of Georgia, proposes this winter to
institute what might be considered an extension of the pilot test program now underway in Suwannee County, wherein swine in Lowndes County, Georgia, will be subjected to a program utilizing a double method of vaccination, 30 days apart, with inactivated vaccine. Later challenge of representative groups of these vaccinated swine will be made in the Department's facilities at Live Oak, Florida, for immunization evaluation.

Your Committee last year recommended that a Swine Disease Section within the United States Department of Agriculture be established, and it is gratifying to note that with the passage of Public Law 87-209 which provides for the establishment of a 12-man Committee to initiate a national hog cholera eradication program in cooperation with the several States, the impetus has been provided which will in effect fulfill the recommendation of our group.

In order to properly coordinate the efforts of the United States Livestock Sanitary Association with those of the Secretary's 12-man Advisory Committee, it is recommended that the Association give consideration to recommending to the Secretary of Agriculture that a representative from this Association serve as a member of the 12-man Advisory Committee.

During the past years, inquiries have been directed to the question of establishing hog cholera free areas within the country. Such status may be realized presently in those States having a limited swine population and whose marketing and transportation problems are such that little exposure to the swine industry exists. Your Committee recognizes that the establishment of such areas throughout the country would enhance a nationwide eradication effort and therefore recommends the following as a suggested format for establishing and maintaining hog cholera free areas:

An "Area" should not include less than a State.

To be declared hog cholera free, the following requirements are to be met within the area:

**Reporting System:**

a. Hog cholera must be a reportable disease.
b. A definite reporting system must be established and in effective operation at least 12 months before the area can be declared free.

**Vaccination:**

a. Prohibition of fully virulent virus or live virus vaccines as immunizing agents against hog cholera; provided that the appropriate State and Federal authorities may authorize the use of these products in supervised research and biologic production.
b. Vaccination permitted only with inactivated (killed) vaccines. (Note: Questions may be raised about including the possibility of using live vaccines of alien host origin, or other products which may in the future be developed with non-virulent properties and which can be used with complete safety. If such products should in the
future be licensed for production, their use could be considered at that time.)

c. All vaccination against hog cholera shall be official (requiring identification and reporting).

d. Regulatory authorities in the area should be notified of all shipments of hog cholera immunizing products, including antiserum, going into the area.

(Note: The Serum-Virus Control Agency should consider methods by which such information could be furnished to the various hog cholera free areas.)

Quarantine:

a. Authority to quarantine, including authority to quarantine on suspicion of hog cholera, and to maintain such quarantine for whatever period may be deemed necessary.

b. Authority to quarantine swine fed raw garbage and to maintain quarantine as long as deemed necessary.

Garbage Cooking:

a. Laws or regulations that clearly define cooked garbage, require that only cooked garbage be fed to swine, and provide penalties for violations.

b. Swine found to be fed raw garbage will be placed under strict quarantine, such quarantine to be continued in force under official veterinary supervision for a period of at least 30 days after proper cooking measures have been instituted; provided that quarantine may be removed if no indication of hog cholera has been observed during that period.

c. Personnel sufficient to provide garbage cooking inspection as frequently and thoroughly as necessary.

Importation of Swine:

a. Importation for purposes other than immediate slaughter must be under permit and either:

(1) Originate in an adjacent hog cholera free State and enter on a veterinary health certificate, such swine to be held in strict isolation and quarantine upon arrival at destination for a period of at least 21 days; or

(2) Have been vaccinated with inactivated (killed) vaccine not less than 21 days nor more than 10 months prior to shipment and move on veterinary health certificate.

b. All swine imported shall be transported in vehicles cleaned, disinfected and bedded under supervision just prior to shipment.
Hog Cholera Free Area may be declared if the above requirements are met, and

a. Twelve months have passed without hog cholera being diagnosed within the area;

b. Twelve months have passed after prohibition of use of fully virulent virus and live virus vaccine within the area, and for importation into the area of feeding or breeding swine so treated;

c. A statistically sound survey of swine within the area fails to reveal any indication of hog cholera.

(Note: Possibilities for accomplishing this purpose include:

1. Inspection;
2. Slaughterhouse testing as a screening device.)

Maintenance of a Hog Cholera Free Area shall include the following:

a. If no outbreaks of hog cholera other than primary outbreaks occur, an area may be maintained as hog cholera free provided that such primary cases are completely eliminated. 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 primary outbreak shall result in revocation of the hog cholera free status.

b. Prompt disposition of infected herds on the farm by supervised burning or burial.

c. Authority and action to inspect premises and conduct traceback investigations.

d. A statistically sound survey of swine within the area fails to reveal any indication of hog cholera.

One of the monumental tasks with which your Committee has been confronted evolves around the establishment of a hog cholera program by which this disease may be eliminated from each of the several States. It is the considered opinion of your Committee that such cannot be set down in specific details to cover the diversified problems existing in each State; however, it is felt that the format for an eradication program can herein be established to provide general guide lines for use in the several States and upon which such an eradication program can be predicated. To this end your Committee recommends the adoption of the following:

1. Educational program.

It is imperative that in launching a hog cholera eradication project an intensified educational program must be effected to fully acquaint all facets of the industry, the profession, and the general public with the benefits to be derived from the eradication of hog cholera and the part that each segment must play in the successful conclusion of such an eradication program. Much of this ground work has been laid by this Association, Livestock Conserva-
tion, Inc., the United States Department of Agriculture, the American Veterinary Medical Association, serum and virus producers, and various agricultural publications. There is much information available on this subject and additional material is presently being developed which will aid in this direction. It cannot be over-emphasized, however, that in obtaining the necessary legislation and cooperation from farmers and industry groups, this is a most important feature and must be strongly pushed in the individual States launching such a project.

2. Vaccination of swine.

In order for a nationwide hog cholera eradication program to succeed, the susceptible swine population must be decreased through accelerated vaccination. It is the opinion of the Committee that virulent hog cholera virus should not be used in an eradication program as has been emphasized by this Association for many years. Therefore, the use of virulent virus should be restricted to authorized usage only in the fields of supervised research and biologic production.

In its report of last year, the Committee advised against the use of serum alone in the treatment of swine at sales markets, recommending in lieu thereof to follow the practice of vaccinating swine simultaneously with modified live virus vaccines and adequate amounts of hog cholera anti-serum except in those cases where based on scientific data the use of vaccine is contra-indicated. In an eradication program, therefore, the use of serum alone must be carefully considered.

In establishing permanent immunity to hog cholera, the ideal is realized when products are administered which effect antibody response within a few hours following inoculation. Consequently, an immunizing agent which confers an immediate response such as that produced with the use of hog cholera anti-serum should be considered for use with those permanent immunity producing products which can be used in conjunction therewith in a vaccination program. This is particularly true when the possibility of exposure to the disease exists.

The use of inactivated vaccines is indicated in unexposed areas or areas subject to little exposure. It must be realized, however, that such vaccines will not produce desired immunity until three weeks have elapsed from time of vaccination, nor can the immunizing properties be expected to last for a period of longer than 10 months.

The efforts of research in the development of more efficient immunizing products must be continued and the products resulting therefrom carefully evaluated for use in eradication programs.


In considering intrastate and interstate movements of swine, your Committee recognizes that the ultimate is the establishment of immunity against hog cholera prior to such movements and that this could best be effected by vaccinating swine at least 21 days prior to any contemplated shipments. This
accomplishment may not be possible in all States and in such event it is recommended that the acceptable methods of vaccination hereinbefore described be employed.

On interstate movements of swine except for immediate slaughter, the following specific methods of vaccination are recommended:

a. Modified live virus vaccine and a minimum dose of at least 20 cc. of hog cholera anti-serum. Animals should have been treated at least 21 days prior to shipment; however they may be shipped so as to arrive at destination within 72 hours from the time of treatment, to be isolated and quarantined at destination for a period of at least 21 days.
b. Modified live virus vaccine alone, not less than 21 days prior to shipment.
c. Killed or inactivated vaccine administered not less than 21 days nor more than 10 months prior to shipment.

4. Identification of Treated Swine.

Because of the many ramifications resulting in the trafficking and marketing of swine, it is imperative that swine vaccinated in an eradication program be properly identified by the administrator of the vaccine at the time of treatment. To this end, your Committee urges that immediate action be initiated by the United States Department of Agriculture toward developing more efficient methods of permanent swine identification than are presently in use.


A report of swine vaccinated against hog cholera should be made promptly to regulatory officials administering eradication programs. Appropriate forms for accomplishing this should be furnished to administrators by the responsible agencies.

6. Reporting Hog Cholera Outbreaks.

Inasmuch as it is imperative in the control or eradication of a disease that existing pockets of infection be known, an efficient system of reporting the existence of hog cholera should be established by regulatory agencies.

7. Quarantine of Suspected or Infected Herds.

All herds known or suspected to be infected with or exposed to hog cholera should be quarantined and no swine allowed to be moved into, through or from the premises except under official supervision.

8. Disposal of Carcasses.

Carcasses of swine which have been condemned or have died from hog cholera should be disposed of promptly by burning, burial, or rendering
under official supervision. Consideration should be given to permitting the movement under official supervision of apparently healthy swine from infected or exposed herds to approved slaughtering establishments for heat processing so as to destroy any virus which may be present.

9. **Disinfection of Infected Premises.**

Infected premises and all vehicles and equipment used in connection therewith shall be thoroughly cleaned and disinfected under supervision. In restocking previously infected premises care should be exercised to preclude infection of such replacements. Consideration must be given to the vaccination status of the replacements and lapse of time since removal of infected animals.

10. **Indemnity.**

The payment of indemnity for swine lost or required to be slaughtered in an official eradication program should be made in accordance with existing laws in the several States; however, it is suggested that in order to obtain a greater degree of participation in the vaccination and eradication programs, consideration be given to the adoption of a sliding scale of indemnification which would permit greater indemnity to participating owners than would be realized by owners not cooperating under the program. The amount of salvage realized from apparently healthy swine in infected herds which are heat processed in approved slaughtering establishments should be deducted from the appraised value of the animals.

11. **Disinfection of Stockyards, Markets and Swine Concentration Points.**

In establishing an eradication program, consideration must be given to the fact that swine concentration points constitute a very definite hazard in the dissemination of hog cholera, and therefore regular cleaning and disinfection practices should be employed at such concentration points to preclude further spread of disease.
1960 OUTBREAK OF STOMATITIS AND LAMENESS OF CATTLE IN TEXAS, OKLAHOMA AND ARKANSAS

LEWIS E. SEAY, D.V.M.*

Stomatitis or inflammation of the mouth of cattle occurs frequently and is widespread. It may appear as a local disease, as a symptom of other diseases or as a secondary condition under a wide variety of circumstances. The lesions in these conditions appear on the same parts of the body of cattle as foot-and-mouth disease and vesicular stomatitis and are very difficult to differentiate during certain stages of the disease (1).

The late Dr. John R. Mohler described a stomatitides of cattle which is similar to the condition which I studied in Texas, Oklahoma and Arkansas in the summer and fall of the year 1960†.

Several veterinarians have described this syndrome and some of the names which have been applied to it are aphthous stomatitis, sore mouth of cattle, noninfectious foot-and-mouth disease, and mycotic aphthous stomatitis (2). Others have expressed the opinion that it was an atypical form of vesicular stomatitis (5).

There was an inclination to believe the latter was possibly correct until many cattle were observed in all stages of the disease and samples submitted to the Vesicular Disease Diagnostic Laboratory, Beltsville, Md. Laboratory reports indicated all of the samples were negative for New Jersey and Indiana types of vesicular stomatitis and seven types of foot-and-mouth disease.

OCCURRENCE AND DISTRIBUTION

Occasional cases of this type stomatitis have been reported and observed in the three-State area for years. The condition has always been reported in the summer and fall, and the affected animals have always been on pastures. However, the first documented extensive outbreak in the area occurred in 1960.

The first two cases were reported in late June, 1960 in a herd in Central Texas near Stephenville. About a week later the condition was reported around Ada, Oklahoma. It was not reported in Arkansas until October of 1960. From the last of July until the first part of September it was widespread along the Red River from Ardmore to Hugo on the Oklahoma side and from Gainesville to Paris on the Texas side. There were a few cattle observed with this syndrome in scattered areas throughout both States. Most of the Arkansas cases were in the Mena Area.

Between June, 1960 and November 15, 1960 investigations were made on 126 premises in the three States. There were approximately 300 cattle on the 126 premises that were clinically affected and about 2,000 that did not show any visible symptoms or lesions.

* Diagnostician and District Veterinarian, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Austin, Texas.  
† With assistance and cooperation of Dr. D. C. Bostwick and Dr. E. M. Joneschill.

365
CLINICAL SYMPTOMS AND LESIONS

The inability to graze and the tendency to spend most of the time lying down were usually the first symptoms observed. In some cases there was a drivel of saliva, extreme congestion of the muzzle and teats with an elevation in temperature of two to four degrees. The temperature usually returned to normal in three to four days. The muzzle and teats became dry and parched in appearance followed by erosions and exfoliation of the superficial layer of the skin. If the mouth is examined in the early stages it will be found red and hot, with congested slightly raised area which change to small pustules. These have often been mistaken for blisters. The pustules erode and develop into ulcers varying in size from one-eighth inch to one inch in diameter. A large irregular indented patch is often formed where several ulcers have coalesced. The lesions are most frequently found on the gums around the incisor teeth, on the dental pad, inside the lips and dorsal anterior part of the tongue, but they may occur on the cheeks, interdental space and on the hard and soft palate. In some cases there was a marked subcutaneous edema of the face, ears and muzzle, similar to that observed in some sheep with blue-tongue.

In animals that have been affected for about a week, there is usually extensive necrosis of the mucosa of the anterior part of the oral cavity and tongue. The lesions will be covered with accumulations of necrotic tissue and the mouth will have an extremely fetid odor. The tongue may be protruding from the mouth one to three inches (2) (3).

In this outbreak a very high percent of the affected animals developed a severe founder type lameness of all four feet. The lameness occurred in some cases simultaneously with the mouth lesions and others two or three days after the stomatitis and occasionally it would be the first symptoms observed. In a few cases lameness was the only symptom seen. There was a marked subcutaneous edema above the hoof but it was never observed extending above the knee or hock. If the animals were observed in the early stages a reddish blue line was very pronounced above the coronary band similar to that seen in sheep with blue-tongue. Affected animals were sensitive to palpation in this region and were extremely reluctant to rise. When they were forced to get up they generally walked on their heels with a stiff gait, and if left alone would soon lie down. A few animals would walk on their toes with a stilted gait. This appeared to be a laminitis type lameness and breaks in the skin around the coronet were not observed, but in a few cases the skin in the cleft of the foot did become fissured and eroded. No vesicles or lesions that looked like freshly ruptured vesicles were observed in the oral cavity or on the feet.

In some cases there was a profuse diarrhea, usually dark in color, with a very offensive odor. The diarrhea was not a common symptom and usually responded to treatment.

The condition affected cattle of either sex and of all ages, but most of the cases were in animals over six months and under two and one-half years of
There was no detected co-relation between the age of the animal and type and severity of lesions. Both young and old cattle were observed with mild and severe cases. Some cases were so mild that they could easily go unnoticed by the owner. In severe cases the animal was greatly depressed, became dehydrated and spent most of the time lying down or standing off by itself with the hind feet drawn under the body and front feet extended.

Almost all of the affected animals made complete recoveries in two to four weeks. However, the ones that were severely lame developed the so-called founder ring on the wall of the foot.

**TREATMENT**

The infected cattle were removed from the pasture and isolated from the herd. A convenient supply of fresh water was provided and the animals were fed soft, nutritious feed, such as bran mashes or ground feed. Mild antiseptics were used to wash the mouth of the more gentle animals. In some extremely dehydrated cases glucose or dextrose was used. Antibiotics and/or antihistamines were used in many cases. Injections of sodium iodide were used on some animals without beneficial results.

**MORBIDITY AND MORTALITY**

The herd morbidity rate was generally low with only a few animals markedly affected, but it was possible for mildly affected animals to go unnoticed. The highest morbidity rate in this outbreak was in a herd of twenty-one steers in Fannin County, Texas, where seventeen were visibly affected without any deaths. There were very few deaths in the outbreak and those that did occur were usually caused by secondary factors or poor animal husbandry. Table I illustrates the low morbidity and mortality rate of animals affected by this condition in 25 herds.

**CAUSE**

The causative agent or agents in this outbreak is still unknown. Field exposure failed to spread the disease from animal to animal and the pattern of the outbreak indicated that it was not spread by animal to animal or man to animal contact. After the vesicular diseases were eliminated by laboratory test the possibility of bluetongue was considered, because symptoms and lesions of cattle in this enzootic were so similar to those of bluetongue in sheep. Blood samples preserved in O.P.G. solution* were submitted to the Animal Disease Research Laboratory at Denver, Colorado, but bluetongue could not be reproduced by inoculating this blood into test sheep and the sheep when challenged later to known bluetongue virus were fully susceptible.

* Potassium Oxalate, Phenol and Glycerine.
### TABLE I

**Herds Typical of 126 Premises Investigated**

<table>
<thead>
<tr>
<th>Herd Number</th>
<th>Cattle in Herd</th>
<th>Number Cattle Affected</th>
<th>Number Recovered</th>
<th>Number Deaths</th>
<th>Age Animal Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>None</td>
<td>10 and 11 months</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>3 months</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>2</td>
<td>2</td>
<td>None</td>
<td>4 years and 2 months</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>None</td>
<td>6 months</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>2</td>
<td>2</td>
<td>None</td>
<td>12 and 18 months</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>2 year bull</td>
</tr>
<tr>
<td>7</td>
<td>155</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>4 year cow</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>None</td>
<td>7 day calf and 2½ year cow</td>
</tr>
<tr>
<td>9</td>
<td>65</td>
<td>2</td>
<td>1</td>
<td>1 cow</td>
<td>1⅔ years and 10 years</td>
</tr>
<tr>
<td>10</td>
<td>52</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>1 year</td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>3 months</td>
</tr>
<tr>
<td>12</td>
<td>40</td>
<td>5</td>
<td>5</td>
<td>None</td>
<td>6 months and 1 year</td>
</tr>
<tr>
<td>13</td>
<td>24</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>6 year cow</td>
</tr>
<tr>
<td>14</td>
<td>35</td>
<td>1</td>
<td>0</td>
<td>1 steer</td>
<td>7 months</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>3 year cow</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>None</td>
<td>4 and 5 months</td>
</tr>
<tr>
<td>17</td>
<td>21</td>
<td>17</td>
<td>17</td>
<td>None</td>
<td>10 and 18 months</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>None</td>
<td>3 and 8 months</td>
</tr>
<tr>
<td>19</td>
<td>50</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>3 year cow</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>7 year cow</td>
</tr>
<tr>
<td>21</td>
<td>25</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>5 year cow</td>
</tr>
<tr>
<td>22</td>
<td>20</td>
<td>8</td>
<td>6</td>
<td>2 cows</td>
<td>6 months and 12 years</td>
</tr>
<tr>
<td>23</td>
<td>18</td>
<td>2</td>
<td>2</td>
<td>None</td>
<td>4 years and 10 years</td>
</tr>
<tr>
<td>24</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>None</td>
<td>7 months</td>
</tr>
<tr>
<td>25</td>
<td>14</td>
<td>4</td>
<td>4</td>
<td>None</td>
<td>6 and 12 months</td>
</tr>
</tbody>
</table>

### CONCLUSIONS

Appears possible that this syndrome was caused by a factor or factors which are contained in the forage since all of the cases were in cattle on pastures. Possibly the syndrome is caused by a mold or molds or a change in the chemical nature of the forage itself. It appears that certain climatic conditions must be present before the condition will occur.

### REFERENCES

REPORT OF THE COMMITTEE ON VESICULAR DISEASES

F. J. Mulhern, Chairman, Falls Church, Virginia; J. J. Callis, Greenport, L. I., New York; A. A. Erdmann, Madison, Wisconsin; R. P. Hanson, Madison, Wisconsin; L. Karstad, Waycross, Georgia; S. H. Madin, Berkeley, California; N. L. Meyer, Alexandria, Virginia; L. O. Mott, Beltsville, Maryland; J. Traum, Berkeley, California.

VESICULAR DISEASES—CURRENT THREAT

It has now been more than two years since the Secretary of Agriculture announced the eradication of vesicular exanthema and five years since the last outbreak. The Committee notes, however, that a relaxation of the controls concerning the use of garbage for swine feed could lead to disaster. The international movement of people and things continues to increase and the possibility of disease producing agents being spread through garbage feeding is greater than ever. Animal Quarantine authorities apprehend thousands of travelers each year bringing in small quantities of prohibited meats. Others carrying such items undoubtedly go undetected. Research at the Plum Island Laboratory has shown that Foot-and-Mouth disease virus is more resistant to heat and chemical destruction than we previously believed. The possibility that smuggled in meats might contain the virus of FMD must be taken into consideration. The Committee recommends that this Association urge the United States Department of Agriculture to strengthen requirements for the importation of livestock and their products to meet the ever increasing danger of accidental introduction of a foreign animal disease. The Committee recommends that this Association take note of this increased danger by asking that all States consider this fact when planning enforcement of garbage cooking laws, particularly in the coastal regions where the garbage from ocean vessels originating in FMD infected countries may illicitly find its way to the garbage feeder. Other States must also use great care. Any piece of meat imported by a foreign traveler and which finds its way into a garbage can anywhere in the United States is a potential threat. This threat can be very greatly reduced by diligent enforcement of garbage cooking laws.

VESICULAR STOMATITIS

There has been an extensive outbreak of New Jersey type vesicular stomatitis during the summer of 1961. In one area the disease was mistakenly diagnosed as "Mycotic Stomatitis" and spread over a wide area and involved hundreds of farms before it was properly identified. There is every reason to believe that an outbreak of Foot-and-Mouth disease might be similarly misdiagnosed.

This Committee recommends that the United States Livestock Association urge every State and Federal official to review procedures for handling
REPORT OF COMMITTEE

reports of disease which involve foot, mouth, or teat lesions. If there is the slightest indication that a contagious, infectious disease exists diagnostic assistance should be requested.

This year has seen the largest number of laboratory confirmed diagnosis of vesicular stomatitis since laboratory testing started. This does not necessarily mean that there have been more cases of vesicular stomatitis but more emphasis has been placed on accurate definition of infected areas by testing tissue and serum specimens from more herds within infected areas.

The first laboratory confirmed case of the year was reported in June from Horry County, South Carolina. Active infection was confirmed in additional counties in South Carolina in June. Several cases were reported in Southern Georgia in July and then in August the disease seemed to spread rapidly Northward in the Appalachian chain of mountains with confirmed cases in Northern Georgia, Eastern Tennessee, and Western North Carolina. In September a large infected area was identified in the rolling lands of the foothills of the Southern Appalachian chain of mountains in East Central Alabama.

Since January, 1961, tissue and/or serum samples have been submitted for laboratory tests from 230 farms in eight States. Laboratory tests confirmed the diagnosis of New Jersey type vesicular stomatitis from 68 farms in Georgia, 45 farms in Alabama, two farms in Tennessee, 20 farms in North Carolina and six farms in South Carolina for a total of 141 confirmed cases since June of this year.

We are still without proof as to the method of spread of this disease. Dr. Vernon H. Lee, entomologist on the staff at the University of Wisconsin, assisted in the investigation of the outbreak. In North Carolina approximately 20 species of insects were trapped in the vicinity of infected premises. These insects are being processed to determine virus content. In his report Doctor Lee indicated a higher than average rainfall in much of the affected area, but its relationship to the outbreak has not been definitely established.

Isolated cases of VS have previously been diagnosed in mountainous areas. During the summer of 1961 more than 50 percent of the cases occurred in the rolling land and foothills of the Appalachian Mountain chain and in the Appalachian and Blue Ridge mountains. Its spread could not be explained by wind direction, temperature patterns or the normal movement of livestock.

Considerable additional intensive investigation will probably be needed to adequately understand the factors affecting the spread of this disease.

RESEARCH OF VESICULAR DISEASES

Vesicular Exanthema.

Vesicular exanthema of swine (VES) has not been known to exist anywhere in the world for several years and was officially declared eradicated in the United States by the United States Department of Agriculture on October 22, 1959 (1). Research on VES at the Plum Island Animal Disease
Laboratory was terminated during 1960; diagnostic capabilities having been achieved for the currently identified types of the disease and sufficient quantities of diagnostic materials having been produced, tested and placed in storage. Existing methods for diagnosis by complement-fixation were improved (2).

Vesicular Stomatitis.

Studies on the structure of vesicular stomatitis (VS) virus were first reported by workers from the Research Institute, Pirbright, England (3). This subject has been studied at other laboratories, and, in general, their findings agree with those developed by the British. There apparently seems to be little question that the virus is rod-shaped with an average diameter of approximately 65 mu. and a total length of approximately 175 mu. (4) (5) (6) (7). Chorioallantoic membranes of chicken embryos infected with VS virus were also studied with the electron microscope and granular areas were observed in cells of the chorionic and allantoic layers. These areas, which are separated from the remaining cytoplasm by a membrane, are thought to contain imbedded virus particles and it is assumed that these areas represent the centers of virus development (4). Similar centers have been demonstrated in infected cultures of swine kidney cells using fluorescent microscopy (8).

In long-term studies conducted on the immunology of VS in cattle and horses, it was observed that complement-fixing antibodies appeared in six to eight days following inoculation and reached a peak within nine to 16 days. Thereafter, they gradually declined and disappeared in 50-110 days. Serum-neutralizing titers were demonstrated in six to eight days postinoculation and continued to increase for four to five weeks. The titers of the neutralizing antibodies remained high but fluctuated irregularly within less than a two-log range for the duration of the test. Most of the recovered animals which no longer showed a complement-fixing titer were susceptible to challenge by inoculation with homologous type virus even though the serum from these animals contained a rather high level of serum-neutralizing antibody. The data developed in this study indicated that the presence of serum-neutralizing antibodies at high levels does not necessarily indicate resistance to infection when inoculated with infective virus (9).

Foot-and-Mouth Disease.

It has been reported that one major vaccine production laboratory in South America is producing substantial quantities of foot-and-mouth disease (FMD) vaccine containing virus grown on monolayers of trypsinized bovine kidney cells. The product is apparently being used with success. This method of virus production is being studied in Italy and experimental lots of vaccine are being produced on a weekly basis (10). Experimental lots of such vaccine are also being produced at Plum Island (11).

Fluorocarbon preparations have been used for purifying antigen of several viruses, including FMD virus. The combination formed between FMD
viral antigens and antibodies may be separated by treatment with this chemical. Virus separated from antibody by this method has been found to be infective (12).

By reorganization of the experimental design commonly used for titration of FMD virus in unweaned mice, methods have been developed which will yield results capable of resolution by analysis of variance. The results achieved depend to a considerable extent upon the strain of mice used. It was also found in this study that randomization of young among the mothers within a mouse strain significantly increases precision while not affecting susceptibility. It was found that the sex of the experimental subject has no influence upon susceptibility. The relative susceptibility of some strains of mice depends to some extent upon the strain of virus being tested. The intramuscular route of inoculation most generally gives a higher endpoint with most all virus strains than the intraperitoneal route and this method of inoculation was concluded to be best for virus detection. The plaque count in monolayers of cultures was determined to be the most accurate method of endpoint estimation (13).

In studies conducted at Plum Island, one strain of mice was studied to determine the age range from which equivalent response to FMD virus could be expected. Using the Rockefeller H strain of mice and type A-119 FMD virus, mice five to 14 days of age were found to be equally susceptible but became resistant after the sixteenth day (14).

In histopathological studies of pregnant and lactating mice infected with type A-119 FMD virus, the major pathological alteration was necrosis of the pancreatic acinar cells, development of laminated intracytoplasmic bodies in some of the pancreatic acinar cells, severe myocardial necrosis, skeletal muscle necrosis, involution of the thymus and the destruction of lymphocytes in the spleen and lymph nodes. It was concluded in these studies that these changes might be due to the direct effect of the virus but were probably influenced considerably by stress induced by pregnancy and lactation (15).

It has been previously reported that FMD virus survived for as long as 50 days in lymph nodes of cured and uncured meat packed in barrels. These studies have been extended and infective virus demonstrated in prescapular lymph nodes removed by surgery from a steer before clinical signs of FMD appeared. Infective virus was also demonstrated in infected lymph nodes removed from an animal five days after regression of disease signs. Hemal nodes from freshly killed and ripened carcasses were shown to contain infective FMD virus and bone marrow from ribs and vertebrae from an infected carcass stored 194 days at 1°C. was also shown to contain infective virus. Swine fed infected bone marrow mixed with bone fragments developed signs of FMD but not when bone marrow alone was fed (16).

In calves experimentally infected with FMD virus viremia exists during the first two days postinoculation and by the fourth day detectable amounts of neutralizing antibody were present, however, in these studies virus was no longer found in the blood at four days. Virus persisted in the kidneys
through at least the sixth day and there was evidence that this virus might be located intracellularly (17).

Relative humidity and temperature have been shown to be important factors when dried FMD virus is exposed to gaseous ethylene oxide for inactivation. When the relative humidity is lower than 40 percent, inactivation could not be relied upon (18).

Under certain conditions, virus of FMD has been shown capable of transmission by air. Animals held in isolation units may be infected by virus carried over a distance of approximately 30 feet from an isolation unit housing infected animals. The susceptibility of the test animals was increased when tongues of the animals were scarified prior to exposure to the air from affected animals (19).

The conglutinating complement absorption technique was adapted to the study of FMD virus systems. This test was found to be sensitive for testing of bovine antisera containing FMD antibodies and more useful than the complement-fixation test for assay of swine serum since in this method the procomplementary effect of swine serum is avoided (20).

The fluid from a vesicle on the hand of a human when placed on tissue cultured cells produced destruction of the cells and a cytopathogenic agent was isolated. Cellular destruction was inhibited by this agent in the presence of "O" type FMD hyperimmune guinea pig serum. This apparently is the first time that FMD virus from a human has been isolated in bovine kidney cells cultured in vitro (21).

A tissue culture color test which makes use of pig kidney cells has been described for the titration of FMD virus and its antibodies. Cultures are prepared in depressions in polyvinyl chloride plates. Phenol red indicator is added to the tissue culture medium and metabolizing kidney cells cause a change in pH media resulting in a change in the color. Virus infected cells do not metabolise, thus there is no change in the color. The color test is a rapid and efficient method of titrating FMD virus and its antibodies and enables large numbers of sera to be assayed readily and accurately (22). An indirect neutralizing test with inactivated viruses of mouse origin has been developed which has been shown to be useful for determining antigen content of inactivated FMD vaccine. The test is based on the observation that formalin-inactivated FMD virus when added to homologous immune serum results in a binding of the neutralizing antibodies of the serum so that when infective virus is added there is little or no further neutralization. The reaction has been designated as the indirect neutralization test and its usefulness depends on quantitative gradation of the reacting components. The author claims that through the use of this test it has been shown that the antigen content of vaccines falls off considerably when the vaccine is placed in storage (23, 24).

REFERENCES


SURVIVAL OF RINDERPEST VIRUS IN EXPERIMENTALLY-INFECTED SWINE

P. D. DELAY, D.V.M.*; W. M. MOULTON, V.M.D†, and S. S. STONE, Ph.D.*

INTRODUCTION

Rinderpest may well be considered as one of the more serious of the animal diseases. It is enzootic in many sections of the world but is not found in the western hemisphere. Thirty-three instances have been cited other than those resulting from military operations, in which the living animal appears to have been incriminated in transmitting rinderpest to rinderpest-free areas (1). Although virulent virus reportedly disappears rapidly from tissues of rinderpest-immune cattle (1), additional data are needed on the viability of the virus in tissues of nonimmunized cattle and in swine.

Hudson and Wongsongarn, in considering the worldwide incidence of rinderpest in pigs, proposed an eastern and western geographic division; Iran and Iraq comprising a boundary between the two areas. “Brief consideration of the geographical distribution of rinderpest suggests that pigs develop the disease in the eastern part of its range but not in the west.” Pigs described as “European” are in areas where this species is not naturally infected with rinderpest (2).

Natural infections of rinderpest in pigs of European origin have never been recognized, according to Scott et al. (3), but experimental rinderpest infection in European swine has been accomplished. Nicolas and Rinjard (4), Robertson (5), Curasson (6), and Carmichael (7), have successfully infected European type pigs with rinderpest virus.

Scott et al. (3) infected pigs of European origin with rinderpest virus by parenteral inoculation, by feeding infected bovine tissues, and by contact.

Nakamura (8) reports slight but characteristic change in the blood picture following inoculation of three pigs with lapinized (L) and three pigs with lapinized-avianized (LA) rinderpest virus. Viremia was seen in all of the pigs receiving L virus and in one pig inoculated with LA virus. Virus neutralizing (VN) and complement-fixing (CF) antibodies were demonstrable in all cases.

This study was designed to measure susceptibility of cross-bred Yorkshire-Tamworth pigs to the Pendik strain of rinderpest and virus survival in tissues of convalescent pigs.

* Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Greenport, Long Island, New York.
† Food and Agriculture Organization of the United Nations, Ankara, Turkey.
MATERIALS AND METHODS

The Pendik strain of rinderpest virus, received from Kenya, East Africa, as accession 214 and passaged twice in cattle at Plum Island Animal Disease Laboratory (P.I.A.D.L.), was used to infect swine. This strain is easily transmitted to cattle by contact. The inoculum, a 50 percent extract of mesenteric lymph nodes from rinderpest-infected cattle, was also used as the C.F. antigen (9). Lymph nodes from normal cattle were similarly treated, and both tissue extracts were adjusted to the same protein nitrogen. The Nakamura III strain was used for V.N. tests in rabbits. Kabete O rinderpest virus, P.I.A.D.L. 2, was used as challenge virus. One ml. of 50 percent spleen suspension of this strain diluted 10^{-5} was lethal for cattle.

Animals Used.

The animals used were as follows:

Swine: Forty-one cross-bred Yorkshire-Tamworth swine, 15 to 19 weeks old, were used for infectivity studies and as controls.

Cattle: Eighteen-month-old grade Herefords were used for virus detection.

Rabbits: Adult New Zealand white rabbits were used for assay of V.N. antibodies.

The buffer used was 5.52 Gm of NaH_{2}PO_{4}, H_{2}O and 9.56 Gm of Na_{2}HPO_{4} per liter, pH 6.8.

Experimental Procedure.

Thirty-one pigs were exposed to rinderpest virus by simultaneous inoculation and feeding. One ml. of Pendik virus containing 10^{4} bovine lethal doses (BLD) suspended in buffer, was inoculated intramuscularly (im) and 10^{4} B.L.D.'s of the virus, suspended in five ml. of milk, was administered per os to each of 31 pigs.

Eight noninoculated control pigs were housed in an adjacent room.

The infectivity of Pendik strain of rinderpest virus was assayed by inoculating each of two heifers i.m. with 1 ml. of the virus diluted 10^{-4}.

Two pigs were injected i.m. with 1 ml. of a 50 percent suspension of mesenteric lymph nodes from a noninoculated steer. These animals were observed daily for 10 days, killed, and examined for lesions.

Serological Tests.

Complement fixation: The procedure was essentially that of Brooksby (10) and Barber et al. (11), using varying dilutions of complement (C') and fixed concentrations of antigen and serum, with overnight fixation at 5 C.

Calculations of C.F. results: With the C' dilution method, a dilution of stock C' was prepared so that a subdilution containing nine-13 percent produced 50 percent hemolysis. Other dilutions were made to contain 20, 30, 45, 67, and 100 percent or undiluted stock C'. Each dilution of C', that is
nine-100 percent, was added to the antigen, serum, antigen plus serum, and buffer solution. Additional buffer was added to keep the volumes equal whenever necessary. Following a fixation period of 18-20 hours at 5 C, sensitized sheep red blood cells (RBC) were added. The tubes were incubated in a 37 C water bath for 30 minutes, centrifuged, and the degree of hemolysis determined spectrophotometrically. The percentage of hemolysis was calculated, converted to probits, and plotted against the logarithm of the percentage of stock C'. The logarithm of the C' for 50 percent hemolysis was determined graphically and converted to the percentage of C'. Thus, with C' alone, an eight percent solution may be required for 50 percent hemolysis and this concentration of C' defined as one hemolytic unit (1CH\textsubscript{50}). This percentage was divided into the percentage of C' required for 50 percent hemolysis, for antigen, for serum, and for serum plus antigen. For example, antigen may require 12 percent C' for 50 percent hemolysis, which would be equivalent to 1.5 units for C' or 0.5 units more than the C' alone. This value (0.5) represents the anticomplementary effect of the antigen and must be subtracted from the number of units fixed by the serum plus antigen. The test sera were treated in the same way to determine anticomplementary activity. The percentage of C' required for 50 percent hemolysis was less than the amount needed with C' alone. This difference precluded use of a correction factor for the sera.

Virus Neutralization Tests.

Virus-neutralizing antibodies of pig sera were assayed in rabbits by a modification of the method of Scott and McLeod (12) as described by Stone and DeLay (13).

Method of Tissue Collection and Preparation.

Methods designed to control external infection and cross-contamination were emphasized because of reported difficulty in containing Pendik strain of rinderpest virus. Noninfected and infected pigs were housed in adjacent isolation rooms. Tissues from noninfected pigs were removed in the same room immediately before tissue collection from infected pigs. Before tissue collection, the animals were removed to a previously decontaminated area, killed, scrubbed in flowing water, and their entire bodies washed with two percent acetic acid. After acid treatment, the bodies were removed to another previously decontaminated room where the abdominal, ventral, thoracic, and cervical areas were heated with a blow torch and covered with a sterile shroud.

Bone marrow, kidney mesenteric lymph nodes, and spleen were taken from swine at seven, 22, 36 and 92 days postinoculation (DPI).

Tissues collected from pigs at a given postinoculation test interval were pooled and prepared as 25 percent suspensions in a chilled high-speed blender. Tests for rinderpest virus were conducted immediately after collection and preparation of the swine tissue by inoculating each of two steers with 15 ml. of the tissue suspensions.
RESULTS

Each of 31 pigs inoculated with and simultaneously fed rinderpest virus developed pyrexia. Twenty-one of the 31 pigs had temperature elevations in excess of 105 F. between the third and seventh DPI (Figure 1). One of the inoculated pigs died at 10 and another at 12 DPI.

No change other than temperature rise was detected in the other animals.

![Maximal temperature graph](image)

**Figure 1.** Maximal temperatures of pigs following infection with rinderpest virus.

**Necropsy Findings.**

Numerous discreet areas of congestion approximately 0.5 cm. in diameter were distributed throughout the skin of the abdomen and extended on the legs to the coronary band. Peyer's patches were unusually prominent; hemorrhagic lesions were observed in the mucosa of the small intestine, cecum, and colon. Diffuse congestion was observed on the mucosa of the urinary bladder and gall bladder. Similar changes in the gall bladder but not in the urinary bladder were observed in necropsy of cattle experimentally infected with rinderpest virus. No lesions were found in the oral cavity, and changes in the Peyer's patches were less severe than those observed in cattle.

**Serologic Response in Swine.**

Complement fixation. The results of the CF tests are shown in Figure 2. Duplicate points are not plotted but are included in the mean represented by the bar. Complement-fixing antibodies were detected in sera from two of three pigs at six DPI in all 31 pigs at 22 and 36 DPI, and in 12 of 15 sera at 92 DPI. There was fixation with normal tissue extracts and serum from one animal at 22 DPI and 36 DPI, respectively; however, the C'
fixing level of the sera was raised fourfold in the presence of rinderpest antigen.

Virus neutralization. The pre-inoculation sera did not contain rinderpest VN antibodies; however, the VN index of all sera collected at six DPI was two and the index of sera from pigs taken at 22, 36, and 92 DPI was greater than three.

Persistence of virus in tissues of rinderpest-convalescent pigs. A pool of kidney, bone marrow, spleen, and mesenteric lymph nodes taken from rinderpest-convalescent swine at six and 36 DPI contained rinderpest virus infective for cattle. Inoculums prepared from tissue of convalescent swine collected at 22 and 92 DPI were not infective; steers given injections of a suspension of these tissues did not show clinical signs and were susceptible to challenge with $10^3$ BLD’s of rinderpest virus (Table I).

**Exposure of Cattle to Rinderpest-Convalescent Swine.**

Two steers in contact with 15 rinderpest-convalescent pigs at 41 DPI did not become infected, and both animals were susceptible and died following inoculation with $10^3$ BLD’s of rinderpest virus.
TABLE I

Tests in Cattle for Infectivity of Tissues from Rinderpest-Convalescent
and Noninfected Pigs

<table>
<thead>
<tr>
<th>Tissues of Pigs Inoculated With Rinderpest Virus</th>
<th>Control Pigs*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI No. Pigs Source of Tissues Results</td>
<td>DPI No. Pigs Source of Tissues Results</td>
</tr>
<tr>
<td>7 3 Pool† Infective</td>
<td>7 1 Pool Noninfective</td>
</tr>
<tr>
<td>22 3 Pool Noninfective</td>
<td>22 1 Pool Noninfective</td>
</tr>
<tr>
<td>36 8 Pool Infective</td>
<td>36 1 Pool Noninfective</td>
</tr>
<tr>
<td>92 15 Pool Noninfective</td>
<td>92 1 Pool Noninfective</td>
</tr>
<tr>
<td>92 Bone Noninfective marrow</td>
<td></td>
</tr>
</tbody>
</table>

DPI = Days postinoculation.

* Received injections of tissues from noninfected steers.
† Kidney, bone marrow, spleen, and mesenteric lymph nodes.

DISCUSSION

Historically, rinderpest has been characterized by certain features in relation to its spread. Circumstantial evidence indicates, according to Scott (1) and Receveur (14), that shipment of cattle carcasses from enzootic areas does not constitute a problem in transmission of rinderpest, particularly if the opportunity for aerosol transmission is excluded. Risk is further decreased if meat exports from enzootic areas are from cattle previously immunized with live attenuated vaccine; however, it was acknowledged that, occasionally, animals do escape vaccination and these animals might develop the disease during export (1). Results of half-life studies prompted Scott to conclude that rinderpest virus is not killed by present methods of storing and transporting beef (15).

Refinements in livestock production and disease control have accompanied political and economic changes that, in turn, influence the direction of international traffic in animals and animal products. Changes in methods of transportation and modification of trade routes enhance opportunity for transmission of animal disease to areas free from a given infection.

Cross-bred Yorkshire-Tamworth swine used in these studies were susceptible to experimental infection with rinderpest virus but did not develop marked clinical signs.

Infected animals without apparent clinical signs are particularly dangerous in relation to transmitting virus.

The recovery of rinderpest virus from convalescent pigs at six and 36 DPI shows that transportation of swine from rinderpest endemic areas may, under certain conditions, contribute to spread of the disease.

Definite conclusions concerning narrow limits beyond which pigs may excrete or carry rinderpest virus must await further study. Data developed in this study indicate that dissemination of virus beyond 92 days is unlikely.
Although less susceptible than cattle, swine react quickly to infection with rinderpest virus and serological methods may be used to detect early infection. The thermal response and lesions in rinderpest-convalescent swine are of limited value in differential diagnosis.

SUMMARY

Each of 31 pigs fed and inoculated with rinderpest virus showed temperature elevation. Two animals died within 13 days following administration of virus.

Rinderpest virus was recovered from tissues of pigs at six and 36, but not at 22 or 92 days after inoculation. Cattle placed in contact with rinderpest convalescent pigs at 41 days after inoculation, did not become visibly infected.

Rinderpest virus-neutralizing antibodies were demonstrated in the sera of pigs at six days and persisted for at least 92 days after inoculation.

Except for the more pronounced involvement of urinary bladders, the lesions in swine were similar to those in cattle experimentally infected with rinderpest virus.

REFERENCES


5. **Robertson, W. A. N.:** Rinderpest in western Australia, 1923 (1924), Melbourne: Green.

6. **Curasson, G.:** Bovine rinderpest (1932), Paris, Vigot Freres.


12. **Scott, G. R., and McLeod, W. G.:** The optimum concentration of lapinized rinderpest virus for the inoculation of rabbits in the production of vaccine. J. Comp. Path. 65 (1955): 236-238.


THE RELATIONSHIP OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS TO EQUINE RHINOPNEUMONITIS VIRUS*

L. E. CARMICHAEL and FRANCES D. BARNES

Because certain similarities in the biological and physical characteristics of infectious bovine rhinotracheitis (IBR) virus (1, 2, 3, 4, 5) and equine rhinopneumonitis (ER) virus (6, 7, 8, 9, 10) suggested that they may be related, studies were made to test this assumption by serological comparisons. Additional observations were made on the effect of a prior inoculation of guinea pigs with ER and IBR virus on antibody responses following a second inoculation with the heterologous virus.

Comparative studies. The IBR virus strain used was originally obtained from Dr. C. J. York, Indianapolis, Indiana. Since then, it has been transferred 25 times in bovine embryonic kidney cells. ER virus was isolated in this laboratory from the liver of an aborted foal which showed typical pathological lesions of equine viral abortion. Initial virus isolations were made in HeLa, swine kidney and embryonic bovine kidney cells. Because tests showed that ER virus growth was best in swine kidney cell cultures, this culture type was used for subsequent propagation. Confirmation of the identity of the virus was made by neutralization tests employing paired antisera supplied by Dr. E. R. Doll, Lexington, Kentucky.

The following serological tests were made for comparisons of ER and IBR viruses: (1) complement-fixation, (2) agar gel-diffusion precipitin, (3) virus neutralization†. Serums were prepared in animals that were exposed to one virus type only, and these included cattle, rabbits, guinea pigs, dogs, and sheep. With the exception of preinoculation and convalescent cattle serums, serums were prepared by multiple inoculations of infectious tissue culture fluid. It was found that IBR virus and ER virus are related by common antigens that cross-react in complement-fixation and gel-diffusion precipitin tests (figure 1). With the exception of cattle serum, immune serums fixed complement with both viruses, although homologous antibody titers were generally eight to 16 times greater than heterologous titers. In contrast, convalescent IBR cattle serums failed to fix complement with either viral antigen. Neutralization tests did not show reciprocal neutralization of either virus; only the homologous virus was neutralized. It, therefore, appeared that the viruses are immunologically distinct, as shown by neutralization tests, but antigenically related, since tests for flocculating-type antibody revealed complete cross-reactions.

* The authors acknowledge with appreciation support from the Co-op Grange League Exchange, Inc.
† Details of the test procedures will be published elsewhere.

Veterinary Virus Research Institute, New York State Veterinary College, Ithaca, New York.
Additional comparisons of ER and IBR viruses were made by observing the antibody response in groups of five guinea pigs that were inoculated in the following manner:

1. One group was inoculated with ER virus and then given a second inoculum of IBR virus three weeks later.
2. A second group was inoculated with IBR virus, and then given a second inoculum of ER virus three weeks later.
3. A third group was given only IBR virus.
4. A fourth group was given only ER virus.

All animals were bled for serum at the time of the first inoculation and then again (groups one and two) prior to the second inoculation. Blood samples were collected again one and two weeks later for serological testing, and each was tested for CF and neutralizing antibodies with both ER and IBR virus antigens.

The antibody response of guinea pigs inoculated as described above showed the following results: (1) A single inoculation of IBR or ER virus evoked low CF antibody titers that were demonstrated only with the homologous antigen. (2) Guinea pigs first inoculated with ER or IBR
virus and then given a second injection of the heterologous virus showed heterotypic CF antibody responses. Heterotypic CF responses were most marked, however, when ER was given subsequent to IBR virus. In addition to the heterotypic responses, anamnestic or secondary-type ER antibody responses occurred in animals that had received a prior inoculation of IBR virus. (3) Neutralizing antibody was not found in animals that received only ER virus. In contrast, animals that were inoculated with IBR virus before or after ER virus inoculation developed neutralizing antibody for ER virus. The results of these observations on the immune responses of guinea pigs appear to illustrate further the relationship between the two viruses; the initially slight reactions that occurred following a single inoculation of virus were intensified in those animals that received a second stimulus by the related antigen. It appeared that the heterotypic CF responses were most marked when ER was given subsequent to IBR virus, however, host factors and the relative sensitivities of the serological procedures may account for this difference.

Similar responses to those described above have been observed in studies on relationships between infectious canine hepatitis and human (11) and bovine (12) adenoviruses, between various serotypes of influenza virus (13), and between virus diarrhea virus of cattle and hog cholera virus (14). Heterotypic antibody responses occurred within each of these groups of viruses, and in the latter instance, noted above, virus diarrhea virus protected swine against lethal doses of hog cholera virus. A possible mechanism of resistance was postulated by the secondary-type antibody response to hog cholera in pigs previously inoculated with virus diarrhea virus. It appears to be a general phenomenon, therefore, that the sequential administration of immunologically different strains of group-related viruses may evoke an increase in heterologous antibody. Furthermore, group reactions of the anamnestic type may occur in animals that have received a prior stimulus by a related viral antigen. The ability of one virus to confer resistance to infection by a related virus probably depends upon the degree of antigenic similarity and also upon the pathogenesis of a particular virus in a given host. In this respect, it would be of interest to study the influence of IBR virus and ER virus on the immune and clinical responses of cattle and horses that had first been inoculated with the heterologous virus.

Since IBR virus has been shown to share biologic characteristics with herpes simplex virus, and probably should be included in the Herpesvirus group (5, 15), it would be of interest to make further comparisons between ER virus, IBR virus and herpes simplex virus. A comparison of certain characteristics of IBR virus and ER virus with herpes simplex virus is shown in Table I. Data regarding many characteristics are lacking, especially as regards the nucleic acid type, viral hemagglutinins, geometric symmetry of ER and IBR viruses, and more extensive serologic comparisons. Evidence thus far gathered, however, would appear to suggest the tentative inclusion of ER virus into the Herpesvirus group, together with IBR virus.
TABLE I
Comparison of Certain Characteristics of IBR and ER Viruses
with Herpesvirus Simplex

<table>
<thead>
<tr>
<th>Character</th>
<th>IBR</th>
<th>ER</th>
<th>Herpes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Ovoid, double membrane</td>
<td>same</td>
<td>same</td>
</tr>
<tr>
<td></td>
<td>surrounding nucleoid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size (millimicrons)</td>
<td>140-155</td>
<td>121-146</td>
<td>120-180</td>
</tr>
<tr>
<td>&quot;double membrane&quot; virus nucleoid</td>
<td>40-45</td>
<td>25-45</td>
<td>30-40</td>
</tr>
<tr>
<td>Symmetry</td>
<td>?</td>
<td>?</td>
<td>Icosahedron, 5:3:2 cubic symmetry</td>
</tr>
<tr>
<td>Sensitivity to lipid solvents (ether, acetone)</td>
<td>inactivated</td>
<td>same</td>
<td>same</td>
</tr>
<tr>
<td>Nucleic acid type*</td>
<td>DNA*</td>
<td>same*</td>
<td>same*</td>
</tr>
<tr>
<td>Hemagglutinin</td>
<td>none reported</td>
<td>same</td>
<td>same</td>
</tr>
<tr>
<td>Group CF antigen</td>
<td>IBR serum reacts with ER virus</td>
<td>ER serum reacts with IBR virus</td>
<td>not reported</td>
</tr>
<tr>
<td>Virus neutralization</td>
<td>homologous virus only</td>
<td>same</td>
<td>same†</td>
</tr>
<tr>
<td>Inclusion body</td>
<td>Type A</td>
<td>same</td>
<td>same</td>
</tr>
</tbody>
</table>

* Increases in cellular DNA Nucleic acid type not reported for purified virus.
† Herpes simplex compared only with IBR virus.

Conclusions. Comparisons of infectious bovine rhinotracheitis (IBR) virus with equine rhinopneumonitis (ER) virus have shown that the two agents are related by a common antigen. Although complement-fixation and gel-diffusion tests showed antigenic cross-reactivity, there was no reciprocal neutralization showing that they are immunologically distinct viruses. Studies on the antibody responses of guinea pigs sequentially inoculated with the two viruses showed heterotypic antibody responses. In addition, prior immunologic experience with either of the viruses appeared to augment the intensity of the antibody response following inoculation with the heterologous virus.

Certain characteristics of IBR and ER viruses were compared with ones described for herpes simplex virus, and these suggest that ER virus, like IBR virus, should probably be included in the Herpesvirus group.

REFERENCES
CHARACTERIZATION OF VIRUS ISOLATES FROM CATTLE*

PETER H. LANGER and KENNETH McENTEET†

Newborn calves used in studies to determine the effects of infectious bovine rhinotracheitis (IBR) virus as well as calves used in studies to determine the efficacy of a triple vaccine became ill with an intercurrent infection characterized by respiratory signs, diarrhea, elevated temperatures and the death of some of these animals. This illness was observed in calves which possessed colostral antibodies against IBR and virus diarrhea (VD) viruses as well as in those which showed an immune response following vaccination with these agents. Calves which did not possess these antibodies also became ill.

In the course of these studies, and in cultures of milk from a cow with mastitis, viruses were isolated from nasal and fecal samples, blood, milk and tissues that had been obtained from sick or dead animals. Because these viruses might account for the illness observed, they were studied.

MATERIALS AND METHODS

Primary bovine fetal kidney cell cultures were prepared after the method of Madin et al. (1) with slight modifications. The cells were initiated in a medium consisting of Hank's basic buffered salt solution (80 percent), 0.5 percent lactalbumin hydrolysate (10 percent of a five percent stock sol.), bovine or horse serum (10 percent) and antibiotics (500 units penicillin, 100 units mycostatin and 100 micrograms of streptomycin per ml.). If a fluid change was required four to five days later, the initiation medium was replaced with a growth medium consisting of Earle's salt solution plus lactalbumin, serum and antibiotics. When the cell sheets were confluent and prior to inoculating the tubes with suspect material or virus, the growth medium was replaced with Parker 199 + 0.5 percent lactalbumin without serum.

Nasal secretions and feces were sampled using dry, sterile cotton swabs which were immediately placed in screw-cap vials containing tissue culture medium. Tissue samples were taken aseptically at time of autopsy and all samples were stored under dry-ice until tested. The tissues were thawed, and ground into a 10 percent suspension in tissue culture medium. Any samples that showed gross contamination or marked cytotoxic effects were passed through a Swinny filter. Following the inoculation of one to two-tenths ml. of these suspensions into each of four tubes, the tubes were incubated at 36 C and examined daily for cytopathic effect (CPE).

* This investigation has been aided by a grant from Nat. Inst. Health, United States Public Health Service.
Veterinary Virus Research Institute,† Department of Veterinary Pathology and Bacteriology, New York State Veterinary College, Cornell University, Ithaca, New York.

389
An isolation was considered to have been made when the same cytopathic effect was noted in two further tissue culture transfers.

A single strain selection of each isolate was made by using the terminal tissue culture tube dilution method. A virus pool was produced following the third terminal dilution. For test purposes, samples were drawn from this pool or from subsequent passages of the virus. However, some of the experimental studies in calves and cows were made prior to this strain selection procedure using low tissue-cultured passages of the virus.

Source of viruses. Isolate 155 was recovered from the spleen, lymph node, kidney and lung of a three week old calf that died as a result of a clinical illness characterized by respiratory signs and diarrhea.

Isolate 531 was recovered from a bronchopneumonic area of lung of a four week old calf which was sacrificed at the peak of an illness characterized by coughing, mucopurulent nasal discharge, elevated temperature and leukocytosis. This calf was born on the farm but had been placed in an isolation unit when four days old to serve as an uninoculated control calf in IBR studies.

Isolate 527 was recovered from the blood of a one week old calf during an illness characterized by elevated temperature, leukopenia and severe diarrhea. This calf was obtained from the same farm as the calf from which isolate 531 was recovered.

Isolate 18 was recovered from the lung of a three week old calf that died during a severe illness characterized by respiratory signs, diarrhea and elevated temperature. This calf came from a group of veal calves.

Isolate 56R was recovered from the mammary secretions of all four quarters of a cow with an acute, bacteria-free case of mastitis.

IsolateSq was recovered from the bronchial lymph node of a cow that died during a herd outbreak of proliferative pneumonia accompanied by elevated temperatures, anorexia and drop in milk production.

Serological tests. Neutralization tests were performed in bovine kidney cell cultures. Test serums were inactivated at 56 °C for 30 minutes.* The test was performed by titrating 10-fold dilutions of virus against undilute serums. Each dilution of virus suspension was mixed with an equal amount of serum, incubated for 20 hours in the refrigerator and inoculated in 0.2 ml. amounts into each of five tissue culture tubes. The tubes were incubated at 36 °C and a final reading was made on the fourth to fifth days, using the hemadsorption reaction with sheep erythrocytes as an index. In the case of virus isolate 527, a final reading based on cytopathic changes was made on the seventh day. Serum titers were expressed as the neutralization index which was computed as the difference between the TCID$_{50}$ of virus and preinoculation serum, and the TCID$_{50}$ of virus and postinoculation serum. Titers were calculated according to the method of Reed and Muench (2).

* In order to minimize the presence of dead virus, virus was harvested at 72 hours as determined by a virus proliferation curve.
CHARACTERIZATION OF VIRUS ISOLATES FROM CATTLE 391

In order to study the neutralizing capacity of raw serum, serums were freshly procured from a calf that had been exposed to virus and was immune, and from a calf that had not been exposed to virus and was not immune, as indicated by the hemadsorption-inhibition test. These serums were not inactivated. The serum-virus mixture was held at +4 C for 20 hours.

Immune rabbit sera were prepared against each isolate. Undilute virus grown in bovine kidney cells was inoculated in one ml. amounts intravenously two times a week for a total of six inoculations. The rabbits were bled out 10 to 14 days after the last inoculation and the serums of two to six rabbits were pooled. All serums were stored at —20 C until tested.

The hemadsorption test was performed according to the method of Vogel and Shelokov (3, 4) 72 to 96 hours following inoculation. At this time, the fluid medium was removed and the cell sheet washed once with a buffered saline solution. To the tube was added 0.2 ml. of a 0.25 percent suspension of erythrocytes. The tubes were allowed to stand at room temperature for three to five minutes at which time a reading was taken. For the hemadsorption-inhibition test, 0.2 ml. of undilute test serum, inactivated at 56 C for 30 minutes was added to the cell sheet after the saline wash and allowed to incubate for a minimum of 15 minutes at room temperature. The sheep erythrocytes were added after this incubation period.

Properties of the virus. Inoculated tissue culture tubes were studied daily and cover slip preparations were fixed in Bouin’s fixative and stained with hematoxylin and eosin stain.

For stability studies ether sensitivity tests were performed by adding ether from a freshly opened can (Squibb, Merck and Mallinckrodt) to undilute tissue culture medium so that a final concentration of 20 percent ether was attained. This mixture was shaken at intervals and held in the refrigerator over night. The ether layer was then drawn off and the tissue culture medium inoculated into bovine kidney cell culture tubes.

Chloroform (Mallinckrodt) sensitivity tests were performed in a similar manner, except that a final concentration of 50 percent chloroform was used. This mixture was centrifuged for 10 minutes at 1,000 rpm and the supernatant was inoculated into bovine kidney cell culture tubes. Untreated samples from the same virus pools were used for the control titrations. A known ether resistant virus (Reo) and ether susceptible virus (parainfluenza 3) were used as ether controls.

For the stability studies involving time and temperature factors, a virus pool was prepared, distributed in screw-cap vials, sealed with paraffin and placed at various temperatures for the appropriate time intervals. Upon completion of a test the vials were stored at —65 C in a mechanical freezer until titered.

Schleicher and Schuell membrane filters with graded pore sizes were used for the size determination studies.

Pathogenicity for animals. Three week old mice were given tissue-cultured virus suspension intranasally. Six blind passages were made from pools of
lung tissue obtained five days after inoculation. Suckling mice less than 24 hours old were inoculated intracerebrally and intraperitoneally and examined daily for three weeks. Guinea pigs were inoculated intraperitoneally, temperatured and examined daily for two weeks. Chick embryos were inoculated via the chorioallantoic membrane (CAM), allantoic and yolk sac routes. Three blind passages were made from pools of material harvested from each source. Tenfold dilutions of pooled samples from each passage were titered in bovine kidney tissue culture tubes.

Twelve calves were used in experimental studies. Eight of these came from a herd of disease-free cattle maintained at the Veterinary Virus Research Institute. Four were derived from other sources, one of these being obtained by caesarian section. The age at time of test ranged from one to 30 days. All were maintained in isolation units. Exposure to virus was intravenously in eight calves and intranasally in four calves.

RESULTS

Properties of isolates. All virus isolations were made in bovine kidney cells. Marked cytopathic effect, which was similar for all isolates, was observed by the fifth day. This consisted of a rounding of cells which were dark and pyknotic and tended to pile up in clumps. Further degeneration was accompanied by a cellular lengthening and stranding which left large spaces in the sheet. Numerous rounded cells were clustered along the edges of these strands. Much extracellular debris was noted at this stage. The majority of the rounded cells eventually fell off the glass but at no time was the entire cell sheet completely detached.

In bovine kidney tissue culture all isolates attained a TCID$_{50}$ of 10$^{5.0}$-5.5 except virus Sq which had a TCID$_{50}$ of 10$^{2.6}$-3.7. In the stained preparations no specific changes were observed. The cytoplasm of affected cells became densely packed with coarsely granular eosinophilic material. Rounded pyknotic cells were scattered among normal appearing cells. Normal and pyknotic nuclei were present in some multinucleated cells. Spherical to oval eosinophilic bodies which were approximately the size of nucleoli and surrounded by halos were present in many cells. Similar inclusions were seen in uninoculated cultures undergoing spontaneous degeneration although fewer in number.

All isolates were found to be ether and chloroform sensitive. Isolate 56R failed to pass through the 150 mu. pore size membrane filter. Isolate 56R is stable at $-65$ C for at least six months. Storage at $-20$ C and $+4$ C for one month resulted in a loss of 4 and 4.7 logs of infectivity respectively. A 1 log loss of infectivity was observed following storage at room temperature (20-25 C) for five days. Storage at 37 C resulted in a loss of 1.4 log at 24 hours, 3.7 logs at 48 hours and 3.2 logs infectivity at 72 hours. No infectivity was found after exposure to 56 C for five minutes.

Pathogenicity. No pneumonia or clinical illness was observed in the three week old mice. No clinical signs were seen in suckling mice, guinea pigs or rabbits.
Virus was recovered in bovine embryonic kidney tissue culture from first and second passage pooled CAM with a TCID₅₀ of 10².1 and 10².5 respectively, and that recovered from first passage pooled allantoic fluids has a TCID₅₀ of 10⁰.8.

The clinical responses in the experimental calves were similar to those observed in field cases. Differences in degree of response appeared to be related to the age of the calf at time of exposure to the virus. The clinical signs observed consisted of elevated temperatures (103-106°F); leukopenia (1925-3750 WBC/cmm) in four of the calves inoculated intravenously; leukocytosis (up to 21,850 WBC/mm) in those receiving the virus intranasally, serous and mucopurulent nasal discharge, conjunctivitis, rapid respiration, diarrhea (watery, white, yellow, brown and frequently flecked with fresh blood), anorexia, and general malaise. Three calves were sacrificed and two died.

Serological findings. Convalescent serums from cattle which had shown a clinical response following inoculation and were refractory upon reinoculation failed to neutralize the virus when the serum-virus mixture was held at 37°C for one hour, 25°C for one hour or 4°C for three hours. Placing the serum-virus mixture at 4°C for 18 hours resulted in a neutralization index of 0 or occasionally less than 50 in replicate titrations. Serums from immune and nonimmune calves which had not been heat inactivated gave a neutralization index of 3,000 and 1,000 respectively. When these serums were tested following inactivation at 56°C for 30 minutes the neutralization index for each was 0.

The findings of the neutralization tests with immune rabbit serums are summarized in Table I. Isolates 56R, 531 and 155 completely crossneutralized with each other. Isolate 18 was not neutralized by 531 immune serum and to a low degree by 56R immune serum. Isolates 527 and Sq each showed

<table>
<thead>
<tr>
<th>Virus</th>
<th>56R</th>
<th>155</th>
<th>531</th>
<th>18</th>
<th>527</th>
<th>Sq</th>
</tr>
</thead>
<tbody>
<tr>
<td>56R</td>
<td>1</td>
<td>75</td>
<td>22</td>
<td>47</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>155</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>531</td>
<td>237</td>
<td>237</td>
<td>237</td>
<td>237</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>.2</td>
<td>145</td>
<td>0</td>
<td>145</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>527</td>
<td>15</td>
<td>N.T.</td>
<td>25</td>
<td>.2</td>
<td>235</td>
<td>0</td>
</tr>
<tr>
<td>Sq</td>
<td>.4</td>
<td>.4</td>
<td>.4</td>
<td>.4</td>
<td>0</td>
<td>.4</td>
</tr>
</tbody>
</table>

* Titers are expressed as the neutralization index which is computed as the difference between the TCID₅₀ of virus and preinoculation serum, and the TCID₅₀ of virus and postinoculation serum.

† N.T. means not tested.
a one way cross. The immune serums from each of these isolates did not neutralize any of the other four isolates nor each other. However, the anti-serums against 56R, 155, 531 and 18 did completely neutralize 527 and Sq viruses.

Hemadsorption tests were conducted with cattle, sheep, guinea pigs, chicken, human 0, rhesus monkey, and vervet monkey erythrocytes. Isolate 527 failed to hemadsorb with any of these erythrocytes. The other five isolates all showed excellent hemadsorption with cattle and sheep erythrocytes but failed to react with any of the other erythrocyte types. Hemadsorption appeared to be immediate and even after 24 hours at room temperature, the erythrocytes could not be dislodged by moderate agitation of the tube.

The hemadsorption-inhibition (HAI) test using sheep erythrocytes as the indicator and undilute test serums gave replicable results.

In two calves exposed to virus 56R and in two calves exposed to virus 155, it was noted that the HAI antibodies against virus 155 always appeared first, i.e., during the third postinoculation week and those against viruses 56R, 531, and 18 after the fourth week. In contrast to the results obtained with immune rabbit serums, the convalescent calf serums obtained from the calves which had been inoculated with isolates 155 and 56R did not inhibit hemadsorption with virus Sq.

There was no inhibition of hemadsorption by IBR, VD, parainfluenza 3, bovine adeno or Reo types I, II, III immune rabbit serums* or IBR and VD immune calf serums when tested against all five hemadsorbing virus isolates.

**DISCUSSION**

Six recently isolated cattle viruses identified as isolates 56R, 155, 531, 18, 527, and Sq have been characterized. Because these strains are serologically related; produce a distinctive, similar cytopathic effect in tissue culture; rapidly hemadsorb cattle and sheep erythrocytes (except strain 527); are all sensitive to ether and chloroform; are nonpathogenic for laboratory animals; show the presence of similar eosinophilic cytoplasmic inclusions in the tissues of naturally and experimentally infected animals; and produce similar clinical effects in experimental calves, they are considered to be similar. Certain strain differences, however, were found. Strain 527 appears to be unique; although it is serologically related to hemadsorbing strains, it does not possess this characteristic. Also, cross-neutralization tests with rabbit serum show other serological differences among various strains and it appears that there may be three different serotypes.

Comparison of basic properties so far studied would indicate that these strains may belong to the myxovirus group. The properties shared in common are: sensitivity to ether and chloroform, comparable size, hemadsorption of erythrocytes, growth in the embryonating chicken egg, presence of a heat-labile inhibitor in normal serum, and stability at —65 C and less stable at higher temperatures.

* The Reo serums were kindly furnished by Dr. A. B. Sabin.
Although the general properties of these strains are similar to those reported for the myxovirus group that includes parainfluenza type 3 which occurs in cattle, differences were observed when some of them were studied in detail. A prime characteristic of the myxoviruses is the ability to hemadsorb a variety of mammalian as well as fowl erythrocytes. In contrast, these cattle strains will react only with cattle and sheep erythrocytes, whether tested at room temperature or following exposure to refrigerator temperatures for varying time intervals. Myxoviruses produce distinctive eosinophilic cytoplasmic inclusion bodies (5). As yet, such inclusions have not been demonstrated for these strains although eosinophilic cytoplasmic inclusions of a different appearance were noted. These unusual inclusions also were seen in uninoculated control cultures, but they were more numerous and always found in inoculated cultures. Also such inclusions have been observed in the majority of the animals from which these viruses were recovered. They have been seen primarily in epithelial cells: alveolar cells of the lung, bronchiolar epithelium, tubular epithelium of the kidney, intestinal mucosa, Hassal’s corpuscles of the thymus and in reticuloendothelial cells of the bronchial lymph nodes. They appeared to be similar to those reported from Scottish calves with pneumonia (6). They also are similar to those observed in hyaline droplet degeneration. Histochemical studies should be conducted to determine the nature of these bodies.

Evidence that this virus may be implicated as the primary cause of illness and death in calves and cows and possibly of economic importance to the cattle industry is: a) all strains were recovered from animals that were showing clinical signs of illness and conversely no isolations could be made from clinically normal or recovered animals, b) virus was recovered from internal organs, blood and milk, as well as nasal secretions and feces, c) convalescent animals showed a rise in titer and d) similar clinical responses were seen when experimental animals were exposed to these viruses.

**SUMMARY**

From calves showing pneumonia and diarrhea and from a cow showing mastitis, six isolations of cytopathogenic viruses were made in tissue-cultured bovine embryonic kidney cells. Inoculation of five of these strains into calves and into udders of cows produced clinical features similar to those seen in animals that furnished these strains. Serological comparisons showed all strains to be related although differences suggested several serotypes might be present.

Sensitivity to ether and chloroform, size, hemadsorption of erythrocytes, growth in the embryonating egg, presence of a heat-labile inhibitor in normal serum, and stability at -65 °C and less stable at higher temperatures are shared in common with the myxovirus group.

**ACKNOWLEDGMENTS**

The authors are grateful to Mrs. Sylvia Gould and Mrs. Shirley Seo for their technical assistance.
REFERENCES


RIFT VALLEY FEVER: A ZOONOSIS

Leslie C. Murphy,* D.V.M. and Bernard C. Easterday,†
D.V.M., M.S., Ph.D.

United States Army Chemical Corps, Fort Detrick, Frederick, Maryland

INTRODUCTION

In recent years a number of animal diseases has broken through the bounds of their natural habitat producing serious epizootics with tremendously high losses in areas far removed from those in which they are commonly found. Examples of these anomalous situations are the discovery of African swine fever in Spain and Portugal and of African horse sickness in the Middle East and adjoining countries. There is reason to believe that other infectious animal diseases may find their way to areas where they do not occur at this time because of the rapidity and extent of communications and transportation. One of the diseases that falls into the latter category is Rift Valley fever, a disease that would probably inflict severe damage among cattle and sheep and would also affect human beings in those areas to which it is foreign. As a first step in preventing its introduction into the United States or in recognizing its presence, should it be inadvertently introduced, a knowledge of its characteristics is most important.

Several sources of information are available to those concerned with preventing the introduction of foreign animal diseases into the United States. In connection with Rift Valley fever specifically, Weiss has published an excellent and rather comprehensive review of the literature on Rift Valley fever after presentation of a paper at the Symposium on Arthropod-borne Virus Diseases of Animals convened at Dakar, Africa, in May 1953 (68). Henning's textbook on Animal Diseases in South Africa (25), the 1954 official report of the United States Livestock Sanitary Association on Foreign Animal Diseases, Their Prevention, Diagnosis and Control (66) and the 1960 edition of Control of Communicable Diseases in Man by the American Public Health Association (2) are other valuable sources of information. The purpose of this paper is not to review in detail the approximately 200 articles on Rift Valley fever but to summarize some of the pertinent facts on this disease as a zoonosis. An outbreak of Rift Valley fever in the United States would be of interest to both veterinary and medical officials since domestic animals and man are susceptible.

Present address:
* Laboratory Animal Section, Virology Research Resources Branch, National Cancer Institute, National Institutes of Health, Bethesda 14, Maryland.
† Department of Veterinary Science, University of Wisconsin, Madison 6, Wisconsin.

397
Rift Valley fever (RVF) is an acute, febrile, insect-borne virus disease primarily of sheep and cattle. Man has also been infected during epizootics of RVF in domestic animals in Africa and from handling infectious materials in the laboratory or performing necropsies of animals dead of the disease.

The first report of RVF and the description of its etiological agent was by Daubney et al. in 1931 (9). These authors first designated the disease as enzootic hepatitis because of the extensive liver damage in infected animals. Since pathological lesions were present in other organs as well as the liver, the disease was named after the Rift Valley in Africa where the first reported epizootic occurred.

Prior to the definitive diagnosis of RVF by Daubney et al. (9), Montgomery in 1912 (43) and Stordy in 1913 (62) in their annual reports briefly described an acute and highly fatal disease of lambs on the government farm at Naivasha and on other farms in the Rift Valley. Montgomery made the suggestion that the disease was a different entity from Nairobi sheep disease.

The epizootic investigated by Daubney et al. (9) was first brought to their attention in July 1930 when a disease occurred that caused heavy mortality in newly born lambs on a farm near Lake Naivasha in Kenya, Africa. The severity of the disease was indicated in the report in that approximately 3,500 lambs and 1,200 ewes had died. Daubney showed by his investigations that: (a) this epizootic was a virus disease affecting sheep, cattle, goats, and man; (b) the virus caused a mortality of more than 95 percent in very young lambs, abortions and a moderate mortality in ewes and cows, and a transient fever accompanied by severe pains in the joints in man; (c) in susceptible domestic animals the characteristic lesion in the liver was a focal necrosis; (d) the disease was not contagious and evidence suggested transmission by a mosquito; and (e) the disease in man had similarities to yellow fever and dengue.

GEOGRAPHIC DISTRIBUTION

Other than accidental laboratory infections in man, RVF has only been reported from the African continent. Evidence of RVF has been found in a wide belt extending from the southern tip of the Union of South Africa northward into Central Africa (28). The first proved epizootic occurred in Kenya (9). Transmission of the virus by mosquitoes and presence of the disease in Uganda was reported by Smithburn et al. (57), when the virus of RVF was isolated from mosquitoes caught in the uninhabited Semliki forest. Prior to this, Findlay et al. (19) had shown that virus-neutralizing antibodies were present in the sera of natives in Uganda, French Sudan, Anglo-Egyptian Sudan, and French Equatorial Africa. Another outbreak of the disease occurred in Kenya in 1933 (7). In the summer of 1950 and 1951 the first reported epizootic occurred in the Union of South Africa (1, 12, 44). The South African epizootic involved the western and southwestern areas of Orange Free State, northern and western areas of Cape Province and the western and southern areas of Transvaal (53). Epizootics reappeared in
the Union of South Africa in 1953 (Fauresmith district), 1955 (Marienthal district) and in the summer of 1956 (Western Orange Free State) (22-24, 68). In 1957 Kokernot et al. (33) found that wild-caught mosquitoes in Zululand carried the virus and Shone (55) reported the presence of antibodies in the sera of cattle in Southern Rhodesia. Kaschula (30) found antibodies in the sera of cattle in the Knysna district of the Union of South Africa, but no evidence of clinical disease other than an occasional abortion in cattle. He suggested that these areas formed truly enzootic areas similar to the Semliki forest. Enzootic areas were described as low, warm areas with high annual rainfall. Similar terrain and climate plus the added factor of sheep farming characterized the epizootic areas. It was reasoned that the disease reached epizootic proportions only in those enzootic areas that were also suitable for sheep, a highly susceptible host (34, 68). The disease halted abruptly with the first frost and with movement of herds to high altitudes (7, 9). In 1960 an outbreak of RVF occurred in humans in the Entebbe area of Africa (70). RVF virus was isolated from five patients who had complained of generalized pain in the limbs, body and eyes.

**ETIOLOGY**

**Strains:**

Strains of Rift Valley fever virus (RVFV) as isolated from their natural environment, i.e., an epizootic in African sheep or cattle, have been called by a variety of terms such as pantropic, viscerotropic, hepatotropic or polytropic. The virus produced extensive necrosis of liver parenchyma and in this sense was frequently referred to as viscerotropic or hepatotropic. Lesions in tissues of mesodermal origin were of questionable RVFV etiology; the term polytropic was proposed as a more accurate term than pantropic which was originally applied to viral pathogenesis of all three germ layers (32). The term “polytropic” has not been found in RVF literature other than in the publication in which it was proposed.

Neurotropic variants (NRVFV) of RVFV have been obtained by intracerebral (IC) passage in young mice (18, 32, 37, 56). MacKenzie and Findlay (37) produced a neurotropic strain of RVFV in a highly susceptible animal, the mouse, by IC passages of mouse-brain material. Mice were first injected intraperitoneally (IP) with human convalescent serum before the IC passages were made. After more than 30 passages, the virus had become “fixed” for nervous tissue and when inoculated IC into mice encephalomyelitis with an absence of liver necrosis resulted. Smithburn (56) showed that the use of immune serum was not required to produce a neurotropic strain. Several strains of RVF virus that were passed in mice by serial IC inoculation became neurotropic in character with a reduction in hepatotropism.

Kitchen (32) reported that, during the first 15 IC passages, the virus retained its hepatotropic character and exhibited no evidence of neuroadaptation. Between the 15th and 20th transfers both neuro- and hepatotropism were observed, but subsequent to the 19th transfer histological
evidence of hepatotropism did not reappear in mice of the line propagated IC. When the neuro-adapted RVFV of the 20th to 50th mouse-brain passages were inoculated into rhesus monkeys both tropisms were present. Less hepatotropism was noted as the brain passages increased and at the 81st passage the virus could not be obtained in the circulating blood.

Weinbren et al. (67) isolated two strains of an agent similar to RVFV from mosquitoes caught in the Lunyo Forest on Entebbe peninsula in Africa. The Lunyo virus strain produced hepatic lesions similar to those produced by RVFV in mice, but there were some histopathological, serological and behavioral differences.

**Morphology:**

Daubney et al. (9) were the first to report that the etiological agent was a virus after repeated cultures made from the liver of naturally and experimentally infected sheep and lambs were negative. These authors showed that the virus passed regularly through Chamberland filters up to the L 11 grade, and occasionally through the L 13 candle. By filtration through gradocol membranes, Broom and Findlay (5) estimated particle size at 23-35 millimicrons. Estimates between 30 and approximately 52 millimicrons for various strains of virus have been obtained by ultracentrifugation (47).

**Stability:**

Weiss has reported in detail the published literature on the chemico-physical properties of RVFV (68). The virus appeared to be most stable within a pH range of seven to eight and below pH six was rapidly inactivated. The virus was stable in undiluted mouse serum heated to 56°C for three hours. At 4°C the virulence of infective blood was retained for eight months. Relatively no loss in titer was found when virus materials were stored at —20°C. Virus suspensions may be dried without much loss of titer.

Andrew and Murphy (3) have studied the stability of RVFV in liquid and frozen lamb sera. RVFV remained viable at 37°C for 21 days and at approximately 25°C for four months, the period of the test. At 4°C and storage under dry ice temperatures very little loss in titer of the lamb sera occurred after one year. A specimen of sheep plasma collected from a natural infection of RVF contained virus in high titer after eight years of storage and shipment under varying conditions of refrigeration (13).

**HOST RANGE**

A wide range of species is susceptible to Rift Valley fever in varying degrees. Findlay (15), Findlay et al. (16) and MacKenzie et al. (38) classed species according to their susceptibility to laboratory infection. They classified lambs, mice, hamsters, and wild rodents as most susceptible, inasmuch as the disease was usually fatal to these animals. It was also fatal to about 50 percent of the second group of animals, sheep and rats. Men, monkeys, cows, goats and grey squirrels experienced severe nonfatal infections and
were placed third. Cats were next with a mild reaction, followed by rabbits, which showed no evidence of infection, although the virus persisted in the blood for several days. In a study of susceptibility among various species of monkeys, Findlay (17) reported differences in susceptibility. Some species of African monkeys were found to be relatively less susceptible than species from India or South America. Ferrets were found to be highly susceptible by Francis and Magill (20). Horses, pigs, mongooses, hedgehogs, tortoises, frogs, and domesticated and wild fowl were listed as nonsusceptible by Findlay (15). Easterday (13) reported on the susceptibility of sheep, goats, cattle, mice, rats, hamsters, and monkeys and the insusceptibility of pigs, guinea pigs, rabbits, and a puppy.

Saddington (50) propagated RVFV on the chorioallantoic membrane of nine- to 10-day-old embryonated eggs. Virus was present in the membrane, amniotic fluid and livers from embryos harvested five days after inoculation. Kaschula (30) propagated both pantropic and neurotropic strains of the virus in the yolk-sac and on the chorioallantoic membrane. Highest titers of virus were obtained at 34 C from eight-day-old embryonated eggs inoculated in the yolk sac and harvested at approximately 48 hours after inoculation.

RVFV has been grown in several types of cell cultures. MacKenzie (36) and Saddington (50) carried RVFV through 12 subcultures in nine- to 10-day-old chick embryo cells suspended in Tyrode’s solution. Endo (14) reported the development of a neurotropic variant by serial passage of RVFV in a Maitland-type tissue culture of embryonic mouse brain. In roller tube tissue culture, Takemori et al. (63-65) demonstrated the cytopathogenic effect of the virus on rat sarcoma cells, human embryo, rat, mouse and swine fibroblasts. Weiss (68) reported cytopathogenesis by NRVFV of lamb kidney cells grown in roller tube cultures. RVFV and NRVFV titrations were made in tissue culture by plaque formation on rat sarcoma cells (63), Chang’s human liver cells (27), and sheep kidney cells (46). Randall et al. (48) propagated RVFV in monkey kidney cells for production of vaccine. In 1961 Easterday (13) reported on the growth of RVFV in Chang human liver cells, HeLa cells, L cells and chicken embryo liver cells.

TRANSMISSION

Natural:

Seasonal aspects of RVF outbreaks coincident with prevalence in low terrain following periods of heavy rainfall led Daubney et al. (9) to conclude that the virus was transmitted naturally by biting insects. Furthermore, they were able to stop the disease by moving flocks to higher altitudes or sheltering animals from mosquitoes. They also found that a lamb inoculated with *Taeniorhynchus brevipalpis* (synonymous with *Mansonia fuscopennata*), although not showing a temperature reaction, was subsequently immune. The mosquito was definitely established as a vector by Smithburn et al. (57) in 1948 when the virus was isolated from three *Aedes* and six *Eretmapodites* species caught in the Semliki forest of Uganda. RVF virus has also been isolated from *Aedes caballus* (24), *Culex theileri* (24), *Aedes circumluteolus*
(33, 67), *Aedes africanus* (67), *Mansonia fuscopennata* (70) and *Mansonia africana* (70). Epidemiological studies in 1948, 1951 and 1953 showed no evidence of person-to-person spread or infection of man by an insect vector (11, 23, 57).

**Experimental:**

RVFV has been transmitted in the laboratory by most of the usual routes of inoculation of infectious materials into susceptible animals. Some of the vectors with which experimental transmission of RVFV has been achieved are *Rhipicephalus appendiculatus* (7), *Eretmapodites chrysogaster* (58), *Aedes aegypti* (13), *Aedes caballus* (24), and *Aedes triseriatus* (13).

The immediate portal through which the virus infects during ingestion of food has not been clearly shown. Daubney et al. (9) failed to transmit the virus by drenching a lamb with infected blood yet Mims (40) showed that mice could become infected during cannibalism. Transmission by contact of infected lambs with susceptible lambs has been shown not to occur (9, 13), nor did lambs become ill by sucking an infected mother (9). Weiss reported that the virus can be spread from infected mice to suckling mice by the handling of an attendant (68).

Easterday (13) has recently confirmed and extended information on the routes by which lambs became infected. Lambs became ill with RVF after IP, intravenous (IV), intranasal (IN), and intradermal (ID) inoculations and after exposure of the conjunctival sac and buccal mucosa. Lambs did not become ill when the virus was placed in gelatin capsules and given by mouth. Exposure of the lambs to aerosols of RVF virus also produced infection and death. Haig et al. (68) found that milk of infected animals contained virus in low titer but this has not been shown to be a factor in the epizootiology of the disease.

**Accidental:**

Exact portals of entry responsible for transmission of the virus in accidental infection of veterinarians and laboratory workers remain somewhat obscure. The first reported infections followed participation in post-mortem examination of infected animals and laboratory exposure in which little or no protective equipment was used (9). Later on, varying degrees of protection, including gloves, surgical masks, and protective garments, were used without success in preventing infections. Accidental infection has resulted from handling contaminated glassware, entering rooms housing infected animals and from routine procedures in virus work. One accidental infection occurred under circumstances that suggested unusual resistance of the virus to drying (20). An individual, whose presumed contact with the virus was scraping and painting the walls of a room which housed infected laboratory animals three months earlier, became infected 15 days after the paint-scraping experience.
Pathogenicity

Man:

RVF infections acquired under natural conditions occurred in humans during each recorded epizootic of sheep and cattle (68). Infections occurred among personnel engaged in post-mortem examination of diseased animals, among laboratory personnel in contact with infectious materials and among others whose occupations involved them with infected animals. Daubney et al. (9) provided the only report of a human experimental infection with RVF virus. An adult male was given lamb plasma containing RVF virus. Symptoms typical of RVF in man were reported without untoward sequelae.

RVF infections in man have been heavily documented from case reports of persons contracting the disease during occupational contact with the virus. A summary of some of these cases is shown in Table 1. Infections were contracted in every laboratory in which work with RVF was carried on, including African, European, Japanese, and American laboratories.

High morbidity among those exposed to the virus was apparent from the earliest reports. Daubney et al. (9) and Findlay (15) reported 100 percent morbidity among personnel following post-mortem experience with infected carcasses. Almost every native engaged in herding sheep during the 1930 epizootic contracted a disease with symptomatic resemblance to RVF. Subsequent to the 1950 outbreak in South Africa, Schulz (53) estimated 70 percent morbidity among approximately 32,000 people assumed to be directly exposed. In contrast to high morbidity, only one human fatality associated with RVF has been reported (54).

Similar in many aspects to influenza and dengue fever, RVF produced a sudden onset with elevated temperature, headache, muscular pain, weakness, sensation of fullness over the liver, rigors, vertigo, photophobia and nausea (68). Elevated temperature frequently occurred in two phases (9, 15). The incubation period was from four to six days. Incapacitation varied from

### Table 1

<table>
<thead>
<tr>
<th>Place</th>
<th>Number of Cases and Occupation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenya, Africa</td>
<td>2 veterinary surgeons, 2 laboratory assistants</td>
<td>(9)</td>
</tr>
<tr>
<td>London</td>
<td>1 pathologist, 1 veterinary surgeon, 3 laboratory assistants</td>
<td>(15, 31)</td>
</tr>
<tr>
<td>United States</td>
<td>1 pathologist, 3 technicians, 2 laboratory assistants, 2 virologists</td>
<td>(20, 31, 49, 54)</td>
</tr>
<tr>
<td>Uganda, Africa</td>
<td>2 pathologists, 6 technicians, 3 animal caretakers</td>
<td>(59)</td>
</tr>
<tr>
<td>Japan</td>
<td>12 laboratory workers</td>
<td>(39)</td>
</tr>
<tr>
<td>South Africa</td>
<td>14 farmers, 7 veterinary surgeons, 2 teachers, 2 research station employees, 1 merchant, 1 diamond worker, 1 technician, 2 natives</td>
<td>(21, 23, 30, 41, 44, 51, 52, 55, 61)</td>
</tr>
</tbody>
</table>

TABLE 1
Summary of Some Confirmed Cases of Rift Valley Fever Infections in Man
inapparent infections to complete debilitation, however, clinically recognizable cases usually required bed rest during the febrile period. Malaise, weakness and complaints of headache and defective vision were reported to persist for several weeks. Visual disturbances have been reported such as photophobia, tenderness of the eyeballs, of pain behind the eyes and retinal damage.

**Sheep, cattle and goats:**

RVF is primarily a disease of sheep and cattle with high mortality in young lambs and abortions in adult sheep and cattle. Alexander (1) and Dickson (12) classified RVF in sheep and cattle according to clinical signs of the disease. They recognized a peracute, an acute, subacute, and a mild or inapparent form. The peracute form was common in very young lambs. An incubation period of about 12 hours was followed by collapse and death within 36 hours in 95 to 100 percent of the infected lambs. During the 24 hours preceding death, lambs were listless, disinclined to eat and sank down soon after being put on their feet. The acute form was commonly encountered in lambs and to a lesser extent in adult sheep. Clinical signs appeared suddenly and included a rapid rise in temperature, vomiting, mucopurulent discharge from the nose, rapid pulse, unsteady gait and abortion in pregnant animals. Death usually followed onset within 24 to 48 hours. The mortality rate was high in lambs and varied from 20 to 30 percent in adult sheep (6).

In adult sheep and cattle, the subacute form was common. Body temperatures rose to 104° to 106° F. and persisted for 24 to 96 hours. There was inappetance and general weakness. Abortion was frequently the only sign in pregnant animals. Milk production decreased rapidly and the mortality rate was low; less than 10 percent in cattle (6). The mild or inapparent form also occurred in adult sheep and cattle. The only sign of disease was a mild febrile reaction and diagnosis could be made only by serological methods. Leucopenia followed the inapparent forms of infection (15).

Experimental infection of sheep, cattle and goats of various ages was followed by a clinical picture similar to natural infections (9, 13, 15, 26). Recent investigations have provided quantitative data on RVF in lambs, sheep, calves, goats, monkeys, and swine (13). Examples of the response obtain after infection of several species of animals are shown in Table 2.

**Monkeys:**

Findlay (17) reported that a febrile, nonfatal form of RVF developed in monkeys within 24 to 96 hours after inoculation. The febrile response persisted for 24 to 120 hours, followed by leucopenia, but other signs of disease were not apparent. Examples of the response in two species of monkeys, *Macaca irus* and *M. mulatta*, are given in table 2 (13).

**Swine:**

Weiss (68) reported that although pigs were considered to be nonsusceptible to RVF, circumstantial evidence in the field indicated abortions in
TABLE 2

Examples of Response in Animals Following Infection With Rift Valley Fever Virus*

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Dose M.I.P.L.D.50</th>
<th>Route</th>
<th>Peak Viremia Log_{10} M.I.P.L.D.50</th>
<th>Viremia Present on Days</th>
<th>Peak Temp. °F.</th>
<th>Log_{10} Present on Dare Days</th>
<th>Survival</th>
<th>Death (D) or Survival (S) on Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb</td>
<td>8-10 weeks</td>
<td>10^{6.5}</td>
<td>I.P.</td>
<td>106.4</td>
<td>2, 3</td>
<td>7.5</td>
<td>1-5</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8-10 weeks</td>
<td>10^{3.5}</td>
<td>I.P.</td>
<td>105.9</td>
<td>2-7</td>
<td>8.2</td>
<td>2-6</td>
<td>D, 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
<td>150</td>
<td>I.P.</td>
<td>106.7</td>
<td>1-2</td>
<td>10.1</td>
<td>2-3</td>
<td>D, 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-12 weeks</td>
<td>15</td>
<td>I.P.</td>
<td>107.4</td>
<td>2-9</td>
<td>6.4</td>
<td>2-8</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 week</td>
<td>2.5</td>
<td>I.P.</td>
<td>106.6</td>
<td>2</td>
<td>9.0</td>
<td>2</td>
<td>D, 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-3 days</td>
<td>32</td>
<td>I.D.</td>
<td>105.8</td>
<td>2, 3, 4, 5</td>
<td>6.0</td>
<td></td>
<td>D, 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-3 days</td>
<td>9</td>
<td>Aerosol</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>D, 6</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>2.5 years</td>
<td>300</td>
<td>I.D.</td>
<td>107.2</td>
<td>3, 4</td>
<td>5.2</td>
<td>3-5</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>300</td>
<td>I.D.</td>
<td>106.8</td>
<td>2-4</td>
<td>7.6</td>
<td>2-4</td>
<td>D, 4</td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td>1-3 days</td>
<td>12</td>
<td>I.D.</td>
<td>105.4</td>
<td></td>
<td>4.8</td>
<td>2-7</td>
<td>D, 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-3 days</td>
<td>1</td>
<td>I.D.</td>
<td>105.0</td>
<td></td>
<td>7.0</td>
<td>2-4</td>
<td>D, 4</td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>1-2 days</td>
<td>10</td>
<td>I.P.</td>
<td>104.0</td>
<td></td>
<td>7.7</td>
<td></td>
<td>D, 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-2 days</td>
<td>100</td>
<td>I.P.</td>
<td>104.0</td>
<td></td>
<td>4.0</td>
<td></td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Rhesus</td>
<td>Adult</td>
<td>150</td>
<td>I.P.</td>
<td>105.4</td>
<td>3, 6</td>
<td>5.5</td>
<td>2-7</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>Adult</td>
<td>150</td>
<td>I.N.</td>
<td>104.6</td>
<td>4, 5, 7, 8</td>
<td>6.4</td>
<td>3-9</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>Adult</td>
<td>3</td>
<td>Aerosol</td>
<td>5, 6, 7</td>
<td>106.0</td>
<td>6.4</td>
<td>4.13</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>Adult</td>
<td>10^6</td>
<td>Aerosol</td>
<td>6-8</td>
<td>106.0</td>
<td>4.8</td>
<td>2-6</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>4 months</td>
<td>150</td>
<td>I.P.</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td>S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 months</td>
<td>1500</td>
<td>I.P.</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td>S</td>
<td></td>
</tr>
</tbody>
</table>

swine in those areas where lambs were dying of the disease. Young pigs appear to be completely refractory to RVF virus (13). Four-month-old Hormel-type pigs inoculated IP with up to 1,500 mouse IPLD_{50} of RVF virus did not have a febrile response, viremia, leucopenia or any signs of illness. Neutralizing antibody was not demonstrable in sera collected on the 21st day after inoculation of the virus.

**PATHOLOGY**

The pathology of RVF infection in various species of animals has been investigated by several workers and reviewed by Weiss (68). The material presented below was summarized from the observations published by these investigators. RVF virus primarily affected the liver which showed characteristic focal necrosis. In lambs, the liver was frequently yellow, rarely enlarged, but always lacking the color of normal liver. It had necrotic foci approximately one millimeter in diameter in association with hemorrhages scattered beneath the capsule. The lesions extended throughout the liver and in peracute cases the normal architecture was sometimes completely lost.

The liver of mature sheep was usually mottled, brown and frequently enlarged. Liver cells degenerated and the lesions accumulated polymorphonuclear leucocytes and histiocytes. Lesions were usually focal and not panlobular, as in lambs.

The spleen in sheep usually showed subcapsular petechiae. The kidneys of lambs showed congestion of the cortical and medullary blood vessels, especially near the boundary zone. They showed cloudy swelling and on occasion the cells of the convoluted tubules lost their ability to retain nuclear stains. Lesions in older sheep most often progressed to tubular degeneration and nephrosis.

The alimentary tract was inflamed in degrees varying from catarrhal to hemorrhagic enteritis. The wall of the gall bladder showed petechiae and was often thickened. Cyanosis was observed in visible mucous membranes and skin, particularly in the udder, scrotum and axillary regions. Subcutaneous tissues were edematous.

In a serial sacrifice experiment in young lambs Easterday (13) observed that at 30 to 40 hours postinoculation of RVF virus, minute (0.5 to one mm.) gray, faintly yellow foci appeared in the parenchyma and beneath the capsule of the liver. During the next 12 hours, these necrotic primary foci enlarged to approximately two to 2.5 mm. and were more rarely seen. They did not coalesce. Just prior to spontaneous death, or in lambs sacrificed when moribund the liver became irregularly congested, focally to extensively hemorrhagic and very soft. The original foci of necrosis were still present but obscured by the massive hepatic necrosis. The serosal surfaces, the endocardium, and the gastrointestinal mucosae remained unchanged until late in the disease when petechiae and ecchymoses became apparent. In some of the late cases, hemorrhages in the adrenal cortices were seen.
The diagnosis of RVF must be confirmed by laboratory procedures such as isolation and identification of the virus, serological tests, and histopathological techniques. Reliance upon symptomatic criteria resulted in delayed recognition of the 1950-1951 outbreak in the Union of South Africa (53). Rift Valley fever may be confused with enterotoxemia, bluetongue, three-day stiff sickness and Wesselsbron (1, 12, 69). Similarities to dengue and yellow fever in humans have also been described (8). The presence of an influenza-like malady among humans coincidentally with an outbreak in lambs, calves, or goats has been a good lead in suspecting RVF infection.

RVFV may be isolated by injection of suspected material into highly susceptible animals as the lamb, calf, mouse, hamster, or ferret. Isolation has also been made by inoculation of infected material into embryonated eggs (30). Whole blood (9), serum (15), throat and nasal washings (20), homogenates of insects (57) and organs such as brain, liver and spleen (15) from suspected sources have been used successfully as inocula.

The virus may be identified by several serological tests as serum neutralization (15), complement-fixation (4), and hemagglutination-inhibition (42).

PROPHYLAXIS AND TREATMENT

Administration of immune serum within 36 hours after exposure to RVF virus has been shown to protect newly born lambs (60). To circumvent this impractical approach to prophylaxis, formalin-treated and attenuated vaccines have been prepared. MacKenzie (35) produced two vaccines: one by inactivation of virus with methylene blue in light and one by inactivation with formalin. A number of investigators studied the attenuation of virus undergoing serial TC passage in mice (68). Kaschula (30) found that attenuated vaccine prepared from 86 mouse passages and 10 egg passages caused abortion in pregnant animals. Live vaccine prepared from virus with 102 mouse passages and 54 egg passages appeared safe for use in cattle and sheep, although it was slightly less immunogenic than with fewer passages. According to Weiss (68) the latter vaccine has been extensively used in Africa with encouraging results. DeTray (10) reported that there are now strong indications that the egg vaccine is not a satisfactory immunizing agent. The virus apparently became modified immunologically by serial passage in eggs. The egg vaccine stimulated serum antibodies which will neutralize the homologous virus but not the virulent field strains. Egg vaccines have been replaced by mouse brain vaccines. These may produce abortion in pregnant ewes or encephalitis in young lambs.

Randall et al. (48) has developed a formalin inactivated vaccine for RVF. The vaccine was produced by inoculating monkey-kidney cell cultures in serum-free medium 199 with RVF virus and incubating 96 to 144 hours at 36°C. Infected cultures were homogenized, clarified by centrifugation and filtered. Material of a high titer was treated with formalin in a final concentration of 1:1000. In addition to the extensive safety tests in laboratory
animals and tissue culture (48), young lambs have been given the vaccine (45). Two lambs from one to three days of age were each given 15 ml. of vaccine by the IP route. A febrile response and viremia did not occur. Approximately three weeks following the administration of the vaccine, each lamb was challenged with $10^4$ MIPLD$_{60}$ of RVF virus given intraperitoneally. No clinical signs referable to RVF were observed, nor was a viremia present. The large vaccine dose was given primarily for the purpose of a safety test of the vaccine and not as an immunization measure.

No specific treatment is available for Rift Valley fever (68).

MEDICAL AND VETERINARY ASPECTS

There is ample evidence for listing Rift Valley fever as one of the zoonoses. Almost without exception individuals coming in close contact with animals infected with RVF or working with the etiological agent in the laboratory have become ill with the disease. Thus, those occupations most likely to be listed as concerned with this zoonosis are farmers, veterinarians, butchers, animal caretakers and others associated with the animal industry in addition to laboratory workers. In the absence of a vaccine for humans prior to the development of one by Randall et al. the investigator of RVF expected to become infected. The immunization program in conjunction with improved safety measures as carried out by recent American investigators has resulted in an absence of human cases of RVF (45). Thus, it appears that an effective vaccine could be available for man if required.

Todd (66) has reviewed the public health aspects of this disease. Information on RVF indicates that for the most part man must come in direct contact with infectious materials to become infected. There are a few reports of individuals visiting laboratories, farms, laboratory animal rooms, and similar places where infected animals have been held who became infected with RVF with aerosol transmission as a possibility. Spread of the disease from person to person by direct contact or infection of man by mosquitoes has not been reported.

Kaschula (29) has reviewed the veterinary and medical problems of RVF and the possibility of the introduction of this disease into the United States. He reports that RVF could probably spread if the virus were introduced at a favorable time and place in this country. Investigations have shown that the virus is stable when protected by a menstruum of animal serum, that viremias persists for periods up to more than a week in many species of animal host and that two species of mosquitoes present in the United States, Aedes aegypti and Aedes triseriatus, are capable of biologically transmitting the virus of RVF. The most likely means of transmission appears to be by arthropods. Insect control measures are indicated. Most reports indicate that the disease is not spread from infected animals to susceptible hosts. Import restrictions generally would not permit entrance of a domestic animal in the viremic phase of the disease. The importation of monkeys from RVF areas should be considered as a possible hazard, since these animals may have a long viremic state and not show clinical signs of disease.
Research is needed on an effective vaccine for protection of the sheep and cattle population should the need arise. The vaccine as used in Africa has been reported to produce abortions in sheep and illness in some lambs. The vaccine produced by Randall et al. did protect susceptible lambs from lethal challenge doses. These vaccine doses were excessive, however, for practical purposes since the administration of the vaccine was for a safety test and not specifically to determine the efficacy of the product as an immunizing agent.

REFERENCES


As announced in the report last year, this Committee has undertaken the project of cataloging and eventually classifying the animal viruses. In doing this, it has been decided to include all animal species except man and other primates. At least two Committees already exist for the purpose of classifying respiratory and entero-viruses of humans. These are more or less supported by the National Institutes of Health. There is also another Committee sponsored by the World Health Organization and the Rockefeller Foundation to cover the arbor viruses. We are endeavoring, then, to fill the gap that exists in the animal field. We feel it is definitely our responsibility as veterinary scientists to do this, not only because we have the best understanding of the animal diseases caused by these viruses, but initially at least, are the ones who will benefit most from such cataloging and classification efforts.

In order to start this work, two sub-committees were established last year to draft suitable procedures which could be followed, if desired, by all virologists working in the field. Two forms have been devised by these sub-committees. The first is a suggested standard protocol for serological identification of animal viruses by the serum-neutralization test. This protocol will provide a base for all research men who wish to compare viruses obtained from different laboratories, or to identify new agents isolated from the field. By use of this procedure, results from different laboratories can be compared far more accurately than has been possible heretofore.

The second is a protocol on which the investigator can provide the data he has collected on a particular virus, including not only serology, but other information such as the species of animal from which the agent was isolated, the disease it may produce, pathology, physical characteristics, etc. (these protocols with explanations are listed below).

After enough of these forms have been submitted, this Committee will endeavor to sort the information from different areas and determine eventually how many different viruses actually have been isolated. All of the information for each different virus will be transferred to McBee or some other suitable punch-card system, and made available to anyone in the world interested in viruses.
REPORT OF COMMITTEE

In order to accomplish this objective, arrangements should be made for facilities to house the information at some university or other suitable institution. Funds will be needed to defray the cost of such an operation. This will include such items as secretarial assistance, printing, postage, etc., as well as a certain amount of necessary travel by Committee members, such as representation by U. S. Livestock Sanitary Association members at meeting of the other Committees referred to above.

In order to process this information adequately, at least one additional meeting of the Committee will be necessary, in addition to the regular fall meeting of the U. S. Livestock Sanitary Association. At least one or two men from this Virus Classification Committee, representing each major animal species, should attend the extra meetings.

The members of the Committee are very pleased with the progress that has been made to date, and with the availability of these forms, we believe additional progress will be rapid. We feel it is a rare opportunity, as veterinary scientists, to be associated with such a project, and we are indeed pleased that the U. S. Livestock Sanitary Association has chosen to lead the way in getting this vital work accomplished.

PROTOCOL FOR RECORDING DATA ON VIRUS

I. Virus name and/or number __________________________________________
   Information from ___________________________ Date ______
   Address __________________________________________

II. Original Source: (Isolation of virus being reported)
   A. Isolated by ___________________________ At ______
   B. Genus and species ___________________________
      Age ___________________________ Sex __________
      Isolated from: (Specify) ___________________________
      Signs of illness ___________________________________________
      ___________________________________________
      ___________________________________________
      ___________________________________________
   C. Date of collection: Day ______ Month ______ Year ______
   D. Specimen collected ___________________________
      ___________________________________________
      ___________________________________________
      ___________________________________________
   E. Location of source when collected ___________________________
      Geographic ___________________________
III. Method of isolation from original source:
Date inoculated ___________________ Diluent used ___________________
A. Host _______________________________________________________
   Age _______ Type of inoculum ________________________________
   Route inoculation _________________________________________
   Cell culture: Type of cell ____________________________________
      Culture vessel __________________________________________
      Medium composition ______________________________________
      Type of inoculum _________________________________ ml. ______
      Manner of recognition: CPE ___________________ Plaques _______
      Other ___________________________________________________
   Freedom from mycoplasma (PPLO) etc. _________________________
      Other ___________________________________________________
B. Validity of isolation:
   Reisolation: Yes _______ No _______ Not tried _______ Date _______
      Isolation by other method _________________________________
      Homologous antibody formation by original source animal:
      ____________________________ _____________________________
      (yes) (test(s) used) (no) (test(s) used) (not tested)
      Other reasons for validity of isolation _________________________
      ____________________________ _____________________________
      ____________________________ _____________________________
      ____________________________ _____________________________
IV. Other isolations ____________________________________________
      Sources __________________________________________________
      Method(s) if different from above ____________________________
V. Properties of virus:
A. Physical: Filterable ___________________ Type(s) filter ____________
      Size ___________________ How estimated ______________________
      Other information _________________________________________
      ____________________________ _____________________________
      ____________________________ _____________________________
      ____________________________ _____________________________
B. Chemical:
      Ether susceptibility ___________________ D.N.A. _________________
      R.N.A. ___________________ D.N.A. __________________
      pH stability ______________________________________________
      Acid production __________________________________________
      Antibiotic sensitivity _____________________________________
      Other __________________________________________________
VI.

TABLE 1

Antigenic Relationship or Lack of Relationship to Other Viruses

<table>
<thead>
<tr>
<th>Antigen of Reported Virus</th>
<th>Antigen of Reference Viruses</th>
<th>Antiserum of Reported Virus or Immune Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H.T.</td>
<td>Protected</td>
</tr>
<tr>
<td></td>
<td>H.O.</td>
<td>Total</td>
</tr>
</tbody>
</table>
| S.N.                     | C.F.                       | H.I.                                       | P.C.

<table>
<thead>
<tr>
<th>Reported virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.N.</td>
</tr>
</tbody>
</table>
VII-A. Biologic properties: *Natural host range*

<table>
<thead>
<tr>
<th>Host</th>
<th>No. Virus Isolations/No. Hosts Tested</th>
<th>No. With Antibody/No. Hosts Tested (Include Type of Antibody)</th>
</tr>
</thead>
</table>

VII-B. *Experimental host range*: Animals; arthropods; other invertebrates; embryonated eggs; cell culture

<table>
<thead>
<tr>
<th>Experimental Host</th>
<th>Passage History Strain Used</th>
<th>Age of Host</th>
<th>Inoculation Route ml.</th>
<th>Evidence of Infection</th>
<th>Titer (logs) (Vol)</th>
</tr>
</thead>
</table>

REMARKS:

VIII. Histo- and gross pathology:

IX. Geographic distribution:
   A. By virus recovery:
   B. From serological surveys:
   C. Other:

X. Remarks and subsequent information:

XI. References:

Do not write below this line

This Catalogue is for the purpose of registering viruses from animals other than primates.

The questionnaire should be filled in with a typewriter, using a relatively new black ribbon and clean type, and erasures avoided. This request is made because a photostatic plate will be made from the filled in questionnaire for transfer to the card to be included in the Catalogue. Please keep within
margins. You should retain a copy of the filled-in questionnaire for reference in case any changes or additions are suggested.

If question cannot be answered for lack of knowledge or because the examination was not made, please state: "not known" or "not done" or "not applicable".

If in answering a question or in giving data it is desired to make reference to a published article or to a personal communication, do so by number and list reference under the corresponding number (See XI). To economize space do not give title of published articles and give only first, or possibly second author’s name.

Some of the questions that may require clarification will be taken up in order:

I. Virus name and/or number. If a record of the virus has been published, use or give reference to the name or designation that was employed in the publication. If no publication has been made, please designate the virus by name or number for at least temporary reference. If the virus is given a name and if more than one strain has been isolated, it is necessary to identify the prototype strain used.*

II. Original source. This refers to the isolation of the virus being reported.

A. Isolated by. Name of individual who isolated virus.

B. Genus and species. Give name of the vertebrate from which the virus was isolated. For arthropods and other invertebrates the genus and species name should be given. For vertebrates the common name may be used but should be followed by the scientific designation.

If the exact age is not known, use terms generally applicable, such as embryo, suckling, nestling, immature, young adult, adult, etc.

Isolated from: Blood, etc., is obviously applicable to vertebrates only.

Signs of illness: Give signs of illness if known. More complete information on the nature of the disease may be recorded under VII-B remarks if further space is needed.

D. Specimen collected. This applies to time of collection in relation to stage of illness if from a diseased animal; i.e., acute chronic, convalescent, death, etc. Specify day. For wild or captive animals, or bird, indicate whether shot, trapped, netted, etc. and for arthropods and other invertebrates whether caught by hand, trapped, kind of bait, etc. If arthropods and invertebrates were collected from animals give species of animals and invertebrates from which collected.

E. Geographical: Refers to country and political sub-division within the country such as State, county, city, town, etc. In addition the approximate latitude, longitude and elevation should be given.

F. Method of storage until inoculated: This should indicate container, storage media, temperature and time.

* By prototype we mean the virus used for comparison.
III. Method of isolation from original source: The questions under this heading should be sufficiently clear and require no explanation. Here, as in all other instances, if there is not sufficient space, additional information may be given at the end of the questionnaire under "remarks" or by reference to published articles.

A. Freedom from mycoplasma (PPLO) etc.: Specify techniques used to insure freedom from non-viral contaminants.

B. Validity of isolation: Applies particularly to new viruses whose identity and disease-producing propensities have not been definitely determined. In such cases evidence should be furnished on the validity of isolation. If the specific questions asked cannot be answered, or the tests were not made, give other reasons why it is believed the isolation was valid, such as repeated isolation from similar or other sources and presence of specific antibody in associated vertebrates.

Homologous antibody formation by original source animal: Will apply to instances where the animal was retained alive or recaptured and it was possible to secure a second or "convalescent" blood sample for testing against the virus isolated.

If the available data indicate that similar isolates have been obtained from other sources or areas the investigator should so enumerate.

IV. Other isolations: Again this applies particularly to newly-reported viruses.

V. Properties of virus: These questions should be sufficiently clear, but if the tests have not been made, be sure to indicate "not done." Further characteristics of the virus which are deemed important, such as susceptibility to temperature changes, desiccation, light, etc. should be recorded under "Other information."

VI. Antigenic Properties:

The purpose of the antigenic studies is to provide criteria which in time may permit grouping of viral agents based on common properties. It is important therefore that each reporter conduct a wide variety of tests designed to reveal relevant antigenic information. Data should be enumerated as indicated or where this is not possible alternative notation should be accompanied by a suitable explanation. In general two types of information are desired:

(1) Information designed to show immunogenic relationships or lack of relationships among isolates, such as indicated by serum neutralization or cross protection tests, and

(2) Information designed to show other antigenic relationships among isolates as indicated by Complement-fixation, hemagglutinin, precipitin reactions, etc.

The following tests are suggested as being most applicable for obtaining pertinent antigenic information:
(1) Serum neutralization (SN): It is suggested that serum neutralization tests be conducted according to the “Suggested protocol for serum neutralization studies with animal viruses” for the sake of uniformity.

Appendix A. Where modifications are necessary such information should be included.

Test results should be recorded as follows: The neutralization endpoint should be expressed as the dilution of serum protecting 50 percent of the cultures against the cytopathic effect of a standard amount of virus.

Results obtained from any technique other than tube assay, such as plaque assay, the use of embryonated eggs or other animal hosts, should be calculated in a form designed to express 50 percent endpoint neutralization.

(2) Cross Protection (CP): Reciprocal cross protection tests should be conducted with each virus under consideration. It is important to recognize that known susceptible animals should be included as controls for each cross challenge.

(3) For other procedures designed to provide additional antigenic information such as hemagglutination inhibition (HI) complement-fixation (CF) precipitin reactions or others give reference to published data or provide information on the conduct of the test.

Data should be recorded as illustrated in Table 1.*

*Ho = homologous titer or titer of an immune serum with its own antigen or virus.

Ht = heterologous titer or titer of an immune serum with another antigen or virus as indicated.

For HI and CF, the titer is the reciprocal of the dilution endpoint of the immune serum.

Controls: Supply evidence showing that animals were devoid of significant antibody prior to production of antisera, and proof that virus was pathogenic for the protection challenge (PC).

VII-A. Biologic properties: Here should be listed all vertebrates, arthropods; and other invertebrates from which the virus has been recovered in nature, as well as results of antibody surveys, giving number tested/number positive. Also under remarks, the type of disease and signs in animals should be given. It may also be well to indicate what is believed to be the most likely cycle in nature. For the viruses on which there is published information, reference to published articles may be made.

VII-B. Experimental host range: Give animals, arthropods, other invertebrates, embryonated eggs, etc., as well as cell cultures (type of cell) that have been tested for susceptibility to infection with the virus. Give passage history of strain used in test: i.e., number of passages in animals (species) or cell cultures. Give evidence of infection. Since it is frequently useful in
classifying the viruses, if record of cell culture is given, indicate whether it produces C.P.E. (cytopathogenic effect and/or plaques). If the information cannot be recorded in the limited space, give references to publications or other reports (See XI). Titers refers to the infectious titer of the inoculum/volume administered to the experimental host.

VIII. Histo- and gross pathology: Give briefly information on lesions produced in both experimentally and naturally infected animals, with notation of inclusion bodies or any other highly characteristic pathological reactions. Indicate histological techniques employed.

IX. Geographic distribution: Designate under “Virus recovery” or under “Serological surveys,” or “other,” as may apply, the continents and countries involved. For more detailed information, for which there is not sufficient space for recording, give references to published data.

X. Remarks and subsequent information: The reporter of this virus is urged to supply the Committee of the virus catalogue with additional information, either direct or through references, as it may become available. This applies to all properties and behavior of the virus such as additional evidence on its antigenic relationships, host range, general ecology, epidemic outbreaks, geographic distribution, etc.

XI. References: List reference as referred to by number in preceding items. Save space by not giving title of published articles and only the name of first or possibly second author.

DEFINITIONS

1. Immuno Type—A virus which is antigenically identical with an immunological prototype.

2. Sub Type—An isolate (virus) which is immunologically similar, with some detectable serological difference.

3. Strain—A virus isolate which has undergone some degree of characterization.

4. Isolate—An uncharacterized virus recovered from any animal source.

Respectfully submitted,

CHARLES J. YORK, Chairman.
A RESOLUTION PROPOSED BY THE COMMITTEE
ON VIRUS RESEARCH

BE IT RESOLVED that the work of the Committee, as reported, be imple-
mented

1. By establishing a repository of virus information at a recognized, impartial institution;

2. That an Executive Secretary be appointed by the Committee to super-
vise the work necessary to maintain this repository. By necessity, the secretary must be a man with some experience in the field of animal viruses, and associated with the institution providing the housing;

3. That funds to carry out this work be obtained from an impartial, non-profit organization of greatest prestige on a national and international level, and with previous experience in this sort of endeavor;

4. That the Executive Board of the United States Livestock Sanitary Association direct the Committee on Virus Research to represent it in this work, and to take the steps necessary to carry out the objectives outlined above;

5. That the name of this Committee be changed to the “Committee on Animal Virus Classification”.

Dr. William R. Hinshaw
United States Army
Chemical Corps Biological Laboratories
Fort Detrick, Maryland

January 10, 1962

Dear Dr. Hinshaw:

I am very glad to have your letter of January 8th.

I am writing this to you as Chairman of the Committee for Animal Virus Classification of the United States Livestock Sanitary Association. Please consider this official authorization from the association to the committee to function in the name of the United States Livestock Sanitary Association in the operation of the project as outlined in the resolution herein quoted. The resolution was approved by the Executive Committee of the association at its meeting in Minneapolis, Thursday, November 2, 1961. The resolution was read before the entire membership present and unanimously adopted the following day.

Yours very truly,

W. L. BENDIX, D. V. M., President,
United States Livestock Sanitary Association
A STANDARD PROTOCOL FOR SEROLOGICAL IDENTIFICATION OF ANIMAL VIRUSES

INTRODUCTION

As pointed out in the report of the Committee on Virus Research, a large number of animal viruses have been isolated in the past few years, due chiefly to the application of tissue culture methods to studies on the etiology of animal diseases. In order to obtain definitive information on the antigenic relationship of these agents, the following procedures are suggested as a basis for virus antigenic studies within a laboratory, and in the exchange of viruses and sera between laboratories. Although tissue culture is the method of study discussed in the protocol, obviously other means of virus propagation, e.g., in embryonated eggs, could also be used where necessary.

While it is recognized that the standard procedures suggested here may not apply in all cases, it is recommended that they be followed initially. Where modifications are necessary, such information should be included as part of the virus history in exchanges of viruses between laboratories, and in publication.

The following steps are recommended in making serological identification of viruses:

A. Single Strain Selection

1. After original isolation and confirmation of the viral nature of the agent, the virus must be subjected to single strain isolation procedures. This step is essential in order to make certain that the isolate represents not a mixture of viruses but a single virus, and to confine the investigation to a definite strain of virus. Two methods have been widely used for this purpose.

   a. Tissue culture tube terminal dilutions—At least four to five tubes should be used for each 10-fold dilution of virus, and the dilutions carried out far enough to insure that an endpoint has been reached. An average of three (3) terminal dilutions should be made. Only one tube showing virus effect at the endpoint should be selected for passage, or for preparing a virus pool after single strain isolation.

   b. Plaque selection—If this method is employed the procedures must take account of the precautions outlined by Dulbecco and Vogt in “Plaque formation and Isolation of Pure Lines with Poliomyelitis Viruses.” J. Exp. Med., 1954, 99, 167-182. One plaque only may be selected for a single line of virus to be considered “pure.” At least three (3) successive plaque isolations should be carried out. In the case of viruses showing the phe-
nomenon of plaque variation, the single line isolation procedures should be carried out for each distinct plaque variant. The final selection of the single strain (or strains) representative of the virus type must be determined on the basis of plaque variant stability, antigenicity, and host response.

2. The isolate(s) must be reinoculated into host species to confirm the identity of the selected strain.

3. Preservation of virus samples: To provide reference material portions of specimens from which the original virus isolation was made must be preserved in sealed phials either at below 

$-50^\circ C$ or in lyophilized form. This procedure should also be followed with samples from early passages initiated before single strain selection, and from a pool of the single strain(s) selected virus.

B. Preparation of Antisera

1. Use of the natural host antiserum should be avoided as far as possible.

2. In the interest of uniformity, whenever feasible, a single species of laboratory animal should be used for antiserum preparation. Rabbits, for practical reasons, offer the best hope of success. Several rabbits should be used for each virus studied. The serum of each animal should be tested prior to immunization to determine that it does not contain non-specific inhibitors for the virus under study. Adequate amounts of pre- and maximum amounts of post-immunization serum should be taken from each animal used for antiserum preparation.

3. Except in instances when, by virtue of virus proliferation in the animal, a single injection of virus produces adequate antibody response virus antigen should be prepared in a cell system different from the one intended for serum neutralization test. If this is not possible a rapid course of inoculation avoiding the intravenous route should be followed.

4. After a course of immunizing inoculations, pre- and post-inoculation serum from each rabbit should be tested separately. A pool of normal pre-inoculation serum, as well as immune serum, should be prepared only from those animals whose presera do not show non-specific inhibition of the test virus, and whose post-sera have a satisfactorily high titer. For practical purposes, the titer should be high enough to dilute out anti-cell antibodies which may cause non-specific interference with virus propagation, and yet have sufficient anti-body remaining for virus neutralization. If there is any doubt in this respect, especially where low virus titers must be used, suitable controls should be included. One such control would be the use of antiserum of rabbits immunized with uninoculated tissue culture cells and fluid prepared from the same species as used for propagating the virus.
5. Sterile samples of pre- and post-immunization serums should be stored in sealed phials either in lyophilized form or at below —10° C.

C. Preparation of tissue cultures

1. Although the plaque assay method is undoubtedly more accurate than terminal dilutions, not all viruses needing identification can be plaqued at the present time. Therefore, in following this standard protocol, it is proposed that tissue culture tubes be used.

2. The exact growth medium for the cells is probably not too important, provided the serum supplement is devoid of virus-inhibiting substances.

3. The maintenance medium should be serum-free (if possible) and optimal for the tissue culture-virus system used.

4. The exact medium used for each virus should be described.

5. Borosilicate glass (Pyrex or Kimax) culture tubes should be used for the cultures.

D. Conduct of serum-neutralization tests

1. Constant amounts of virus with varying dilutions of serum is the system of choice.
   a. A final concentration of 100 TCID_{50} of virus, with a suggested range of 50 to 200, should be employed.
   b. All serum should be heat treated for 30 min. at 56° C prior to testing.
   c. The dilutions of test sera, both normal and immune, should be made in a suitable medium which will not adversely alter the constituents of the tissue culture medium. The maintenance medium is frequently employed for this purpose. Ten-fold dilutions of serum should be used wherever possible. If the serum titers are too low for this, 2-fold dilutions should be substituted. The dilution used should be specified for each virus system.

   (An example of the 10-fold serum dilution procedure would be to draw up 0.2 ml. of test serum and add it to 0.8 ml. of diluent, equaling a 1:5 dilution. The pipette is discarded after mixing. Use a new pipette to draw up 0.3 ml. of diluted serum; add 0.2 ml. to next tube containing 1.8 ml. of diluent, equaling 1:50 dilution of serum. Discard remaining 0.1 ml. of 1:5 serum with a pipette, or put back into Tube No. one. After sufficient dilutions are prepared, add equal amounts of diluted virus to the serum tubes, or add the serum to tubes already containing the correct amount of virus. If virus is added to serum dilution tubes, care should be taken to start with the tube containing the highest dilu-
tion of serum. A fresh pipette should be used for each set of serum tubes. The final concentration of virus and the final dilution of serum should take into account the dilutions resulting when the virus and serum are added together. Failure to discard pipettes between dilutions, not adding serum and virus together in correct sequence, inadequate mixing of the serum or the serum-virus mixtures, using the incorrect volume of virus and serum when mixing, are all factors that can cause variation in results, and hence create difficulties in making comparisons between laboratories.)

d. The serum-virus mixtures should be incubated at 25° to 27° C. (a controlled room temperature) for two hours. Other procedures may be used if this does not fit the stability and neutralization characteristics of the virus studied. Again, where different techniques are used, they should be included as part of the virus history.

e. The serum-virus mixture should be added in 0.2 ml. amounts to each tissue culture tube, using four to six tubes for each serum dilution. Each tube should contain 1.8 to 2.0 ml. of tissue culture fluid which is not to be included in any dilution calculation.

f. Inoculated culture tubes should be incubated at 35° to 36° C. or, if necessary, at other temperatures compatible with the cell-virus system used.

g. Virus control titrations should be conducted at the same time and under the same experimental conditions as the neutralization tests.

h. The Reed-Muench formula is suggested for determining neutralization (as well as virus) endpoints. Care should be taken to use the same logarithmic base as for the dilution factor employed.

E. Interpretation of test

1. Complete reciprocal cross-neutralization test should be performed with existing viruses, to determine exact relationships.

2. Whether viruses are related, unrelated, or antigenically identical depends on interpretation of test results. The formula offered by Archetti and Horsfall (J. Exp. Med., 92, 1950, pp. 441-462) is to be used as a basis for determining these factors. Other methods to supplement this may be used, if desired.
CONSTITUTION AND BY-LAWS

OF THE

UNITED STATES LIVESTOCK SANITARY ASSOCIATION

ARTICLE I—NAME

The name of this Association shall be "The United States Livestock Sanitary Association."

ARTICLE II—PURPOSE

The purpose of this Association shall be the study of livestock sanitary science, milk and meat hygiene, and the dissemination of information relating thereto, the unification so far as possible of the laws, regulations, policies and methods pertaining to milk and meat hygiene, and to the prevention, control and eradication of transmissible livestock diseases; to maintain co-ordination among the various livestock regulatory organizations, and to serve as livestock sanitary science clearing house between this Association and the following: The livestock owner, the livestock sanitarian, the milk and meat hygienist, the veterinary practitioner, the transportation and stock yard companies, the milk and meat producing and distributing companies, and various other interested agencies. The word "livestock" as herein used shall be understood to include poultry.

ARTICLE III—MEMBERSHIP

There shall be three kinds of members—Official and Individual and Non-Voting Junior.

OFFICIAL MEMBERSHIP

The livestock sanitary departments of each state also the United States, and the Canadian, Cuban and Mexican governments, Puerto Rico, the Virgin Islands and Los Angeles County, California shall be eligible to official membership in this Association and be represented on the Executive Committee by the livestock sanitary executive official.

INDIVIDUAL MEMBERSHIP

Any person engaged in livestock sanitary work for Federal, provincial, state, county or municipal governments and any other person interested in livestock sanitation or milk and meat hygiene may be elected to individual membership.
JUNIOR NON-VOTING MEMBERSHIP

Students in agriculture, medicine, veterinary medicine, vocational agriculture or any 4-H Club member as well as future farmers under 21 years of age are eligible to election as junior non-voting members.

ARTICLE IV—MEETINGS

The meetings of this Association shall be annual and special.

ARTICLE V—OFFICERS

The officers of this Association shall be: President, President-Elect, First Vice-President, Second Vice-President, Secretary-Treasurer, and an Executive Committee.

EXECUTIVE COMMITTEE

The Executive Committee shall be composed of the executive officer representing the livestock sanitary departments of the various States, the Director of Livestock Regulatory Programs of the United States Department of Agriculture, the Veterinary Director General of Canada, the executive regulatory officer of Cuba, Mexico, Puerto Rico, the Virgin Islands, Los Angeles County, California, the elective officers of this Association and eight delegates at large representing the livestock industry including poultry.

No more than two delegates from each of the four districts of the United States shall be elected. 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, North Dakota, Ohio, South Dakota, and Wisconsin; the Southern, comprising the States of Alabama, Arkansas, Georgia, Florida, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia, Puerto Rico and the Virgin Islands; and the Western district consisting of the States of Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming. It shall be the duty of the Committee on Nominations to canvass the membership of this Association and select eight (8) nominees for delegates at large. Said nominees must be selected from and represent the livestock industry, including poultry. No more than two (2) delegates at large shall be elected from each of the four designated areas or districts, nominations from the floor of the convention may be made for additional nominees by districts and shall be bona fide residents of the respective district for which they are nominated. Such delegates shall be elected at the time and place as are the elected officers of this Association.
CONSTITUTION AND BY-LAWS

The Executive Committee shall constitute the administrative body of this Association and shall determine its activities and policies.

All recommendations and reports of officers and committees shall be referred for consideration to the Executive Committee.

The President-Elect shall be ex-officio chairman of the Executive Committee.

The Executive Committee shall elect yearly a Secretary-Treasurer for the Association. The Secretary-Treasurer shall receive such salary and allowance as may be fixed by the Executive Committee.

The Executive Committee shall cause to be audited annually or oftener if deemed necessary, the receipts and disbursements of the Secretary-Treasurer, and shall have authority to hear and determine all complaints filed before it in writing relative to the conduct of any member; and shall have authority to accept or reject applications for individual membership properly placed before them. Three negative votes shall disqualify for such membership.

ARTICLE VI—PROGRAM COMMITTEE

The President, the Chairman of the Executive Committee and the Secretary-Treasurer and the Chairman of the respective committees shall constitute the Program Committee. It shall be the duty of the officers of the Program Committee to make the necessary arrangements and provide the program for the annual and special meetings.

ARTICLE VII—DUTIES OF OFFICERS

1. President: It shall be the duty of the president to preside at all meetings of this Association; to appoint all committees excepting the Executive and Officer faction of the Program Committee; to call special meetings of the Association whenever he considers the holding of such meetings necessary for the good of the livestock industry or upon the written request of five members of the Executive Committee. The president shall be an ex-officio member of all committees.

2. President-Elect: The president-elect shall be chairman of the Executive Committee. In the absence of the president, he shall preside at the meetings of the Association. In the event of the absence, disability or resignation of the president he shall perform all duties of the president. He shall be an ex-officio member of the Executive and Program Committees.

3. First Vice-President: The first vice-president shall assume the duties of the president in the event of the absence, disability or resignation of the president and president-elect. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability or resignation of the president-elect. He shall be an ex-officio member of the Executive Committee.
4. Second Vice-President: The second vice-president shall assume the duties of the president in the event of the absence, disability or resignation of the president, president-elect and first vice-president. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability or resignation of the president-elect and first vice-president. He shall be an ex-officio member of the Executive Committee.

5. Secretary-Treasurer. The Secretary-Treasurer shall keep an accurate record of the proceedings of the Association. Whenever authorized so to do by the Executive Committee he shall publish said proceedings and distribute them to the members of the Association. The Secretary-Treasurer shall also keep an accurate record of the proceedings of the Executive Committee and shall furnish a copy to each member of said Executive Committee. He shall forward to each Executive Committee member a copy of each regulation approved by the Association. He shall keep an accurate account of all Association moneys received and disbursed. He shall also present to the Chairman of the Executive Committee a list giving the name, occupation and address of each applicant for individual membership for the approval of the Executive Committee. He shall perform such other duties as may be authorized and prescribed by the Executive Committee. He shall be ex-officio secretary of the Executive Committee, also an ex-officio member and secretary of the Program Committee. He shall be bonded for not less than ten thousand dollars.

ARTICLE VIII—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 and further provided that the amendment has received the approval of the Executive Committee.

BY-LAWS

ARTICLE I—ORDER OF BUSINESS

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

A suspension of the By-laws may be made by a two-thirds majority 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. The Application shall give the name, occupation and address of the applicant and shall be accompanied by a fee of five dollars ($5.00), which amount shall include the membership dues for one year. Applications shall be presented in proper form to the Secretary-Treasurer, who shall in turn submit them to the Executive Committee.

An individual member may be expelled for cause by the Executive Committee.

**Article III—Meetings**

The annual meetings shall unless otherwise determined not less than thirty (30) days in advance by a majority of the members of the Executive Committee, be held at Chicago, Illinois, during the time of the International Livestock Exposition. The place for holding the meetings in Chicago as well as the duration of said meetings shall be determined by the Officer Members of the Program Committee of the Association.

The place for holding special meetings shall be determined by the President with due regard to the wishes of the members of the Executive Committee, the subject matter to be considered, accessibility, and the information to be obtained. The notice of time and place of holding a special meeting shall be mailed to the members at least thirty days prior to the date fixed for the special meeting.

**Article IV—Quorum**

Twenty-five members of the Association shall constitute a quorum.

Twenty members of the Executive Committee shall constitute a quorum.

**Article V—Dues**

The dues for individual membership in this Association shall be five dollars ($5.00) per annum, payable in advance (on or before January 1st of each year) to the Secretary-Treasurer of the Association.

The dues for non-voting junior members shall be three dollars ($3.00) per annum, payable (on or before January 1st of each year) to the Secretary-Treasurer of this Association.

The dues for official memberships shall be one hundred dollars ($100.00) each per annum, payable in advance (on or before January 1st each year) to the Secretary-Treasurer of this Association.
OFFICERS, CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS FOR 1962

HOWARD W. DUNNE
Chairman

E. P. POPE
Secretary
4th ANNUAL MEETING

CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS

OCTOBER 30-31, 1961

MINNEAPOLIS, MINNESOTA

J. W. NEWBERNE, Chairman, Indianapolis, Indiana
E. P. POPE, Secretary, Madison, Wisconsin

SALMONELLA SYMPOSIUM—Moderator, PHILIP R. EDWARDS.
1. Laboratory Procedures for the Isolation of Salmonella from Human and Animal Food Products, MILDRED M. GALTON 434
2. Occurrence and Distribution of Salmonella in Animals in the United States, ALICE B. MORAN 441
3. Salmonella Organisms Isolated from Feed Ingredients, B. S. POMEROY, et al. 449
5. Industry's Role in Reducing the Incidence of Salmonella in Animal Feeds, C. F. NIVEN, Jr. 453
7. Methods of Investigating the International Spread of Salmonellae, K. W. NEWELL 464
8. Discussion 470
9. Summary, PHILIP R. EDWARDS 475

GENERAL SESSION
1. The Diagnosis of Hog Cholera, H. W. DUNNE 478
2. Differential Diagnosis of Bovine Virus Diseases, CHARLES J. YORK 485
3. The Organization and Function of the Veterinary Medical Laboratory, V. B. ROBINSON 493
4. Diagnosis of Canine Diseases, J. H. SAUTTER, et al. 499
5. Some Aspects in the Diagnosis of Disease in Wildlife, C. M. HERMAN 512
6. Protecting Laboratory Personnel Against Rabies, E. S. TIERKEL 269
7. The National Animal Disease Laboratory, E. E. WEDMAN 518
8. Comments on the Laboratory Diagnosis of Leptospirosis in Domestic Animals with an Outline of Some Procedures, EARL E. ROTH, et al. 520
9. Hard Tissue Lesions Associated with Malnutrition, W. S. MONLUX 535
10. The Nature of Serological Reactions with the Complement Fixation Test for Ornithosis in Avian Serums, Y. SHIMIZU and R. A. BANKOWSKI 542
LABORATORY PROCEDURES FOR THE ISOLATION OF SALMONELLA FROM HUMAN AND ANIMAL FOOD PRODUCTS

MILDRED M. GALTON*

Since members of the genus, Salmonella, were first recognized in the late 19th Century, and their widespread distribution as a cause of human and animal disease was established, numerous methods for the isolation of these organisms from clinical specimens have been developed. As the epidemiology of salmonellosis began to unfold, it became apparent that foods played an important part in the chain of infection. With the need for detection of salmonellae in foods, microbiologists naturally applied methods that had been found satisfactory for recovery from human and animal fecal samples, only to find in many instances that these procedures were inadequate.

In this report, the development of methods for the isolation of salmonellae from clinical specimens will be discussed as well as some of the problems encountered in the isolation of these organisms from foods and certain of the methods developed for the solution of these problems. In addition, a suggested procedure that has proved effective for detection of salmonellae in food products will be presented.

Development of media for isolation from fecal samples: A satisfactory specimen is the first essential in any laboratory examination. It is generally agreed that freshly passed stool specimens are the most desirable. However, equally satisfactory results may be obtained from rectal swab specimens during the acute phase of illness when immediate inoculation of culture media is possible. If this is not possible, a preservative medium which will prevent the multiplication of the normal intestinal flora with little or no effect on the pathogens should be used. The buffered glycerol-saline solutions (1, 2, 3) are perhaps the most reliable for this purpose. Feces should be added to the preservative in a proportion of 1:10.

Another useful method recommended by Lie Kian (4, 5), Dold and Ketterer (6) and Bailey and Bynoe (7) when samples must be shipped, consists of drying the stool specimen on filter paper or blotting paper.

The use of special liquid enrichment mediums is always indicated in the examination for asymptomatic carriers as the numbers of salmonellae present may be small. The two mediums that have been used most widely are tetrathionate broth of Mueller as modified by Kauffman (8) and selenite broth of Leifson (9). However, Thomson (10) found that ordinary nutrient broth was often as effective or superior to selenite broth as an enrichment for the isolation of salmonellae from feces. Smith (11) observed that direct plating was superior to selenite and tetrathionate enrichments for the isolation

* From the Department of Health, Education, and Welfare, Public Health Service, C.D.C., Epidemiology Branch, Veterinary Public Health Laboratory Unit, Atlanta, Georgia.
LAB PROCEDURES FOR ISOLATION OF SALMONELLA

of *S. cholerae suis* and *S. abortus-ovis* and later recommended Brilliant Green MacConkey Broth for isolation of the former type (12). He concluded that the selenite and tetrathionate broths were too toxic for these types. Similarly, Banwart and Ayres (13) found that tetrathionate broth inhibited the growth of *S. paratyphi*.

Several modifications of tetrathionate broth have been recommended to suppress the growth of Proteus. Galton, *et al.* (14), found that addition of 0.125 mg. of sodium sulfathiazole per 100 ml. of tetrathionate suppressed the multiplication of Proteus from canine fecal swabs. More recently, Jameson (15) reported that the addition of one percent sodium lauryl sulfate and a bismuth sulfite solution to tetrathionate broth favored the selective inhibition of Proteus and allowed active multiplication of salmonellae from sewer swabs.

Numerous plating media have been developed for the isolation of salmonellae and other enteric pathogens. These include the differential media such as Endo's agar and eosin-methylene blue agar which distinguish the lactose and non-lactose fermenting organisms and inhibit gram positive organisms. In addition, certain differential media incorporate bile salts which makes them slightly selective and suppresses the spreading of proteus as MacConkey's agar (16) and Leifson's desoxycholate agar (17). However, the more selective plating media are more effective in the isolation of salmonellae from enrichment broths or by direct plating since they are designed to inhibit many strains of the normal intestinal flora. The most widely known are bismuth sulfite agar (18, 19), desoxycholate citrate agar (17), SS Agar, MacConkey brilliant green agar (12), and the brilliant green-phenol red agar (BG) of Kristensen, Lester and Jurgens (20).

Although salmonellae may be isolated on all these media, the BG agar, when properly prepared, is more inhibitive for other enteric organisms and salmonella colonies are detected with greater ease. In our experience and others (13, 21, 22), the use of BG agar is indicated for maximum yield of salmonellae, other than *S. typhi*, following enrichment from clinical specimens and foods.

*Problems in the isolation of salmonellae from foods and feed ingredients*

Many of the problems raised by the rapid development of food processing in the detection of salmonella in prepared foods were discussed at a symposium in New York in 1955 (23). It was pointed out that the commonly used media for the isolation of salmonellae were designed primarily for feces or other clinical specimens. Obviously, the addition of comparatively large amounts of organic material, as food samples, may have an adverse affect on the selectivity and enrichment quality of these media. Slocum (23) observed that direct pre-enrichment procedures to encourage development of salmonellae prior to selective enrichment were more feasible than modification of the medium composition for optimum selectivity with each of a wide variety of foods. The value of a lactose pre-enrichment for detecting small numbers of salmonellae in dried egg products has recently been reported by North (24). Earlier, Silliker and Taylor (25) noted the inhibitory
effect of egg albumen. North attributed the success of this method to the restoration of a larger number of salmonellae to a state of active growth from a state of reduced viability or attenuation caused by drying or freezing of the products during processing. The value of nutrient broth as a primary medium was noted earlier by Thomson (10) in the isolation of *S. paratyphi* B from a sample of flour for nearly a year, whereas cultures from the same samples in selenite broth were negative after five months. Thomson (26) also observed that salmonellae usually could be recovered more frequently from small rather than from large inocula. In this connection he noted that as the dilution of feces increased up to 1:1000, coliforms progressively decreased and the salmonellae appeared in purer culture on selective plating media. He was of the opinion that the advantage of selenite broth and tetrathionate broth was primarily due to the fact that they are fluid media rather than to their composition. McCoy (27) was also able to recover salmonellae from higher dilutions of bone meal from tetrathionate enrichment broth when these organisms appeared to be absent from the larger inoculum. He attributes this to the probable presence of salmonella in such material in clumps and the dispersion of these clumps during dilution.

Recently, other workers have made considerable progress in the development of methods and media, particularly adapted to the search for salmonellae in foods. Selenite broth has been modified by the addition of cystine (28), brilliant green (29) and sulfapyridine (22). Byrne, et al. (30), found that rehydration for dilution of dried eggs and egg products in distilled water and inoculation of this suspension into multiple tubes of selenite-cystine broth favored the isolation of salmonellae. Similar enhancement of salmonella growth was obtained from dried yeast when an aqueous suspension was incubated at 30° C for 24 hours prior to inoculation of selenite or tetrathionate broth.

Our interest was focused on the problem of salmonella in foods during our investigation of two outbreaks of salmonellosis in dogs in an animal hospital. Their foods were examined and salmonellae were isolated from pork and beef livers purchased from a local abattoir for dog food (14). Shortly thereafter, a search for salmonella in retail meat products in Jacksonville, Florida, was commenced (31). Concurrently, dehydrated dog meals obtained in sealed packages on the retail market were also examined (32). More recently, similar studies have been conducted in the Atlanta area on meat products for human consumption (33), animal feeds and feed ingredients (34).

Early in the studies on fresh pork sausage, it was noted that the number of salmonella positive specimens increased with an increase in the amount of inoculum up to a point. However, an excessive amount of meat in the tetrathionate broth inhibited its selective action. In a series of comparative tests, the most desirable amount of inoculum and the ratio of this to enrichment was found to be 30 gms. of sample in 100 ml. of tetrathionate broth.

After incubation, however, the enrichment broth invariably had a heavy layer of fat and particles of meat as a top layer on the broth. This made it
difficult to obtain a satisfactory loopful of material for inoculation of the brilliant green agar plates. It seemed apparent, also, that the poor dispersion would markedly limit the effectiveness of the enrichment. Various methods and materials were tried to overcome this; the simplest and most effective was found to be the addition of a wetting agent, Tergitol No. 7. This detergent appears to enhance salmonella growth within a wide range of dilutions. The amount to be added was determined by titration and the minimum amount required to provide for emulsification of the fat and to give a distinct head of foam was selected. On repeated titrations, six ml. of a 10 percent solution of tergitol added to the 30 gm. portion of sausage in 100 ml. of tetrathionate broth was satisfactory. This same concentration was found to be satisfactory for isolation of salmonellae from dog meal. It should be pointed out, however, that for different foods, a preliminary titration would be desirable. No evidence of inhibition of salmonellae was noted with concentrations of tergitol up to 0.7 percent; rather, there was an apparent promotion of growth. Similar findings were obtained in tests with Tween 80 but in titrating, it was difficult to determine the end point as no distinct head of foam appeared.

Another problem with these meat samples was the heavy overgrowth of Pseudomonas and coliform organisms on the BG agar. It was established by in vitro tests that there was a wide difference in sensitivity to sulfonamides of these contaminating organisms and salmonella. Of these drugs, sulfadiazine proved most effective. A concentration of 8-16 mg. of sodium sulfadiazine per 100 ml. of medium effectively inhibited Pseudomonas, markedly suppressed coliform organisms but no evidence of inhibition of salmonellae was noted. The procedures suggested have evolved from the above mentioned studies.

**PROCEDURE**

Care should be taken in the collection of samples to avoid contamination. Packaged food presents no problem if unbroken packages can be obtained. For bulk samples of human foods, animal feed and feed ingredients, 16 ounce screwcapped refrigerator jars, one half or one pint ice cream cartons or small plastic bags may be used, depending upon the type of sample and method of transport to the laboratory. Sterile tongue depressors may be used to fill these containers.

A 30 gm. portion of the sample should be weighed and transferred to a 16-ounce screwcapped refrigerator jar containing 100 ml. of tetrathionate enrichment broth with brilliant green (one ml. of a 1:1,000 solution added). Then six ml. (or the amount determined by preliminary titration) of a 10 percent solution of Tergitol No. 7 should be added. With the top tightened securely, the jar should be shaken vigorously to disperse the sample. For samples of dried products, a second vigorous shaking is needed after about an hour followed by overnight incubation at 37 C. A high recovery rate will be obtained if in addition duplicate cultures are prepared in tetrathionate from each sample, particularly if small numbers of salmonellae are present.
It is desirable, also, to place another 30 gm. sample in 100 ml. of nutrient broth, add tergitol and shake well before incubation.

After incubation a generous loopful of tetrathionate enrichment is streaked onto a BG agar plate containing eight mg. of sodium sulfadiazine per 100 ml. (BGS). Then 1:100 and 1:1,000 dilutions are prepared from the tetrathionate enrichment in buffered distilled water. A generous loopful of each of these dilutions should be streaked to BGS plates. In addition, a large loopful of the 1:1,000 dilution should be streaked to a bismuth sulfite agar plate (WB). The BGS plates should be incubated overnight at 37°C and suspicious colonies picked to triple sugar iron agar (TSI). On the BGS agar, salmonella colonies are usually transparent pink to deep fuscia. If the plate has a predominance of lactose fermenting colonies, the salmonellae may be masked and appear as transparent brownish colonies with little change in the color of the medium. Close observation by transmitted light will reveal the brownish appearance of these colonies. The WB plates should be incubated for 48 hours at 37°C. This medium is used only to search for Arizona organisms as recommended by Edwards (35). Some Arizona strains are slow lactose fermenters and can be detected on the BGS plates. However, others that have also been found in food and in cases of food infection ferment lactose rapidly and would be missed. Both Arizona strains and salmonellae produce similar typical black colonies on WB agar. Edwards has noted that Arizona and salmonellae have the common ability to produce both \( \text{H}_2\text{S} \) and lysine decarboxylase, not shared by other enteric bacteria. To determine this, he has developed a lysine-iron-agar to be inoculated from black colonies on WB agar. By this method, rapid lactose fermenting Arizona strains may be detected.

In summary, the tetrathionate brilliant green broth with an optimum proportion of sample to enrichment and tergitol added to aid in emulsification has been used successfully to isolate salmonellae from a variety of human and animal food products when streaked to Brilliant Green Agar containing sodium sulfadiazine. Further, the observation has been confirmed that when 1:100 and 1:1,000 dilutions of the tetrathionate brilliant green enrichment are prepared and streaked to Brilliant Green Sulfadiazine Agar, the number of colonies of lactose fermenting organisms on the plate decrease and better isolation of the salmonella colonies is obtained. The use of nutrient broth with tergitol has been suggested because of the inhibitory action of tetrathionate broth on certain serotypes of salmonella. Although it has been well established that when small numbers of salmonellae are present, more positive samples may be obtained by the use of several different enrichment and plating media, or even multiple portions of the sample into the same medium, it has been found that this simple procedure can be applied successfully to a wide variety of food products.
REFERENCES


OCCURRENCE AND DISTRIBUTION OF SALMONELLA IN ANIMALS IN THE UNITED STATES

ALICE B. MORAN*

In the past four and one-half years some 6,216 cultures of *Salmonella* from animals have been serotyped in the Enteric Laboratory at the Communicable Disease Center under a cooperative agreement with the United States Department of Agriculture. This rather large number of cultures which came from more than 35 different animal species included 86 different serotypes. The rate of occurrence of these serotypes varied greatly. Twenty-three of them were seen one time only. On the other hand, 15 others comprise 82 percent of the total cultures. These are *S. typhi-murium*, *S. pullorum*, *S. cholerae-suis variety Kunzendorf*, *S. heidelberg*, *S. anatum*, *S. enteritidis*, *S. newport*, *S. san-diego*, *S. gallinarum*, *S. infantis*, *S. chester*, *S. st-paul*, *S. muenchen*, *S. derby*, and *S. blockley*.

*S. typhi-murium*, of which there were 1,385 cultures, was by far the most common and accounted for 22 percent of all *Salmonella* serotypes encountered. The list of animal sources of this organism testifies to its wide dispersal in nature: it was found in turkeys (612), chickens (282), pigeons and doves (67), ducks (9), geese (22), and "other birds," a category that includes such birds as the cockatoo, hoatzin, hornbill and parrot (22), in swine (45), sheep (11), cattle (185), horses (49), cats (2), dogs (9), mice (40), monkeys (4), minks (7), guinea pigs (12), foxes (1), nutrias (2), chinchillas (1), skunks (1), opossums (1), and reptiles (1). No other *Salmonella* type is seen as often nor from such widely scattered sources. Taking the period as a whole, *S. typhi-murium* cultures from turkeys outnumbered those from chickens by about two to one, and the same proportion holds as regards percentage of *S. typhi-murium* in the two species: 25 percent of the cultures from turkeys were this organism compared with 12 percent of the cultures from chickens. Most of the cultures from pigeons were *S. typhi-murium*, and of these nearly all were the Copenhagen variety, a variety that has been associated with pigeons for a long time. More than half the cultures from ducks and most of the cultures from geese were *S. typhi-murium*, as were three-quarters of the cultures from mice and half the cultures in the categories listed as "other birds" and "sheep and goats." In swine, the cultures of this organism represent nine percent of the total; 42 percent of the cultures from cattle were *S. typhi-murium*, as were 21 percent of the cultures from horses.

The serotype that ranked second in incidence, *S. pullorum*, was practically speaking, confined to the avian species. The 1,010 cultures identified represent 16 percent of the total *Salmonella* cultures serotyped. All but five of

* United States Department of Agriculture, Agricultural Research Service, Animal Disease Eradication Division, currently assigned to the Communicable Disease Center, Enteric Laboratories, Atlanta 22, Georgia.
<table>
<thead>
<tr>
<th>ALICE B. MORAN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>164</th>
<th>3</th>
<th>166</th>
<th>167</th>
<th>168</th>
<th>169</th>
<th>170</th>
<th>171</th>
<th>172</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other Animals</td>
<td>102</td>
<td>1</td>
<td>32</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Minks</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkeys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horses</td>
<td>1</td>
<td></td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Cattle</td>
<td>1</td>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sheep and Goats</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Swine</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other Birds</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Quails</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pheasants</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Geese</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ducks</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pigeons and Doves</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chickens</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Turkeys</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Salmonella

January 1957–July 1961

Occurrence and Distribution of Salmonella Serotypes in U.S.

**TABLE 1**
<table>
<thead>
<tr>
<th>Strain</th>
<th>Occurrences</th>
<th>Cases</th>
<th>Mortality</th>
<th>Hospitalization</th>
<th>Salmonellae</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corvallis</td>
<td>22</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td>Cubana</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Derby</td>
<td>52</td>
<td>18</td>
<td>4</td>
<td>2</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>Detroit</td>
<td>1</td>
<td>1</td>
<td>50</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Dublin</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Duesseldorf</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>63</td>
<td>25</td>
<td>50</td>
<td>99</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Florida</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Gallinarum</td>
<td>22</td>
<td>184</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>Gaminara</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>Give</td>
<td>38</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>Habana</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>Hamilton</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>Hartford</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>159</td>
<td>127</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Illinois</td>
<td>2</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Indiana</td>
<td>6</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Infantis</td>
<td>78</td>
<td>94</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Java</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Javanian</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Johannesburg</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Kentucky</td>
<td>20</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Litchfield</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Livingstone</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Lomita</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Luciana</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Madelia</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Manhattan</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Manila</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Meleagridis</td>
<td>47</td>
<td>16</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>
### TABLE 1—Continued

*Occurrence and Distribution of Salmonella Serotypes in U. S.*

January 1957—July 1961

<table>
<thead>
<tr>
<th>Salmonella</th>
<th>Turkeys</th>
<th>Chickens</th>
<th>Pigeons and Doves</th>
<th>Ducks</th>
<th>Geese</th>
<th>Pheasants</th>
<th>Quails</th>
<th>Other Birds</th>
<th>Swine</th>
<th>Sheep and Goats</th>
<th>Cattle</th>
<th>Horses</th>
<th>Cats</th>
<th>Dogs</th>
<th>Mice and Rats</th>
<th>Monkeys</th>
<th>Minks</th>
<th>Other Animals</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miami</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Minnesota</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Mississippi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Montevideo</td>
<td>13</td>
<td>23</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Muenchen</td>
<td>95</td>
<td>16</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>114</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Brunswick</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>New Haw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Newington</td>
<td>12</td>
<td>8</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Newport</td>
<td>165</td>
<td>6</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
<td>3</td>
<td>3</td>
<td>57</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>255</td>
</tr>
<tr>
<td>Norwich</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nottingham</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oranienburg</td>
<td>6</td>
<td>15</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Orion</td>
<td>20</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>Oslo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Panama</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Pensacola</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Poona</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Pullorum</td>
<td>14</td>
<td>987</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>2</td>
<td></td>
<td>3</td>
<td></td>
<td>1,010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reading</td>
<td>26</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>Rubislaw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Location</td>
<td>1911</td>
<td>1912</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>------</td>
<td>------</td>
<td>-------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St. Paul</td>
<td>114</td>
<td>18</td>
<td>132</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Diego</td>
<td>215</td>
<td>10</td>
<td>225</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saphra</td>
<td>38</td>
<td>14</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schwarzengrund</td>
<td>22</td>
<td>5</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Senftenberg</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simsbury</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stanley</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taksony</td>
<td>1</td>
<td>26</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tennessee</td>
<td>50</td>
<td>30</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomasville</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thompson</td>
<td>21</td>
<td>42</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhi-murium</td>
<td>612</td>
<td>282</td>
<td>894</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhi-suis</td>
<td>22</td>
<td>45</td>
<td>67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uno</td>
<td>21</td>
<td>42</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urbana</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worthington</td>
<td>10</td>
<td>18</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2,450</td>
<td>2,266</td>
<td>4,716</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
these were of avian origin, and predictably the largest number came from chickens, actually more than 97 percent of the total.

*S. cholerae-suis* variety *Kunzendorf* (H$_2$S+) comprised six percent of the total cultures; 353 of the 378 cultures were from swine. The remaining cultures came from chickens (12), pheasants (1), cattle (4), dogs (1), monkeys (2), minks (3), skunks (1) and a loris (1); the loris is a variety of lemur. H$_2$S—negative cultures of *S. cholerae-suis* are very rare in the United States; no more than one or two have been seen in this entire period of four and one-half years.

For ease in following the Table, I shall continue the discussion of these more important *Salmonella* serotypes in alphabetical order.

*S. anatum*, comprising approximately four percent of the total cultures occurred in 12 different species. Of the 291 cultures, 176 were from turkeys, but there were a fair number from chickens (59) and some 26 from cattle as well. In lesser numbers the organism was seen in ducks (1), quails (5), other birds (7), swine (8), horses (3), dogs (1), monkeys (1), minks (3) and reptiles (1).

*S. blockley*, comprising one percent of the total was seen 103 times; 82 of these isolations were from chickens, 16 were from turkeys, four were from swine and one from a pigeon. In comparison with the 14 other more common types which have been recognized for 20 years or more, *S. blockley* has a relatively recent history. It was first seen in 1955 and since then has established itself as one of the more frequently encountered serotypes.

*S. chester* comprised over two percent of the total cultures and was largely confined to poultry. Of the 164 cultures of this organism serotyped, 106 were from turkeys and 52 from chickens. Three cultures were seen from “other birds,” two from mice and one from a rabbit.

*S. derby*, seen 113 times, and comprising around one percent of the total was more widely distributed; 52 of the cultures came from turkeys, 17 from chickens, one from ducks, four from quails, two from other birds, 27 from swine, two from sheep and goats, one from cattle, two from dogs, one from monkeys and four from minks.

*S. enteritidis* comprised four percent of the total cultures; 99 of these came from horses, 63 from turkeys, 50 from cattle, 25 from chickens, 10 from mice and rats, six from guinea pigs, two from chinchillas, one from a seal and one from a pheasant. The rather large number of cultures from horses needs a word of clarification, for many of these represent repeated cultures from animals that were carriers.

*S. gallinarum* was entirely confined to poultry and predominated in chickens; 184 of the 206 cultures were from chickens; the remaining 22 were from turkeys.

*S. heidelberg* comprised over four percent of the total and was found in eight animal species. Of the 294 cultures of this organism, 159 came from turkeys, 127 from chickens, two each from swine and cattle and one each from geese, quails, dogs and minks.
S. infantis comprised three percent of the total cultures. It was distributed in nine animal species although cultures from poultry predominated; 94 of the 186 cultures being from chickens and 78 from turkeys. Three cultures each were seen from horses and mice, two each from swine, dogs and minks and one each from sheep and cattle.

S. muenchen was seen 114 times and comprised approximately one percent of the total; 95 of these cultures were from turkeys, 16 from chickens, one from other birds and one each from swine and cattle.

S. newport comprised four percent of the total cultures and was distributed in 18 species. It ranked next to S. typhi-murium in width of dispersal (S. typhi-murium occurred in over 22 species). There were a total of 255 cultures of S. newport and more came from turkeys than from any other source. There were 165 cultures from turkeys. There was also a rather large number from cattle (57) and in smaller numbers S. newport was seen in chickens (6), ducks (1), pheasants (1), quails (1), swine (3), sheep and goats (3), horses (3), cats (1), dogs (4), monkeys (3), minks 2, skunks (1), elephants (1), a rhinoceros (1), a kangaroo (1) and a sloth (1).

S. st-paul was largely confined to poultry. Of the 136 cultures representing two percent of the total, 114 were from turkeys, 18 were from chickens, two were from horses and two from monkeys.

S. san-diego was largely confined to turkeys. The 229 cultures of this organism represent three percent of the total; 215 of them were from turkeys, 10 were from chickens and four were from cattle.

Time does not permit a discussion of the 71 remaining Salmonella serotypes, nor in any case would it be wise to do so. "Those who try to exhaust the subject," said Oscar Wilde, "end by exhausting the listener." At any rate the mimeographed tables that have been distributed tell the story.

The greatest variety of Salmonella serotypes occurred in turkeys (57); 17 of these averaged less than one identification per year and 28 averaged less than two identifications per year. There were 52 different serotypes that occurred in chickens. In this case 18 of them averaged less than one identification per year and 28 averaged less than two. Many of the Salmonella cultures from poultry came from apparently healthy animals that are reactors to the pullorum test, and are submitted for culture under the National Poultry and Turkey Improvement Plans, and furthermore are isolated from the intestines, not from the internal organs. This doubtless plays a part in the large number and variety of Salmonella serotypes found in turkeys and chickens. One might speculate on what the picture might be if pigeons, which had but three different serotypes were subjected to similar scrutiny, or ducks which had six different serotypes, or geese which had three.

There were 35 different Salmonella serotypes found in cattle but the numbers of each were small except in six cases. S. anatum, S. enteritidis, S. newport, and S. typhi-murium have already been discussed. The other two are S. dublin, a serotype that, as far as is known, occurs in the United States only in the West; the other serotype is S. taksony, a type that was seen only twice in other species.
In swine there occurred 25 different serotypes. The only three that occurred in notable numbers, *S. cholerae-suis*, *S. derby* and *S. typhi-murium*, have already been discussed. By and large, these cultures came from sick animals. Later in this program Mrs. Galton will report on work done in her Unit on isolations of *Salmonella* from hogs before slaughter and after. Although the cultures were serotyped in the Enteric Laboratory at the C.D.C. they are not included in the material that makes up the tables.

Dogs were next to swine in variety of serotypes seen. There were 23 different types out of a total of 51 cultures.

The other animal species had fewer serotypes and I shall not take time to discuss each one here.

The material presented perhaps reflects in a general way the *Salmonella* situation in animals in the United States, but it will not be wise to draw too sweeping conclusions from it. Although the numbers of cultures received for serotyping show a general upward trend each year, it is not possible for a worker in a reference laboratory to say whether this is due to an increase in isolations, to better recognition of *Salmonella* species or to an increasing tendency to take advantage of the serotyping service available. The latter probably plays a large part. You members of the audience know better than anyone whether you are at present isolating more *Salmonella* organisms than ever before, and if so whether this is due to improved isolation techniques or whether salmonellosis is increasing in the United States, and I for one would be glad to hear your opinions.

**TABLE 2**

*Tabulation of Salmonella Serotypes From Species Under “Other Animals” in Table 1*

<table>
<thead>
<tr>
<th>Salmonella</th>
<th>Galena Pike</th>
<th>Rabbits</th>
<th>Seabrooks</th>
<th>Foxes</th>
<th>Nutrias</th>
<th>Chincilliars</th>
<th>Shanghai</th>
<th>Opsoaura</th>
<th>Elephants</th>
<th>Rhinoceros</th>
<th>Kangaroo</th>
<th>Goatia</th>
<th>Loris</th>
<th>Sloth</th>
<th>Seals</th>
<th>Reptiles</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blukwa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chester</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cholerae-suis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v. Kunzendorf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detroit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dublin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enteritisidis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Give</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montevideo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Brunswick</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newport</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oranienburg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhi-murium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urbana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 1
- 2
- 1
- 1
Salmonella infections continue to be important problems facing the livestock and poultry industries. The presence of Salmonella organisms in feedstuffs, particularly animal by-products, has been reported in this country as well as Europe. A recent survey (3) conducted by the Animal Disease Eradication Division of the U.S.D.A. has reviewed the problem of feedstuffs being contaminated with Salmonella and other pathogenic organisms. In the past 25 years a total of over 100 serotypes have been isolated from chickens and turkeys and other avian species in the United States. During this period more than 50 serotypes have been isolated from poultry in Minnesota. In some of the outbreaks of Salmonella infections in chicks and poults there have not been satisfactory explanations of the sources of the organisms, and in some cases, the hatcheries were unjustly considered the primary source of the infection.

The bacteriological examination of young chickens and turkeys and other farm animals submitted to diagnostic laboratories, usually includes the examination of the intestinal tract. Over the years Salmonella organisms were commonly isolated from the intestinal tract. In the diagnostic laboratory in Minnesota in 1960 approximately 50 percent of the isolations from young poults were made only from the intestinal tract. There is very little difference, when compared with the results encountered in 1943, when bacteriological examination of the intestinal tract was first included in the routine diagnostic examination.

The reports of Boyer et al. (1) and Watkins et al. (2) stimulated this study which began in 1959 to determine the incidence of salmonellas in protein supplements used in poultry and livestock feeds in this area.

The samples were secured in cooperation with the quality control laboratories of local feed concerns and directly from rendering plants in Minnesota. The feed ingredients originated from 22 States and Canada. The Salmonella isolates were submitted to Dr. P. R. Edwards, Communicable Disease Center, Enteric Bacteriological Laboratories, Atlanta, Georgia, for serotyping.

Procedures for the Isolation of Salmonella from Feedstuffs:

1) Size of Sample.

A twenty to thirty gm. sample of the feedstuff was inoculated into a 300 ml. Erlenmeyer flask containing 100 ml. of selective enrichment broth.
Selenite F broth was routinely used. Following inoculation, the flask was gently rotated to insure uniform mixing, and incubated for 18-24 hours at 37° C.

2) Plating on Differential Media.

Two bulging loopfuls from the Selenite F broth flask were streaked on differential media such as Brilliant Green agar or S.S. agar. The plates were incubated at 37° C for 18-24 hours.

3) Selection of Colonies from Differential Media.

Colonies typical of salmonellas and Arizonas were transferred to Triple Sugar Iron agar (TSI) and these tubes were incubated for 24 hours at 37° C. The tubes of TSI that showed evidence of Salmonella were further identified by use of the urea test to eliminate the Proteus strains. The suspected Salmonella cultures were identified by the use of fermentable media and other biological tests. The Salmonella cultures were sent to Dr. Edwards for final classification.

Results:

The samples of the feed ingredients originated from processing plants in 22 States and Canada. Samples from six States and Canada were negative;

<table>
<thead>
<tr>
<th>Feed Ingredients</th>
<th>No. Samples</th>
<th>No. Salmonella Isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat Scraps</td>
<td>257</td>
<td>83</td>
</tr>
<tr>
<td>Meat, Bone Meal</td>
<td>221</td>
<td>32</td>
</tr>
<tr>
<td>Fish Meal, Solubles</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>Poultry By-Products</td>
<td>45</td>
<td>11</td>
</tr>
<tr>
<td>Feather Meal</td>
<td>39</td>
<td>12</td>
</tr>
<tr>
<td>Blood Meal</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Tankage</td>
<td>171</td>
<td>23</td>
</tr>
<tr>
<td>Meat, Bone and Poultry By-Products</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Miscellaneous Ingredients</td>
<td>157</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>980</strong></td>
<td><strong>175</strong></td>
</tr>
</tbody>
</table>

However, only a few samples were obtained from sources in these States. The results of the bacteriological examination and types of feed ingredients examined are given in Table 1. The group of miscellaneous products which were cultured included samples of soya bean oil meal, corn meal, and complete feeds. The samples of ingredients that were not specifically identified, as to the type of animal by-product, when they were received from the quality control laboratories are listed as material not identified. A total of 980 samples were examined bacteriologically for salmonellas and 175 samples were found contaminated with the organisms.
The results of the serotyping revealed that 43 serotypes and six unidentified serotypes were isolated from the ingredients. The distribution of serotypes by groups were as follows: Group A, 0; B, 6; C, 11; D, 1; E, 10;

**TABLE 2**  
*Frequency of Occurrence of Salmonella Serotypes Isolated From Feed Ingredients*

<table>
<thead>
<tr>
<th>Salmonella Serotype</th>
<th>No of Times Isolated</th>
<th>Salmonella Serotype</th>
<th>No of Times Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alachua</td>
<td>2</td>
<td>Meleagridis</td>
<td>2</td>
</tr>
<tr>
<td>Anatum</td>
<td>6</td>
<td>Minnesota</td>
<td>2</td>
</tr>
<tr>
<td>Bareilly</td>
<td>1</td>
<td>Montevideo</td>
<td>15</td>
</tr>
<tr>
<td>Binza</td>
<td>7</td>
<td>Muenchen</td>
<td>1</td>
</tr>
<tr>
<td>Blockley</td>
<td>1</td>
<td>Newport</td>
<td>2</td>
</tr>
<tr>
<td>Branderup</td>
<td>1</td>
<td>Ohio</td>
<td>4</td>
</tr>
<tr>
<td>Bredeney</td>
<td>16</td>
<td>Oranienburg</td>
<td>11</td>
</tr>
<tr>
<td>California</td>
<td>3</td>
<td>Orion</td>
<td>1</td>
</tr>
<tr>
<td>Cerro</td>
<td>4</td>
<td>Schwarzengrund</td>
<td>11</td>
</tr>
<tr>
<td>Champaign</td>
<td>3</td>
<td>Senftenberg</td>
<td>26</td>
</tr>
<tr>
<td>Cubana</td>
<td>9</td>
<td>Siegburg</td>
<td>2</td>
</tr>
<tr>
<td>Derby</td>
<td>7</td>
<td>Taksony</td>
<td>3</td>
</tr>
<tr>
<td>Enteritidisa</td>
<td>2</td>
<td>Tennessee</td>
<td>6</td>
</tr>
<tr>
<td>Gaminara</td>
<td>1</td>
<td>Thomasville</td>
<td>1</td>
</tr>
<tr>
<td>Illinois</td>
<td>8</td>
<td>Thompson</td>
<td>1</td>
</tr>
<tr>
<td>Infantis</td>
<td>15</td>
<td>Typhi-murium</td>
<td>5</td>
</tr>
<tr>
<td>Java</td>
<td>1</td>
<td>Typhi-murium-var.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Copenhagen</td>
<td>1</td>
</tr>
<tr>
<td>Johannesburg</td>
<td>4</td>
<td>Urbana</td>
<td>2</td>
</tr>
<tr>
<td>Kentucky</td>
<td>8</td>
<td>Westhampton</td>
<td>1</td>
</tr>
<tr>
<td>Lexington</td>
<td>9</td>
<td>Worthington</td>
<td>14</td>
</tr>
<tr>
<td>Livingstone</td>
<td>1</td>
<td>Unidentified</td>
<td>7</td>
</tr>
<tr>
<td>Madelia</td>
<td>1</td>
<td>Witchita</td>
<td>1</td>
</tr>
</tbody>
</table>

Miscellaneous, 15. The number and specific serotypes of the salmonellas isolated from feed ingredients are given in Table 2.

**Discussion:**

Over the years a number of different selective enrichment broths and differential plating media have been used in the bacteriological examination of reactors to the serological tests for pullorum disease and paratyphoid infections. No attempt was made in this study to compare the various laboratory procedures. The procedure and media used in this study had proved satisfactory. If additional procedures, media and samples were done, the number of positive isolations may have been increased. Some samples were contaminated with Proteus and other rapidly growing organisms, consequently the salmonellas may have been missed.

Many of the Salmonella serotypes isolated from livestock and poultry have been isolated from feed ingredients. It is recognized that only in a few instances the serotypes encountered in the feed were specifically isolated from
animals or poultry receiving the feed. Usually the time lag between the laboratory studies and field investigations is such that samples of the feed ingredients are not available from the feed manufacturer. Also, feed additives which are included in complete feeds may interfere in the isolation of Salmonella from the feeds.

If continued progress is going to be made in reducing the incidence of paratyphoid infections in poultry and livestock, every effort must be made to eliminate the contamination of feed ingredients with Salmonella. This will require cooperative efforts to develop sanitation programs for all areas that involve storage, handling, processing, and manufacturing of feed ingredients and complete feeds.

Summary:
1) The bacteriological examination of 980 samples of animal by-products from 22 States indicated Salmonella contamination of 175 samples.
2) Forty-three Salmonella serotypes were identified. Many of these serotypes have been isolated from poultry and livestock submitted to diagnostic laboratories.
3) Additional investigation is essential to develop sanitation standards which may be used by various phases of the feed industry to minimize contamination of feedstuffs with Salmonella and other pathogenic organisms.

REFERENCES
INDUSTRY'S ROLE IN REDUCING THE INCIDENCE OF SALMONELLA IN ANIMAL FEEDS

C. F. NIVEN, JR.

Chicago, Illinois

That animal feeds, and particularly animal feed constituents of animal origin, frequently are contaminated with salmonellae is now well recognized. Pomeroy has adequately reviewed this subject and, therefore, it will not be necessary to substantiate the data already presented. On the other hand, in spite of the numerous reports in the literature as to the high incidence of salmonellae in animal feed constituents, information concerning their actual significance in human and animal health appears to be inadequate.

In an attempt to obtain additional information concerning both the incidence and significance of salmonellae in meat animals and animal by-products, the American Meat Institute Foundation instituted several years ago a rather extensive research program on this subject. A preliminary report on the research thus far conducted has been published (Leistner, et al. 1961). In this research, investigations have been made on the incidence of salmonellae in pig faeces at the farm level, as well as at the time of slaughter, in commercial feeds, rendered animal by-products intended to be used as feed constituents, and mesenteric lymph nodes of hogs immediately after slaughter. Also, investigations have been made on the incidence of bedding material obtained from trucks used to transport pigs from farm to market, and in holding pen deposits used by both the stock yards and packing establishments.

In the laboratory, conventional methods were employed to detect salmonellae in these samples. Routinely, both selenite-F enrichment broth and tetrathionate broth were used as enrichment media. The enrichment cultures were then surface plated onto brilliant green agar. Suspicious colonies were isolated and the pure cultures were identified by using a series of physiological characteristics, and finally were identified as to serotype through the courtesy of the Illinois State Department of Health.

We found the methods employed to be reasonably satisfactory for the detection of salmonellae in the different types of samples examined, with the exception of pig faeces. Table I presents a conservative estimate as to the minimum numbers of salmonellae per gram of specimen that could be detected by the methods employed. Selenite enrichment broth appeared to be slightly superior for the examination of rendered animal by-products. On the other hand, tetrathionate broth seemed to be superior for the examination of faecal samples. When both enrichment media were employed, a 35 percent increase in positive findings resulted, perhaps due mainly to a doubling of the size of sample tested. Our results indicate that some of the serotypes were more easily detectable in one or the other of the enrichment media.

American Meat Institute Foundation, The University of Chicago.

453
TABLE I

Minimum Numbers of Salmonellae Detectable by Methods Employed

<table>
<thead>
<tr>
<th>Sample</th>
<th>Salmonellae/Gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig faeces</td>
<td>20</td>
</tr>
<tr>
<td>Commercial feeds</td>
<td>2</td>
</tr>
<tr>
<td>Meat meals</td>
<td>1</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Results of our investigations definitely confirmed that a relatively high percentage of rendered animal by-products used as feed constituents contain salmonellae. Approximately 61 percent of the samples examined were found to be positive. Of particular interest to us was the apparent high correlation between the total bacterial count of these samples and the presence or absence of salmonellae. These results ultimately may throw more light on the exact mode of entry of salmonellae into the samples.

Thus far, limited investigations upon the incidence of salmonellae in commercial feeds indicate that a much lower percentage contain salmonellae in detectable quantities. Only three of the 23 samples examined were found to be positive.

Of even greater interest is the rather low incidence of salmonellae in pig faeces obtained at the farm level. Among 75 samples examined, only two were found to be positive. These samples were obtained from farms located throughout the midwest. If it is assumed that pigs are constantly consuming feeds which contain salmonellae, the rather low incidence of these organisms in their intestines may indicate that little or no growth occurs in the intestinal tract in the healthy pig. It should be remembered, however, that the techniques employed were less adequate in detecting small numbers of salmonellae in faeces than in other samples examined.

In direct contrast, the incidence of salmonellae in the colon contents of pigs at the time of slaughter was considerably higher than that found at the farm level. Among those pigs which had proceeded to market through the regular channels, approximately 18 percent had detectable quantities of salmonellae in the colon contents immediately after slaughter. Of even more interest was the fact that so-called “special” pigs showed a much higher incidence of salmonellae in their colon contents after slaughter. These “special” pigs include sows, boars and culls which ordinarily are held for much longer periods of time in holding pens throughout the marketing channels prior to slaughter. 59 percent of such pigs were found to contain salmonellae in their colon contents after slaughter. We feel that these findings are a direct reflection of the sanitary conditions of holding pens. Approximately 94 percent of holding pen deposits examined by us were positive for salmonellae. These results generally confirm those already reported by Galton, Smith, McElrath and Hardy (1954).
INDUSTRY'S ROLE IN REDUCING OF SALMONELLA

TABLE II
Numbers of Samples in which Common Salmonella Serotypes were Detected

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>Rendered Meals</th>
<th>Pens</th>
<th>Pig Faeces</th>
<th>Lymph Nodes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. derby</td>
<td>7</td>
<td>22</td>
<td>35</td>
<td>4</td>
<td>68</td>
</tr>
<tr>
<td>S. anatum</td>
<td>8</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>49</td>
</tr>
<tr>
<td>S. senftenberg</td>
<td>14</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>14</td>
</tr>
<tr>
<td>S. newport</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>—</td>
<td>12</td>
</tr>
<tr>
<td>S. montevideo</td>
<td>11</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>11</td>
</tr>
<tr>
<td>S. infantis</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>—</td>
<td>10</td>
</tr>
</tbody>
</table>

Table II summarizes the most common Salmonella serotypes detected in the different specimens examined in our study. It should be noted that the two most common serotypes found in rendered animal meals were not detected from any other source. On the other hand, there was a reasonably close correlation between the common serotypes found in holding pens and the colon contents of pigs at the time of slaughter. These results indicate that the Salmonella detected in the colon contents at the time of slaughter do, indeed, originate from the holding pens. On the other hand, the results throw little light upon the significance of salmonellae infection in rendered animal by-products. The two most common serotypes found in rendered animal by-products, namely S. senftenberg and S. montevideo, were not found in any other specimen examined.

Quantitative determination of numbers of salmonellae per gram of sample is tedious and time-consuming. We attempted to estimate the relative Salmonella population levels in meat meals, holding pen deposits and the colon contents of pigs at the time of slaughter. Both direct plating on brilliant green agar, and the most probable numbers technique were employed for these studies. Results presented in Table III indicate that meat meals invariably contain relatively low numbers of salmonellae. On the other hand, holding pen deposits may contain reasonably high numbers. Also, some of the so-called “special” pigs had relatively high numbers of salmonellae in their colon contents at the time of slaughter.

TABLE III
Estimation of Salmonella Population in Samples Examined

<table>
<thead>
<tr>
<th></th>
<th>Meat Meals</th>
<th>Holding Pens</th>
<th>Pig Faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples estimated</td>
<td>55</td>
<td>5</td>
<td>115</td>
</tr>
<tr>
<td>Samples having &gt;1000/gram</td>
<td>0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Samples having &lt;1000/gram</td>
<td>26</td>
<td>3</td>
<td>46</td>
</tr>
<tr>
<td>Negative samples</td>
<td>29</td>
<td>0</td>
<td>63</td>
</tr>
</tbody>
</table>
Whenever a problem confronts an industry or public health agencies, it appears that we must proceed through several stages before an ultimate solution is attained. First, and perhaps the most important, is recognition of the problem. With respect to the high incidence of salmonellae in rendered animal by-products, industry has already proceeded through this first stage.

Next comes the realization of the significance and importance of the problem. We must know what the potential human and animal health hazards are. Is the problem of economic significance? Industry is now well advanced into this second stage. It should be realized, however, that more conclusive evidence as to the significance of salmonellae in animal feeds in this country is badly needed. Do salmonellosis outbreaks in farm animals frequently result from consumption of animal feeds containing small numbers of salmonellae? Can a carrier state in the animal be established? Is it of economic importance as far as animal health is concerned? Is the problem of importance in human health? These questions cannot be adequately answered at this time. We need considerably more information concerning the epidemiology of salmonellosis outbreaks in poultry flocks and meat animals, with special emphasis on whether or not animal feeds are the original source of the pathogens. That occasional salmonellosis outbreaks in animals can originate from infected feeds is well established, especially in the European literature. However, we do not seem to know the proportion of such outbreaks in the United States which can be traced directly back to feeds.

The third stage to be confronted in a problem involves the searching for alternative measures to solve or circumvent the problem. Regardless of their possible significance, industry must face the fact that pathogens of any kind are undesirable and should be eliminated from their products. It should be realized that most rendering establishments in the United States were designed with an eye to engineering efficiency because of high labor costs. Such designs often are not compatible with desirable hygienic procedures. Employees often have duties to perform having to do with handling both the raw and rendered products. Frequently, there is no physical separation between the areas handling the raw and rendered product. Numerous other violations of good hygienic practice may be witnessed in such establishments. But we should keep in mind that such violations were considered good operating procedures long before a Salmonella problem was known to exist.

The fourth stage involves ultimate solution. In some rendering establishments, this has apparently already been accomplished through the employment of a sustained and effective sanitation program in conjunction with a routine testing program of the rendered product, to determine its effectiveness.

Under the joint auspices of the American Meat Institute and the National Renderers Association, industry representatives currently are developing a sanitation guide which we anticipate will be adopted and put into practice throughout the industry. By following the guide, we believe that a great deal can be accomplished in reducing the incidence of salmonellae and other pathogens in rendered animal by-products.
Because of obsolescence, a sustained sanitation program probably will not be effective in all plants. As a result, we have been investigating the feasibility of a terminal heating procedure that could be used to assure freedom from salmonellae. Before we can determine whether such a procedure will be effective and economical, such physical measures as specific heat and heat conductance of rendered animal by-products must be established. Obviously, any new rendering establishment should be designed to facilitate an effective sanitation program, and yet be compatible with economical operating procedures. We are convinced that such can be accomplished, especially knowing the fact that the rendering procedure essentially sterilizes the product, and we are merely faced with a problem of recontamination. In the meantime, it is necessary to do our best under existing conditions. Complete elimination of salmonellae in rendered animal by-products can be accomplished, but it is not an easy task.

REFERENCES


FINDINGS AND RECOMMENDATIONS OF THE UNITED STATES DEPARTMENT OF AGRICULTURE TASK FORCE ON SALMONELLA IN ANIMAL BY-PRODUCTS AND FEEDS

E. E. WEDMAN, D.V.M., M.P.H.*

Numerous reports have been made in both domestic and foreign literature that pathogenic microorganisms are found in livestock by-products, poultry, and fish (1-30). The genus *Salmonella* specifically has been reported in many of these products.

Resolutions were received from the American Association of Avian Pathologists, the Committee on Salmonellosis and Related Enteric Diseases of the National Plans Conference and the North Central States Poultry Disease Conference to study the problem of pathogenic organisms in animal by-products and livestock rations. As a result of these resolutions, the Agricultural Research Service of the United States Department of Agriculture initiated a study to survey the problem. The study was designed to gather information on the occurrence of pathogenic organisms in animal by-products and rations, factors responsible for their occurrence, their real or potential disease threat to the nation's domestic livestock and poultry, and to determine what measures could be taken to eliminate or minimize the problem.

**METHODS**

A special committee composed of members of the Animal Disease Eradication Division of the United States Department of Agriculture was named to study the spread of *Salmonella* and other disease-causing organisms in poultry and livestock by means of animal by-products. Initially, a review of the literature was prepared to document published material on the subject. Secondly, meetings were held between committee members and interested groups to determine policies and procedures for gathering the desired information. Working through the veterinarians in charge of each State and their respective staff veterinarians, the Animal Disease Eradication Division made 1,100 contacts throughout the country to accumulate information on the occurrence of pathogenic organisms in animal by-products, various routes of their dissemination, their real or potential disease threat to the country's poultry and domestic livestock, and to determine what measures could be taken to eliminate the problem. These contacts included laboratories and extension services of colleges and universities, public health and private industry laboratories, regulatory agencies, veterinary practitioner groups, rendering groups, animal by-product processors, feed manufacturing and processing groups, meat packers and allied groups, fur bearing animal raisers, and biological and drug manufacturers.

* Doctor Wedman is Chief Veterinarian, Diagnostic Services, Animal Disease Eradication Division, National Animal Disease Laboratory, Ames, Iowa.

458
FINDINGS AND RECOMMENDATIONS ON SALMONELLA 459

RESULTS

The study revealed that a wide variety of animal by-products and rations were contaminated with pathogenic organisms (Table 1). The Salmonella spp.

<p>| Microorganisms Other Than Salmonella Isolated From Animal By-Products and Feeds |
|-----------------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th>Product</th>
<th>No. of Samples Cultured</th>
<th>No. of Samples Positive</th>
<th>Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry feed</td>
<td>4</td>
<td>2</td>
<td>Candida albicans</td>
</tr>
<tr>
<td>Bone and fish meal</td>
<td>300</td>
<td>5</td>
<td>Bacillus anthracis</td>
</tr>
<tr>
<td>Pork livers</td>
<td>1</td>
<td>1</td>
<td>Mycobacterium tuberculosis var. avian</td>
</tr>
<tr>
<td>Horse meat</td>
<td>1</td>
<td>1</td>
<td>Klebsiella sp.</td>
</tr>
<tr>
<td>Horse liver</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Clostridium botulinum sp.</td>
</tr>
<tr>
<td>Packing house offal</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Clostridium botulinum sp.</td>
</tr>
<tr>
<td>Complete feeds</td>
<td>30</td>
<td>17</td>
<td>Staphylococcus sp. throughout</td>
</tr>
<tr>
<td>Turkey offal</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Erysipelothrix rhusiopathiae</td>
</tr>
<tr>
<td>Wool</td>
<td>50</td>
<td>3</td>
<td>Bacillus anthracis</td>
</tr>
<tr>
<td>Bone meal</td>
<td>10</td>
<td>2</td>
<td>Bacillus anthracis</td>
</tr>
<tr>
<td>Imported goat hair</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Bacillus anthracis</td>
</tr>
<tr>
<td>Imported goat hair</td>
<td>8</td>
<td>4</td>
<td>Bacillus anthracis</td>
</tr>
<tr>
<td>Poultry by-products</td>
<td>3</td>
<td>3</td>
<td>Newcastle Disease Virus (mesogenic)</td>
</tr>
</tbody>
</table>

TABLE 1

were the most frequently reported pathogens. Results of attempted isolations from 5,712 samples were gathered. Of the 5,712 samples, 718 yielded Salmonella. Results were received from 31 states and involved 14 various animal by-products and rations (Table 2). Sixty-two different serotypes were recorded. The most frequently occurring serotypes were S. montevideo, S. senftenberg, S. typhimurium, S. cubana, S. infantis and S. oranienburg (Table 3).

The majority of the contributors to this study felt that recontamination of the product was the most important single factor responsible for Salmonella spp. in animal by-products and rations. It was also reflected by the consensus of the contributors to this study, that rodents were the most probable sources of contamination. Although other animals, wild birds, and human handlers of the products were suggested as possibilities. There was little definitive evidence that pointed to animal by-products and rations as sources of offending organisms responsible for field occurrences of the disease.

SUMMARY AND CONCLUSIONS

Due to the fact that minimum evidence of contaminated feed was responsible for actual disease, the United States Department of Agriculture study
TABLE 2
Recovery of Salmonella From Animal By-Products and Complete Rations*

<table>
<thead>
<tr>
<th>By-Product</th>
<th>Samples Cultured for Salmonella</th>
<th>Samples Containing Salmonella</th>
<th>Number of States Reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal by-products†</td>
<td>1090</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Blood meal</td>
<td>17</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Bone meal</td>
<td>46</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Calf milk replacer</td>
<td>33</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Complete feeds</td>
<td>1415</td>
<td>71</td>
<td>15</td>
</tr>
<tr>
<td>Dog food</td>
<td>143</td>
<td>27</td>
<td>5</td>
</tr>
<tr>
<td>Dried buttermilk and whey</td>
<td>6</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Egg products‡</td>
<td>142</td>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td>Fat</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Feather meal</td>
<td>524</td>
<td>35</td>
<td>9</td>
</tr>
<tr>
<td>Fish meal</td>
<td>164</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Laboratory animal feeds§</td>
<td>Unknown</td>
<td>158</td>
<td>1</td>
</tr>
<tr>
<td>Livers</td>
<td>26</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Meat scraps</td>
<td>874</td>
<td>276</td>
<td>18</td>
</tr>
<tr>
<td>Meat scraps and bone meal</td>
<td>326</td>
<td>86</td>
<td>9</td>
</tr>
<tr>
<td>Meat meal</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Poultry by-products</td>
<td>141</td>
<td>47</td>
<td>10</td>
</tr>
<tr>
<td>Poultry feeds</td>
<td>403</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Products (miscellaneous animal protein)</td>
<td>31</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Swine supplements</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Tankage</td>
<td>316</td>
<td>46</td>
<td>8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>5712</strong></td>
<td><strong>718</strong></td>
<td><strong>31</strong></td>
</tr>
</tbody>
</table>

* A breakdown on the number and types of Salmonella recovered from each by-product and ration is available on request.
† Information as to the type of animal by-product was not available.
‡ Egg concentrate, frozen whites, dried whole eggs, frozen yolk, dried yolk.
§ Neither attempted isolations nor successful isolations are shown in the total at the end of the table since number of attempted recoveries was not known.

Group concluded there was not cause for alarm concerning this problem although causes for concern were definitely apparent. These causes included the potential disease problem arising from the "cycling" of a number of Salmonella serotypes from farms to processing plants and back to farms by animal by-products incorporated in rations. In addition the fact that known pathogenic Salmonella serotypes were reported as some of the most frequently recovered organisms from animal by-products and rations caused concern.

The study group concluded that additional work is needed to promote more uniform methods of Salmonella isolation from animal by-products and rations and to learn more about the relative pathogenicity of these serotypes. It was also felt that the number of organisms in by-products necessary to create a disease threat or establish a carrier state in so-called healthy population needs further study.
Nevertheless, the study group felt that realistic, workable measures to minimize the chance for exposure of livestock and poultry to Salmonella organisms occurring in animal by-products and rations are desirable and essential. It was evidenced by the information received in this study that industry and laboratories throughout the country were interested in endeavoring to assure a product free of these organisms when it leaves the plant. Research in this area is being conducted by industry to study current procedures and environmental factors associated with contamination of these products. Additional meetings between members of the Animal Disease Eradication Division, United States Department of Agriculture and industry have been held since this study to discuss the possibilities of a proposed
sanitary code. Further meetings are planned. The purpose of the sanitary code would be to recommend industry practices designed to produce a product free from Salmonella. Industry has indicated a willingness to place into effect whatever measures are necessary once it is shown that such methods are effective and practical. In addition, meetings are scheduled with various workers in the field of Salmonella to discuss uniform methods of isolating these organisms from animal by-products and rations.

REFERENCES

27. POMEROY, B. S., Head, Department of Bacteriology and Hygiene, University of Minnesota, St. Paul, Minnesota, Personal communication, 1961.
METHODS OF INVESTIGATING THE INTERNATIONAL SPREAD OF SALMONELLAE

KENNETH W. NEWELL, M.B., Ch.B., D.P.H.*

New Orleans, Louisiana

It is well known that Salmonellae do spread internationally. This is not spread so much by people as by things.

There are a number of well documented examples such as the wartime dried egg outbreaks in the United Kingdom, the post war fish meal outbreaks in Europe, the Swedish Meat outbreak, and the Paratyphoid B outbreaks due to frozen egg in England and Wales.

These are only examples and we have no means of knowing whether they are increasing or decreasing. It is probable that with the increasing trade in food products they are increasing.

These examples were major outbreaks—medical catastrophes—so large that it would be strange if we had failed to recognize them. However, it is probable that there have been an equal number, equally large, which have possibly been recognized but not fully traced. This is not so strange. We have so little knowledge of the vehicles responsible.

In addition to these disease explosions, it is suspected that many of our small sporadic, endemic, isolated human cases are also part of national or international outbreaks although we do not connect or recognize them. This is also understandable. Many of the symptoms of Salmonellosis are so mild that the patient does not go to the doctor—if he does it is possibly not worth a full investigation—if it is investigated it may not be reported. Even if, exceptionally, all of these things happen, the time lag may be too great for a reasonable investigation.

Using this line of reasoning I think it would be generally agreed that

1. There are large numbers of unrecognized national outbreaks.
2. That many known national outbreaks are not adequately investigated, and traced back to the animal and to the source of animal infection.
3. That some national outbreaks are unrecognized as international ones.

This is not very satisfactory. Obviously something can be done to pay more attention to the grouping of human (indicator) cases of the same type. Known outbreaks can be investigated more thoroughly—back to the animal and how the animal was infected. We can also examine more carefully the known vehicles of Salmonella spread.

But this is not enough and is unlikely to result in the quick discovery of new vehicles or to an adequate control of this illness.

* Professor of Epidemiology, Department of Tropical Medicine and Public Health, School of Medicine, Tulane University, New Orleans, Louisiana.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All salmonellae</td>
<td>428</td>
<td>122</td>
<td>120</td>
<td>104</td>
<td>262</td>
<td>506</td>
<td>748</td>
<td>689</td>
<td>908</td>
<td>1393</td>
<td>2089</td>
<td>1711</td>
<td>2142</td>
<td>3171</td>
<td>3576</td>
<td>3833</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>234</td>
<td>38</td>
<td>65</td>
<td>35</td>
<td>108</td>
<td>185</td>
<td>319</td>
<td>573</td>
<td>473</td>
<td>663</td>
<td>1053</td>
<td>1593</td>
<td>1236</td>
<td>1604</td>
<td>2438</td>
<td>3038</td>
<td>4276</td>
</tr>
<tr>
<td></td>
<td>(Percent of total)</td>
<td>(55)</td>
<td>(31)</td>
<td>(54)</td>
<td>(34)</td>
<td>(41)</td>
<td>(41)</td>
<td>(63)</td>
<td>(77)</td>
<td>(69)</td>
<td>(73)</td>
<td>(76)</td>
<td>(76)</td>
<td>(72)</td>
<td>(75)</td>
<td>(77)</td>
<td>(85)</td>
</tr>
<tr>
<td>Other salmonellae</td>
<td>194</td>
<td>84</td>
<td>55</td>
<td>69</td>
<td>154</td>
<td>269</td>
<td>187</td>
<td>175</td>
<td>216</td>
<td>245</td>
<td>340</td>
<td>496</td>
<td>475</td>
<td>538</td>
<td>733</td>
<td>538</td>
<td>1107</td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>16</td>
<td>77</td>
<td></td>
</tr>
</tbody>
</table>

I consider three further things are necessary:

1. When a new vehicle is found, there should be some international body which is willing to support an international investigation.

2. Every encouragement should be given to the speedy and complete collection and publication of national (or regional) Salmonella isolation figures classified in a standard and meaningful way.

3. We should use accepted Epidemiological techniques with this material nationally and internationally.

I do not consider any of these three things to be impossible and I want to emphasize this with an example.

One Epidemiological method is a breakdown of information by TIME, PLACE, and PERSONS.

If one uses TIME alone, and the reported number of Salmonella incidents (or isolations) from human sources, one cannot compare the incidence or true prevalence within or between countries. The differences in diagnosis, investigation and reporting are too great. However, a change in the proportions of incidents due to certain groups of Salmonella types may be meaningful. This may show a trend or change in time which may be of importance.

Table 1 shows the reported Salmonella incidents in England and Wales from 1923-1955 subdivided into those due to *S. typhimurium* and those due to "other Salmonellae." Both groups show a regular and steady increase over the years and much of this increase is certainly due to better laboratories and reporting.

Independent of this change there was another change from 1940-1944. The proportion of "other Salmonellae" increased dramatically. It is probable that this reflected the wartime dried egg outbreaks.

**TABLE 2**

**Salmonella Incidents in England and Wales and Northern Ireland in 1952 and 1955***

<table>
<thead>
<tr>
<th>Year</th>
<th>England and Wales</th>
<th>Northern Ireland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salmonella Incidents Per Million People</td>
<td>Percentage of Incidents Due to <em>S. Typhimurium</em></td>
</tr>
<tr>
<td>1952</td>
<td>46</td>
<td>75</td>
</tr>
<tr>
<td>1955</td>
<td>80</td>
<td>79</td>
</tr>
</tbody>
</table>

* Information for England derived from: Great Britain, Public Health Laboratory Service (1954, 1956) Information for Northern Ireland obtained from the Northern Ireland Hospitals Authority, Central Laboratory, Belfast; the reports of the Minister of Health and Local Government, Northern Ireland, for 1952 and 1955; and the annual reports of the Medical Officers of Health, County Councils and County Borough Councils.

In Table 2 England and Wales and Northern Ireland are compared. The rate of Salmonella incidents show opposite trends between 1952 and 1955.
and even if these rates are open to suspicion, the proportional changes of type in Northern Ireland make it seem highly probable that some real change did take place.

TABLE 3

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All salmonellae</td>
<td>94</td>
<td>80</td>
<td>60</td>
<td>56</td>
<td>123</td>
<td>39</td>
</tr>
<tr>
<td>(Percent of total)</td>
<td>(84)</td>
<td>(80)</td>
<td>(78)</td>
<td>(77)</td>
<td>(43)</td>
<td>(38)</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>79</td>
<td>64</td>
<td>47</td>
<td>43</td>
<td>53</td>
<td>15</td>
</tr>
<tr>
<td>(Percent of total)</td>
<td>(84)</td>
<td>(80)</td>
<td>(78)</td>
<td>(77)</td>
<td>(43)</td>
<td>(38)</td>
</tr>
<tr>
<td>Other salmonellae</td>
<td>15</td>
<td>16</td>
<td>13</td>
<td>13</td>
<td>70</td>
<td>24</td>
</tr>
<tr>
<td>(Percent of total)</td>
<td>(16)</td>
<td>(20)</td>
<td>(22)</td>
<td>(23)</td>
<td>(57)</td>
<td>(62)</td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>35</td>
<td>11</td>
</tr>
<tr>
<td>(Percent of total)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
<td>(29)</td>
<td>(28)</td>
</tr>
</tbody>
</table>

* Information supplied by Dr. C. R. Murdock, Northern Ireland Hospitals Authority, Central Laboratory, Belfast.

What these changes were is shown in Table 3. In 1955 there was an increase in isolations of *S. typhimurium* but at the same time there was an even greater percentage increase in “other Salmonellae” (especially *S. heidelberg*).

This sort of information is meaningful and useful. It shows a marked change and indicates a problem worth investigating. But it does not show vehicle or source.

But by using the same type of information and bringing in PLACE as well as TIME, it is possible to come to some pretty shrewd guesses.

Using again as my example, *S. heidelberg* and the Salmonellae reported in the medical literature from different countries, one can make a table of time like the following:

First reported outbreaks (*S. heidelberg*)

- England and Wales: 1953
- Australia: 1953
- U.S.A. (Massachusetts): 1954
- Canada: 1954
- Northern Ireland: 1955
- France: 1956

These differences may not appear to be major ones but a range of three years is quite a considerable time for a world outbreak.

From this, or a similar table, it is possible to do one of two things. One can compare those countries with a similar experience in time and find a possible factor in common—or one can compare widely differing areas in time and try to find a factor with the same time spacing. For many reasons the second course is the easier one. If one has a choice, it is preferable to
pick two areas as similar as possible, but differing in time, in order to let the number of factors which differ be as small as possible.

This type of comparison was done for England and Wales and Northern Ireland from 1952-1956. These countries have many economic connections and common food sources and yet they differed in time (England and Wales 1953, Northern Ireland 1955) in their experience with S. heidelberg. There were some additional existing clues. S. heidelberg was thought to be connected with a meat product.

Now meat products are one of the major differences between the two countries. England and Wales import large quantities of carcass meat from Australia and other sources while meat imports were stopped in Northern Ireland in 1952. It was then an exporter.

If Northern Ireland meat exports were responsible for the outbreaks in England one would expect the time gap to be in the opposite direction. Therefore, this explanation is unlikely. The next possibility is that the domestic animals used for meat in the two countries were infected at different times. A common feeding stuff could result in such a spacing and it was responsible to search for one with a similar time gap.

There was one which fitted in exactly. One particular feeding stuff from one source (due to a change in customs regulations) was imported into England in small quantities in 1951 and in very large quantities in 1952. None was imported into Northern Ireland until a large shipment arrived from February to May in 1955. (Five to seven months before the first outbreaks occurred.) This sort of time lag within a country is to be expected.

Further investigation brought to light the fact that the country of origin of this product had the first major outbreak of S. heidelberg in 1950 and that there was adequate opportunity for contamination of the feeding stuff.

This investigation was carried out retrospectively and none of the feeding stuff was available for bacteriological examination.

The conclusion that this feeding stuff was related to the outbreak was unproved. However, as an example, it is meant to show what can be done to help to concentrate attention upon possible vehicles worth examining. There is no necessity for this type of investigation to be grossly retrospective.

What is necessary (and I put forward here a strong plea) is that national, local and international results of Salmonella findings by local or reference laboratories be put together in a meaningful form and be made available for study at frequent intervals. We are not here so interested in history, and so reports that are years late are in some senses useless. This applies to most countries in the world and to the United States of America.

REFERENCES
DISCUSSION

Question: Were salmonella carriers detected among the workers exposed to the heavy infestation of salmonellae in packing plants?

Mrs. Galton: We have not cultured samples from workers in the abattoirs in any recent studies. S. typhi was found during one survey of fecal samples from workers in one plant in Florida, but no other salmonella types.

Doctor Edwards: Miss Janie Morris of the Georgia State Health Department once looked into this question and in the beginning it seemed as though there was a very high percentage of carriers among abattoir workers, and this is what occasioned the surveys which she made. The first few samples seemed to have a lot of positives among them but, later, investigation indicated that there was not a significant number of carriers among abattoir workers.

Doctor Newell: I looked into this problem in northern Ireland in 1957 and 1958, and we did a control study of people who were exposed, we thought excessively exposed, to salmonellosis by working in meat processing plants and by abattoir workers, and compared this with a group of people who had clerical jobs in the same institutions and had a clinical history of a three-day illness. We could find no difference between these two groups of workers. However, if we continued to examine the families of these two sets of workers, there was a gross and significant difference between the families of people who had had a high exposure and people who had not. From memory, this was something of the order of four times as great in exposed workers. This tied in with some of the investigations which were done by Colindale on reported cases of salmonellosis where there was found to be a very high rate in groups of people who were reported to have salmonellosis. The most frequently reported occupational group was the families of these handlers, not the handlers themselves. If the food handlers were infected because of their occupation, their lack of reported illness may indicate clinical immunity, or clinical protection, of people who have repeated infections who do have exposure but this does not apply to the families.

Question: In talking with Doctor Heather, of the Texas State Health Department, regarding his work with dog foods, he did not find salmonellae in pelleted dog foods. Have you found any?

Mrs. Galton: No, we have not found salmonella in the pellet or the biscuit type of dog food.

Doctor Edwards: I think it is extremely dangerous to generalize on the pathogenicity of any certain salmonella type. This is something you may not know very much about. Perhaps as much is dependent upon the particular strain or culture of the organism as upon the serotype as regards its capacity to produce disease. Then, of course, there is always the question of numbers.

Doctor Ellis: In regard to the isolation of Salmonella from animal feeds, Doctor Pomeroy, I think up to the time I left Florida, we had examined
approximately 1,200. Now, these feeds represented all types of samples. When we talk about the number of isolations of Salmonellas we make from so many feed samples, I think we must take into consideration that many feeds are pure cereal grain feeds and do not contain bone meal, meat scraps, etc., and so when you are doing a general survey of all feeds, you will find as we did, I believe, less samples positive. In the 1,200 that we examined, less than one percent.

Dr. B. S. Pomeroy: I disagree with the gentleman's remarks here, but I certainly agree that if we are going to make progress from here on out, we should take a strong look at our rendering industries as well as blenders of feed stuffs. We have been working with a blender here, locally, and he has this problem that he gets foodstuffs from all over the country to blend through his plant. His plant is an automatic plant except for the time that the protein goes into the hopper and comes out blended. I think there is an answer to it. I think the crude protein materials can be properly prepared and arrive on the market Salmonella free. It certainly has been clearly demonstrated in other countries of the world that this can be accomplished, but how soon it can be accomplished in the United States, I am not here to predict.

Dr. W. L. Sippel: I do not think this question is quite as simple as has been postulated. I am sure that all of us will agree that it is desirable to have feed samples free of Salmonella. However, when you approach a man who has a feed factory or a rendering plant and you tell him he has to get rid of the Salmonella, he is, first, going to ask you "how," and, second how much is it going to cost? And when you tell him that it is going to involve quite a considerable expenditure, he is going to think of a reason why he cannot do this. It evolves, ultimately, to a court case and who is going to get up and testify that these Salmonella are all pathogenic as has been alluded to (and what happens then). This is really the crux of the matter. It is the pathogenicity of Salmonella as a broad and all inclusive term and I think this is where we need some additional answers at this time.

Dr. P. R. Edwards: Thank you, Doctor Sippel. I think your remarks very clearly bear out the contention that I have always had that economics possibly are much more forcible than the consideration of public health. I was quite interested in your comments. Doctor Newell said that he would like to criticize his own regime and I think he is justified in this. I realize that it is often very difficult to investigate these incidents epidemiologically, and yet, if you remember the slides which I used this morning, you will note that the number of incidents from general outbreaks, through family outbreaks, and to sporadic cases increased tremendously. Actually, I doubt that there are very many real sporadic cases of Salmonella infection. Surely, most of these involve either a family or more than one family. We see this repeatedly in the cultures that come to us for diagnosis. Within a given State, there will be Salmonella cultures originating in several cities within the State, and you will have a focus of one type in one city, a second type in a second city, and a third type in a third city. This happens repeatedly. Now, obviously, these are not sporadic cases. This is a marked indication of our lack of epidemio-
logical investigation of these happenings. I was very pleased that Doctor Newell singled out *S. heidelberg* for particular mention since it has undergone such a dramatic change in this country. We never saw any cultures of *S. heidelberg* except the old stock cultures for many years, and, now, it is one of our common types and is widely distributed throughout the country in both man and animals. I would like to ask Doctor Bartram of the Food and Drug Administration to say something about their activities at this time. They are rather deeply involved in salmonellosis and investigation of foods. Would you do that, please, Dr. Bartram?

**Doctor Bartram:** I came to this meeting to sit with my ears open and my mouth closed. However, I am glad to have the opportunity to say a few words about salmonella. Not because I think I know anything about it. I am quite certain that I do not. I can recall thirty years ago when I was teaching, that I placed great stress on the fact that we did not have a salmonella problem in this country. I was wrong. If I had been told six months ago that Hollandaise sauce was a possible vehicle for salmonella infections, I would have certainly laughed at the individual who would have dared to make such a prediction. We can run through, I think, a large number of food products in much the same way. We have had dried yeast, and who would have thought of that as a likely source of salmonella infections? There were some outbreaks due to an infant feeding formula which was used as a substitute for milk. There are a number of foods that we can mention. In the discussions this morning, someone mentioned, “Where do we go from here?” The perennial question that I am asking, and I think all of us need to, is “What is next that will appear as a vehicle for salmonella infections?” With specific regard to the subject of animal feeds, which has been the subject of discussion here today, let me say that we are deeply concerned with them in the Food and Drug Administration. I am very much afraid that in the final analysis, animal feeds, if I have correctly read the cosmetic, food and drug act, will fall within our jurisdiction if it is necessary that something must be done. We have had a number of discussions concerning their role and the possible need for action on our part. We are trying to keep abreast of the information which is available and that is primarily the reason I am here. I think we need the type of information that has been discussed here today very much. What is the importance of this high incidence of contamination in the animal feed products to the animals themselves. I think if asked to state specifically my own personal feelings at the present time, I could certainly say that somehow we must, as Doctor Niven said, “break this chain.” Doctor Durbin, Head of our Veterinary Branch in Food and Drug, was very sorry that he could not be here to attend this meeting. However, he specifically wanted to say to this group that we are in need of information as to whether this is something that we must get into, and so I know that I speak very definitely for him. He would welcome any comments, any suggestions, and any information that you might have. I may add a fervent amen to the conclusions and proposals that have been made and I certainly hope that a symposium, such as this, is only the beginning of a group effort to reach some conclusion on
this problem. There seems to be no question but that the incidence of salmonella is increasing and, certainly, it is something that we must tackle together.

Dr. P. R. Edwards: Thank you, Doctor Bartram.

Question: Addressed to Doctor Pomeroy about typhimurium infection.

Dr. B. S. Pomeroy: Since 1943 our basic laboratory procedures in Minnesota have not changed. We have cultured the intestinal tracts of turkeys in the laboratory routinely, using that as an indicator system. As far as the incidence of S. typhimurium is concerned, I believe that our decrease in the incidence of S. typhimurium down to the low level of about 10 percent is due to an intensified program to eliminate the typhimurium infected flocks from our over-all salmonella program. I believe this is tied in precisely with the efforts Doctor Hinshaw initiated in California in the 1940's and which carried over into the 1950's. This is purely a reflection of pressure on the part of the industry to eliminate typhimurium infections in our adult breeding stock.

Question: About serological testing.

Doctor Pomeroy: The final analysis of whether a flock is infected is not on the basis of serology but on the basis of isolation of the organism from the host. A flock may have some serological reactors in it but if we cannot isolate the Salmonella from that flock, we consider it a negative flock. I might explain our program here in Minnesota. At the present time, we test our turkey flocks with pullorum antigen and typhimurium antigen, and any reactors are submitted to a bacteriological test. If we isolate ANY Salmonella, whether it be pullorum, typhimurium, or another salmonella type, that flock must go through a series of serological tests to have a negative test and negative bacteriology before it can be used as an official flock under an official program. Fortunately, or unfortunately, this procedure was started before we realized that we were feeding the Salmonellae back to the flocks probably as fast as we were trying to eliminate them. What concerns me right now is whether we are out in left field in putting this much pressure on the flocks because of the fact that feedstuffs, potentially, may feed back to our flocks Salmonellae and thus cause serological response in these birds and then we reisolate the organisms from the intestinal tract.

Question: What I meant, Doctor Pomeroy, was that you often pick up S. typhimurium with S. pullorum antigen though you are not using S. typhimurium antigen. Do you also have a tendency to pick up other Group B Salmonella with typhimurium antigen?

Doctor Pomeroy: Yes, we do. We isolate Group B serotypes from a lot of birds that react to the typhimurium test. Our typhimurium program works good if we only would designate the birds from the flocks in which we get typhimurium because the incidence has gone down terrifically but we still get a lot of other Group B types.

Question: Are you prepared with any figures as to the percentage of your salmonellae isolations from clinical cases that are submitted as a result of serological testing?
Doctor Pomeroy: Well, our source of the material in the laboratory is of two types—one is in the young poult's under six weeks of age which are obviously brought to the laboratory because the owner feels that he has got an above normal loss in that flock. The second, of course, is the birds that react to the serological test under the control program. The thing that bothers me is the fact that 50 percent of the isolates come from the intestinal tract only. So if we wanted to make our data look good, and we eliminated the intestinal isolates, we could say we are making tremendous progress in the Salmonella control program. But in about half of the infected lots, the Salmonellae are found only in the intestinal tract. The point then is just what are they doing potentially in that flock? Obviously they have come to the laboratory because they are having a higher than normal loss. The only thing we may isolate are salmonellae. This is what concerns me a little bit in interpreting these results.

Dr. P. R. Edwards: All of these Salmonella types contain common antigens. It is true that the major antigens are determined chemically by the relative composition of the carbohydrates of the cell wall, but they all contain common antigens and you can expect some cross reactions between any of them. I have an idea, and this is more supposition than anything else, that the avian species may not react in exactly the same way as a rabbit. The rabbit perhaps gives you a little cleaner production of agglutinin for your major antigen. But even if we try to use rabbit sera and do not dilute it properly, you cannot tell one group from another. That is the reason why all these sera for grouping have to be standardized and used in given dilutions in order to obtain significant results. As to the intestinal carrier, while I certainly am not a poultry pathologist and I am sure many of you could answer this question better than I, certainly such a bird constitutes a focus of infection and a method of perpetuation of the organism and, as such, I should say should be eliminated just as though she were infected. In fact, she is more of a menace than the bird which has it in its internal organs because it is probably going to die where the other one is going to perpetuate the infection.
SUMMARY SALMONELLA SYMPOSIUM

PHILIP R. EDWARDS*

Atlanta, Georgia

SUMMARY

The discussants seem to have taken most of my closing remarks from my mouth. However, I will go over them again. I think that in considering this problem, we must go back and consider the changes in the food habits of our human population and the changes in the feeding practices in animals. I think that these changes are really quite comparable. The food for man is no longer prepared basically at home in small lots and the same thing can be said for animals. When I was a boy, hogs were fed corn from the farm and some 40 percent digester tankage from a single supplier. Today, you have the products of multiple suppliers going into these food products and this, of course, increases the chance that the organisms will be present in the food. This is exactly the same as whether you eat a couple of coddled eggs for breakfast or whether you have some egg product that is composed of hundreds of thousands of eggs, one of which might have had billions and billions of Salmonellae in it. There is one thing that worries me a bit, and this has been brought out to a certain extent today and perhaps not so clearly as it should have been, and this is the question of the presence of S. typhimurium in man, in animals, in egg products, and in feed. Of about 30,000 cultures from man and animals that we examined at CDC, about 17 percent were S. typhimurium. As you could see from the figures which Miss Moran presented this morning, it does not make very much difference if you separate the cultures from man and the cultures from animals; they come out about the same because the animal cultures of S. typhimurium, I think, were around 20 to 22 percent. We have not examined very many cultures from egg products but of 151 which I calculated, 18.6 percent of the cultures isolated were of the type S. typhimurium. In Great Britain, the situation is somewhat different. Doctor Newell showed us a slide which went up to 1955 and in a fairly recent publication covering years from 1955 to about 1958, it was stated that of approximately 13,000 cultures isolated from man in England and Wales, 75 percent were S. typhimurium. In the British Isles and in northern Europe, they have always had a larger S. typhimurium problem relative to other Salmonellae than we have. Although in the figures published by the Colindale Laboratories in 1961, the percentage of S. typhimurium had decreased enormously, it is stated in one of the publications from the Colindale Laboratory that over a period of years, the

* Chief, Enteric Bacteriology Unit, Department of Health, Education and Welfare, Public Health Service, Bureau of State Services, Communicable Disease Center, Laboratory Branch, Atlanta, Georgia.
numbers of Salmonellae other than *S. typhimurium* have increased five times. So perhaps they are encountering, or will encounter eventually, the same situation that we have. Now while you have *S. typhimurium* in, let us say, 20 percent in man, 20 percent in animals, about 18 percent in egg products, and in Britain you have 70 to 75 percent when you find that of 851 cultures from feeds and feed ingredients which came to CDC for typing, 1.7 percent were *S. typhimurium*. This, to me, seems significant. I do not know just what the significance is unless it is the fact that *S. typhimurium* has a tendency to be more invasive than many of our other Salmonella types and yet, as you will remember I said this morning, I felt we were treading on dangerous ground when we made the assumption that one Salmonella serotype was more invasive or more pathogenic than another. The low incidence of *S. typhimurium* in feeds, as compared to its presence in animals and man, suggests that there are many sources of Salmonellae other than contaminated feeds. We find a lot of what one might call exotic types in foods and feeds. Exotic is not a good word, of course, but if we can use exotic in its broad sense, I think it will express my meaning. We are finding the presence of such types in man and in animals. They appear, of course, in much smaller numbers than they do in the feeds. But here again, we come back to what Doctor Newell spoke of a few moments ago about the time lapse between the appearance of an organism in the feed and in human infection. The changing incidence of Salmonella types is a most impressive thing if one follows it throughout the years. I am sure you are all familiar with the nationwide episode of *S. reading* infection which took place a few years ago. It was really never satisfactorily explained. This organism went up in incidence, down in incidence. It never reached its former very low level probably because a lot of carriers were created and we occasionally find it in man and animals today, but it was a quick thing—up and down. On the other hand, *S. infantis*, *S. heidelberg*, and *S. enteritidis* have been increasing rather dramatically in numbers and they seem to not be going up and down but on a gradual rise and apparently are well established and here to stay.

In regard to human salmonellosis, I think the danger lies not so much in the presence of Salmonellae in one product or in another product. I think that the danger lies in their introduction into the area of food preparation, whether this be in a commercial institution or in the home. Salmonellae introduced in one vehicle may be thoroughly cooked and killed. In the process of the preparation of this contaminated vehicle, the contamination enters the kitchen, perhaps the food handlers ingest small numbers of these and become asymptomatic carriers, your utensils become contaminated, and, of course, it has been shown that utensils contaminated from one source give rise to salmonella infection due to another food prepared in the same utensils. So the introduction into the area of food preparation, I think, is where the real danger lies. As to the steps that should be taken, I am in complete agreement with Doctor Newell that salmonellosis in man and animals should be a reportable disease and this will require the close cooperation of public and private agencies dealing with animal and human health, the practicing
physician, the practicing veterinarian, the health officer, the veterinary health inspector, the sanitary, and the laboratory worker. Not only should they be made reportable diseases, there should be a mechanism for the prompt dissemination of this information and they should be reported on as nearly a current basis as possible. Mrs. Galton has made some small start on that at CDC and I hope that this can be continued and enlarged and gradually the material from the states can be incorporated in it. And now, of course, we come to the question of compulsory control and inspection and what rulings should be made, what ordinances should be adopted, regarding the presence of Salmonella in human and animal food. Well, I personally feel that it is probably better not to pass any such ordinances or make any such rulings unless they can and will be enforced, and the difficulties of enforcement at the present time are rather large. If we think of foods, the animal foods, and organic fertilizers, of course, should be included in this list, and we know the rather large organization that is presently maintained in the State experiment stations for the chemical examination of feeds and fertilizers. We have to consider that this must be extended to bacteriological examination, and furthermore, that these bacteriological examinations would have to be done in larger numbers and on a continuing basis. I think that the number of samples that would have to be examined would be larger than is presently done to keep them honest as far as the chemical composition of their products. And then, of course, we come to the possibilities of voluntary improvement of which Doctor Niven spoke this morning and I think we can go far in this way. Of course, it is not my place to say what we should do about this, whether we should try to adopt compulsory regulations or not, but I think that these things we should be thinking about and decide in what direction we must go in order to minimize the chain of infection from animals to man.

Dr. J. W. Newberne: I should like to express the sincere appreciation of the Conference of Veterinary Laboratory Diagnosticians for the excellent job Doctor Edwards has done here in moderating this symposium. Also, I should like to thank Mrs. Galton who has contributed much of her time and talent to organizing the symposium. The information disseminated here today certainly contributes toward a better grasp of the many faceted problem we have confronting us. As an expression of our appreciation, I would like to give all of the participants in this symposium a round of applause, after which time we will be dismissed.
THE DIAGNOSIS OF HOG CHOLERA

H. W. Dunne*

University Park, Pennsylvania

Much has been said about the need for a test for hog cholera upon which to base an eradication program. There are some who contend that no program should be initiated until a diagnostic test has been devised.

Everyone is in agreement that a single test, accurate, fast and with red and green light decisiveness would be of considerable aid to any eradication program. In the brucellosis program a single test has been used with considerable success. Only by serology could adequate detection be accomplished. In hog cholera, however, evidence of the disease is not hidden. The virus produces specific lesions and blood changes which make possible a diagnosis by means other than a single test. It is the purpose of this paper to present both long established data and new data not previously published to confirm the opinion held by many diagnosticians that hog cholera can be diagnosed accurately and quickly with data obtainable from the infected herd. Although an attempt will be made to simplify the diagnosis in this report by concentrating on two tests and on lesions, attention must be drawn to the fact that all obtainable information, history, clinical signs, lesions and tests can be important in arriving at a diagnosis, particularly in borderline cases.

The occurrence of a leukopenia in hog cholera infected pigs was established by Lewis and Shope (1929). For many reasons, the test was not completely accepted at that time as being diagnostic of hog cholera. Over the years it has received sporadic support but enough inadequacies prevailed to cast doubt on its usefulness. Discrepancies existed as to what constituted a normal leukocyte count. There was no agreement as to what was a sufficiently low count to be considered significant in the diagnosis of hog cholera.

Leukocyte Counts on Normal Pigs

During the past several years experimentation with hog cholera in swine at Michigan State University and Pennsylvania State University has provided a wealth of information on the numbers of leukocytes in the blood of normal and hog cholera-infected pigs. Total white blood cell (TWBC) counts were taken on 1,671 normal pigs six to 12 weeks of age. Not included were counts of TWBC of pigs five weeks of age or younger for normally they often are less than 12,000 and occasionally as low as 5,000. Pigs were from a variety of sources. The only common factors in all were that they were not vaccinated for hog cholera, had no previous exposure to hog cholera, were from hog cholera-susceptible sows and appeared to be in a normal state of good health.

* Department of Veterinary Science, Pennsylvania State University.

Authorized for publication as journal series No. 2615 of the Pennsylvania Agricultural Experiment Station, University Park.
In Figure 1, it becomes quite obvious that some "normal" pigs were anything but normal. Pigs with TWBC determinations of 40,000 or over comprised 3.5 percent of the total counted. It is quite doubtful that many of these pigs could be classified as normal. Another group including 11.5 percent of the total counted ranged between 30,000 and 40,000. Some of these pigs undoubtedly must have been normal. Others may have had a low grade bacterial infection. It is also recognized that leukocyte determinations made after an animal has eaten, may be several thousand higher than those on the same animal before it has eaten. If counts of 40,000 or over are to be considered as abnormal (3.5 percent) in establishing a normal range, it is statistically sound to consider the 0.5 percent under 10,000 as abnormal. Thus the normal range of TWBC for pigs six to 12 weeks of age would be 10,000 to 40,000 with a mean of a little over 21,000. Of particular importance is the sharp drop to 10,000. Only seven counts in 1,671 "normal" pigs fell below 10,000. In pigs of this age (six weeks to 12 weeks), it seems obvious that any leukocyte counts below 10,000 would indicate a definite leukopenia. From 10,000 to 13,000 TWBC (5.3 percent of total normal counts) would fall into a range suspicious of leukopenia.

Leukocyte Counts on Pigs Experimentally Infected with Hog Cholera

Leukocyte determinations were made on all pigs experimentally infected with hog cholera virus. Sometimes several counts were made on one pig.
Only those counts were listed in the results if the animal's temperature was 105°F or above at the time the count was taken. Although many pigs with hog cholera have temperatures ranging above 104°F but less than 105°F it was felt that those with temperatures of 105°F or more would be most likely to have a leukopenia, particularly in the more chronic cases. Approximately 35.0 percent of the counts were taken more than eight days after the first temperature rise to 105°F or more. Several field strains of the virus of hog cholera, including variant strains, some with high and some with low pathogenicity, were used in the experiment. Methods of infection were varied and included subcutaneous, intramuscular and intracranial injections, tracheal inhalation, tonsillar swab and contact exposure. Secondary infection was not uncommon. A number of pigs were in experimental "breaks" following serum block simulating field situations.

In Figure 2 it can be seen that 53.5 percent of the total counts fall below 10,000 WBC. Above 10,000 but less than 13,000 (in the suspicious range) was 16.3 percent of the total counts. Approximately 30 percent of all counts from hog cholera-infected pigs had leukocyte counts in a normal range or higher.

The degree of overlapping of leukocyte counts from normal (Figure 1) and infected pigs (Figure 2) is shown in Figure 3 where the one graph is
superimposed upon the other. Attention is drawn to the line between 9,000 and 10,000. Only rarely does the TWBC fall below 10,000 in normal pigs six weeks of age or older, whereas 53.5 percent of the TWBC from hog cholera-infected pigs were below 10,000.

Terminal leukocyte counts made on 753 moribund animals (Figure 4) such as might commonly be brought to the laboratory showed that only 43.9 percent of the total counts from moribund pigs were below 10,000. Again 16.2 percent were in the suspicious range between 10,000 and 13,000. This points out that animals near death are less likely to have a leukopenia than those still able to move easily.

From the above data it is obvious that leukopenia is a common reaction in pigs infected with hog cholera and quite diagnostic when observed but that leukocyte determinations should be made on more than one animal. At least three and preferably six counts should be taken before concluding that a leukopenia does not exist. Serious consideration should be given to obtaining oxalated samples from several sick animals or to conducting the leukocyte count at the herd site.

Histological Lesions in the Brain

The second diagnostic test which is widely used is the histological examination of the brain. First described by Brunschwiler (1925) and later confirmed by Rohrer (1930) the lesions consist of vascular and perivascular
infiltration with endothelial swelling. Seventy-five percent of the cases were positive for these findings. Seifried (1931) described a mononuclear infiltration of the perivascular spaces. Helmboldt and Jungherr (1950) found lesions to be primarily vascular and perivascular cuffs and microgliosis. They also noted hyalinization of the vascular wall. In approximately 200 experimental cases of hog cholera in our laboratories there has been involvement of the medulla in more than 95 percent of the animals. Absence of the lesion is most likely to be noted in peracute cases. Although there is some question as to whether the cells are present as the result of infiltration or of endothelial proliferation (we feel that latter is correct) there is little doubt that the increase in cells about the vessels has considerable diagnostic significance. When these brain lesions are present with a leukopenia the diagnosis is positive hog cholera. Either one alone should be considered strongly suspicious of hog cholera and confirmed by the demonstration of diagnostically significant gross lesions upon post-mortem.

Gross Lesions of Hog Cholera

Certain gross lesions of hog cholera are diagnostically significant. Although all of the lesions listed below are not often seen in one animal, any of the specific lesions in addition to lesions of a septicemia and one of the above tests would be considered positive for hog cholera. Lesions indicating a
septicemia, of course, are primarily hemorrhages. These are constant in the kidney—petechial or ecchymotic—in subcapsular, cortical or medullar areas but occur with frequency in the skin, the urinary bladder, larynx and other mucosal tissues. The six lesions with diagnostic significance are as follows:

1. Peripheral Hemorrhage of the Lymph Nodes. This accumulation of erythrocytes in the periphery of the lymph node is apparently the result of vessel rupture within the node. It occurs most frequently in the mesenteric lymph nodes (47 percent incidence) but may be seen in any of the lymph nodes. More commonly in other areas the nodes have a marbled appearance as the result of hemorrhage.

2. Infarction of the Spleen (27 percent incidence). This is diagnostic but must not be confused with the discoloration associated with adsorption of sulfides from the digestive tract.

3. "Button" Ulcers. These result from infarction of submucosal vessels. Although occasionally they may be confused with other localized areas of intestinal necrosis, the true "button" ulcer has high diagnostic significance.

4. Focal Necrosis of the Gall Bladder. Long considered to be hemorrhages of the gall bladder, these lesions also are due to infarction and have high diagnostic significance.

5. Ulcers of the Prepuce. Never described before, these lesions were first observed by Doctor Kradel of our staff and are seen infrequently in animals dying of causes other than hog cholera. Strongly resembling the "button" ulcers of the intestine they also are associated with infarction and occur frequently in the male pigs dying of hog cholera.

6. Rib Lesions. The result of a metabolic disturbance in the acute stages of infection the rib lesion occurs during a time when blood phosphorus is high and blood calcium low. The lesion site is the costochondral junction and it is characterized by an irregular widening of the epiphyseal line. This is a failure of the mature cartilage cells to calcify. In chronic cases calcification occurs belatedly and the rib continues to grow. The result is a transverse line of calcification across the rib at variable distances—as much as one-half inch—below a normal appearing epiphyseal line.

Discussion

It is well to recognize that no single test is ever 100 percent effective. None of the tests or lesions described here can be considered pathognomonic. However, they can be considered diagnostically significant in that each is seen almost exclusively in hog cholera. The occurrence of a leukopenia and perivascular cuffing in a diseased pig that does not have hog cholera would be a rarity. The occurrence of two of the named gross lesions in conjunction with leukopenia or perivascular cuffing of the brain also would be extremely rare in pigs infected with anything but hog cholera. While borderline situations will arise regardless of the type of test system used, we feel that this system of diagnosis is relatively foolproof if the effort is made to provide ample data, particularly in the area of leukocyte counts. If indemnities are
to be paid in an eradication program, it would behoove the government authorities—State or Federal—to send men into the field in any questionable situation to get an adequate number of blood samples or blood counts to alleviate any doubt in diagnosis.

REFERENCES


DIFFERENTIAL DIAGNOSIS OF BOVINE VIRUS DISEASES

CHARLES J. YORK, D.V.M., Ph.D.*

Indianapolis, Indiana

In considering the subject of differential diagnosis of bovine virus diseases, the implication might be that all virus diseases of cattle should be included. This is an impossibility for one paper, and not entirely necessary. In many situations, the signs of illness and lesions are such as to readily establish the infection present. Furthermore, many of the virus diseases of cattle are not indigenous to North America, and need not be considered here. Adequate descriptions of foreign virus diseases can be found elsewhere. During the last few years, however, several virus infections have been described in the United States of America, all of them exhibiting respiratory signs as part, at least, of the disease syndrome. Some of these are relatively new; others were known previously and fit this category as a result of new information. Because of the involvement of the respiratory tract, differential diagnosis between them is more difficult.

The virus infections considered in this discussion are: Infectious Bovine Rhinotracheitis; Virus Diarrhea-Mucosal Disease; Sporadic Mucosal Disease; Malignant Catarrhal Fever; Bovine Parainfluenza 3; Pneumo-enteritis; Ulcerative Stomatitis.

The virus diarrhea-mucosal disease is represented here as the infection of known viral etiology represented by C 24 V prototype virus. It usually occurs on a herd basis. The sporadic mucosal disease is presented here, for the sake of differentiation, as a disease similar to severe cases of the above-mentioned virus diarrhea-mucosal disease but differing in that only a few animals seem to be affected in a herd, and while thought to be of viral origin, has not as yet been proven as such. The pneumo-enteritis infection is a new condition described publicly for the first time in another paper at this session of the United States Livestock Sanitary Association. Because research is constantly bringing new information and new viruses to the foreground, this list, as presented, is obviously complete only as of today. Several points to be mentioned are controversial, and probably not everyone will agree with the degree of emphasis placed on the various differentiating criteria.

In order to emphasize the similarities and differences for the various signs of illness that may be exhibited by each of the infections mentioned, a series of tables has been prepared which may be used as a guide for diagnosis. Obviously, in such tables it is impossible to note all the features that may be observed and, more specifically, the range of severity of each of the signs of illness listed. It should be pointed out that in most instances for all diseases,

* Research Division, Pitman-Moore Company, Div. of The Dow Chemical Company.
### TABLE II

*Clinical Signs of Illness Differing Among Certain Bovine Virus Diseases*

<table>
<thead>
<tr>
<th>Sign of Illness</th>
<th>Infectious Bovine Rhinotracheitis</th>
<th>Virus Diarrhea</th>
<th>Sporadic Mucosal Disease</th>
<th>Malignant Catarhal Fever</th>
<th>Para-influenza 3</th>
<th>Pneumoniaenteritis</th>
<th>Ulcerative Stomatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypersalivation</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Variable</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Lacrimation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Variable</td>
<td>Variable</td>
<td>No</td>
</tr>
<tr>
<td>Corneal Opacity</td>
<td>No</td>
<td>10%</td>
<td>Yes</td>
<td>Severe</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>No</td>
<td>Yes</td>
<td>Severe</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Laminitis</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Vaginitis</td>
<td>Yes</td>
<td>No</td>
<td>Occasional</td>
<td>Variable</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Coughing</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Abortions</td>
<td>?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td>Sign of Illness</td>
<td>Infectious Bovine Rhinotracheitis</td>
<td>Virus Infection</td>
<td>Sporadic Mucosal Disease</td>
<td>Virus Infection Malignant Catarrhal Fever</td>
<td>Para-influenza 3</td>
<td>Pneumonenteritis</td>
<td>Ulcerative Stomatitis</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------------------</td>
<td>-----------------</td>
<td>--------------------------</td>
<td>------------------------------------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Temperature</td>
<td>104° F. +</td>
<td>104° F. +</td>
<td>103° F. +</td>
<td>105° F. +</td>
<td>104° F. +</td>
<td>104° F. +</td>
<td>Normal</td>
</tr>
<tr>
<td>Respiration</td>
<td>Polypnea to Dysspnea</td>
<td>Polynoe</td>
<td>Dysspnea</td>
<td>Dysspnea</td>
<td>Polypnea</td>
<td>Polypnea</td>
<td>Normal</td>
</tr>
<tr>
<td>Anorexia</td>
<td>Yes</td>
<td>Partial</td>
<td>Yes</td>
<td>Yes</td>
<td>Variable</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Depression</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Nasal Discharge</td>
<td>Serous</td>
<td>Serous</td>
<td>Mucopurulent</td>
<td>Mucopurulent</td>
<td>Serous to Mucoid</td>
<td>Serous</td>
<td>None</td>
</tr>
<tr>
<td>Nasal Hyperemia</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Severe</td>
<td>Variable</td>
<td>Variable</td>
<td>None</td>
</tr>
<tr>
<td>Decrease in Milk Production</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
## TABLE III

*Gross Pathology Observed in Certain Bovine Virus Diseases*

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Infections Bovine Rhinotracheitis</th>
<th>Virus Diarrhea</th>
<th>Sporadic Mucosal Disease</th>
<th>Virus Infection Malignant Catarrhal Fever</th>
<th>Para-Infuenza 3</th>
<th>Pneumoperitonsitis</th>
<th>Ulcerative Stomatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal Hyperemia</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Serofibrinous Exudate</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Low</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Oral Erosions</td>
<td>No</td>
<td>Yes</td>
<td>Severe Hyperemia</td>
<td>Severe Erosions (Necrotic)</td>
<td>No</td>
<td>No</td>
<td>Yes Erosions</td>
</tr>
<tr>
<td>Muzzle Appearance</td>
<td>No</td>
<td>Hyperemia</td>
<td>Severe Hyperemia and Erosions</td>
<td>Severe Hyperemia and Erosions (Necrotic)</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>G. I. Erosions</td>
<td>No</td>
<td>Yes</td>
<td>Severe Hyperemia</td>
<td>Severe Erosions (Necrotic)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>C.N.S.</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Morbidity</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>?</td>
</tr>
<tr>
<td>Mortality</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>?</td>
<td>0</td>
</tr>
<tr>
<td>Occurrence</td>
<td>Herd</td>
<td>Herd</td>
<td>Sporadic</td>
<td>Sporadic</td>
<td>Herd</td>
<td>Herd</td>
<td>?</td>
</tr>
</tbody>
</table>
TABLE IV
Isolation and Serology of Certain Bovine Viruses

<table>
<thead>
<tr>
<th>Tests</th>
<th>Infectious Bovine Rhinostrachitis</th>
<th>Virus Infection</th>
<th>Sporadic Mucosal Disease</th>
<th>Malignant Catarrhal Fever</th>
<th>Para-Influenza 3</th>
<th>Pneumonitis</th>
<th>Ulcerative Stomatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bovine, Dog, Porcine Kidney; Hela Cells; etc.</td>
<td>Bovine Kidney (Some strains only)</td>
<td>?</td>
<td>Bovine Thyroid</td>
<td>Bovine and Monkey Kidney</td>
<td>Bovine Testicular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytology</td>
<td>Bovine Kidney</td>
<td>?</td>
<td>Syncytium Formation</td>
<td>Rounded Degenerated Cells</td>
<td>Large Strands; Scattered Granular Cells</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>5 Days</td>
<td>Dark, Granular Vacuoles Stranding 6-8 Days</td>
<td>7 Days</td>
<td></td>
<td>5 Days</td>
<td>7-9 Days</td>
<td></td>
</tr>
<tr>
<td>Serology</td>
<td>Serum Neutralization (S.N.)</td>
<td>Serum Neutralization</td>
<td>?</td>
<td>Serum Neutralization</td>
<td>Comp. Fix. S.N.</td>
<td>Hemag. Inhib. Inhibition</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S.N.?</td>
<td>Hemadsorption</td>
<td></td>
</tr>
</tbody>
</table>

* Fluorocarbon extraction probably necessary.
the severity varies tremendously from one outbreak to another and even from one animal to another in the same herd. Thus, we have situations in which only a few animals may show gross signs of illness characteristic of a given disease, with most of a herd showing only very mild or inapparent infection. In other circumstances, a high percentage may show "typical" lesions which would readily pinpoint the infection present. Hence, in an effort to determine the probable etiology of a virus infection, one should never look at just one or two animals in a herd, but, wherever possible, examine a number of them and add up the composite information obtained. Furthermore, the age of the animal plays a very important role. In general, the very young animal—that is, one to three weeks old—often exhibits a wide variety of lesions regardless of the virus infection, even though a number of these lesions may not be observed in the older animal. This is especially true of lesions of an erosive nature affecting the mucosal tissues. For example, infectious bovine rhinotracheitis does not ordinarily produce lesions in the oral or GI tract of the mature animal, but at times does produce such lesions in the young animal, thus causing confusion with virus diarrhea or other similar infections.

In listing the features for a differential diagnosis, Table I presents all the signs of illness that are, with few exceptions, common to the diseases listed. As mentioned above, undoubtedly considerable variation does occur for each of these signs of illness, but the range in itself is not sufficiently constant to be of any material assistance in reaching a diagnosis.

Table II lists a number of signs of illness that are normally different for the various infections, and which, if present, are an aid in reaching a decision. Again, the same caution must be stated, that is, that variations from animal to animal can be observed.

Table III includes the more common lesions which might be produced by the several virus infections. The ones in this table are limited to those which might readily be discernible in an ordinary gross field post-mortem examination. Obviously, with the assistance of a trained pathologist, an analysis of the histopathology is often of material aid in arriving at a decision. However, it must be pointed out that not infrequently, the lesions are such that while the pathology may be suggestive, in itself it is often not the deciding factor in a diagnosis. These observations, coupled with the other signs of illness listed in Tables I and II, are most important, and it should be stated that the final decision as to the cause of illness in the field rests with the practicing veterinarian or field man making on-the-spot observations.

As additional assistance, however, in resolving this problem, various laboratory tests are listed in Table IV. While it is obviously not possible for the man in the field to conduct such tests, if the appropriate specimens are collected and submitted to the laboratory, the findings are often significant in pinpointing the cause of infection in a herd outbreak of disease. Here the interpretation of the laboratory results is most important in outlining the findings and coordinating them with the history of the disease in the herd. Several of these are important enough to warrant additional comments.
1. The specimens to be collected for isolation attempts:

While, with the exception of the disease labelled Sporadic Mucosal Disease, viruses have been isolated from all of these infections, such isolations are not always readily obtained. Even if a virus is isolated from the specimens, it does not necessarily indicate that it is the cause of the illness observed. This can be made more definite if repeated successful isolations are made from different animals in the same herd, or from other herds showing similar signs of illness. Furthermore, if acute and convalescent serum samples were obtained from the same animals from which the virus was isolated, a change in antibody content from negative to a positive titer against the virus isolated is presumptive evidence that infection with this agent was actually occurring at the time the illness was observed. Once a virus has been isolated, further evidence that the virus isolated was probably the cause of illness rests on its identification. This can only be done with the use of previously prepared specific immune sera. If the virus turns out to be one already described as producing the type of illness in the herd in question, then the chances are good that it was involved in the outbreak from which the specimens were submitted.

2. Serology:

A number of ways of conducting neutralization tests or other serological procedures have been adequately described elsewhere. However, the single largest error that is introduced by various laboratories trying to conduct serology as an aid in diagnosis is the failure to obtain paired serum samples. Unless samples are collected during the acute phase of illness, and another set of serum samples obtained from the same animals two to three weeks later, it is virtually impossible to prove that the antibodies demonstrated for that particular virus had anything to do with the disease under investigation. Only if there is a sharp rise in titer from the acute to the convalescent sample, or a complete change from negative to positive, does the antibody titer have any significance. Any attempt to place significance on the height of the antibody titer of a single serum specimen is meaningless.

Occasionally, other circumstances do exist in which a slightly different approach to serology may be of value. If, in a herd outbreak where the opportunity does not exist for the collection of paired serum samples, several samples are obtained from animals not yet ill, several samples from animals acutely ill and several from animals obviously recovering or known to have just recovered from the illness, then tests on these single samples may have some validity, if the results turn out as follows. Obviously, the sera from those not yet ill should be negative against the suspected virus; the acute phase samples, negative or with very low titer; while those recovered should demonstrate strongly positive titers. However, in a herd where there has been a high incidence of inapparent infection, or one of long duration or poor management observation, then such a procedure is of no value. If the pattern is such that the infection is gradually going through a herd, this procedure is often of value. If only definite convalescent samples can be obtained, then the only recourse is to check the serum samples against every
possible known virus. If, by chance, these serum samples are positive against only one of the known viruses, this may be considered suggestive evidence that the recent infection observed might have been due to this particular virus.

While differential diagnosis of a number of bovine virus diseases is not easy, if all of the features discussed in these tables are considered as a group, it is felt that enough information exists at the present time to allow for an adequate diagnosis when such infections are encountered in the field.
The organizational structure is vital to the successful function of any institution, and the veterinary medical laboratory is no exception. A maximum expression of scientific talents, the proper utilization of physical facilities, the morale of personnel, and many other factors are influenced by the character of the organization. All of these enter into the over-all effectiveness of the laboratory.

In spite of the importance of the subject, it was with considerable misgivings that the preparation of this paper was undertaken. Everyone here has been associated with laboratory work in animal diseases, and many have had a part in the design of physical facilities and the formation of an organization. Being aware of this, one would not dare pose as an expert in this field. Instead, some basic points of mutual interest will be mentioned and certain possibilities of general laboratory organization presented.

A discussion on the organizational structure of a veterinary medical laboratory is difficult without considering the basic philosophy underlying the objectives and policies of these institutions. In the interest of expediency, a general objective might be suggested as, "the application of the best scientific procedures in current use to the study of animal diseases for definitive characterization and identification." It might be added that these efforts are to be directed toward making the greatest possible contribution to the health of the current animal population in the area served by the laboratory. Further exploration of the objective might suggest an urgency for the maximum amount of information about an animal disease problem in the shortest possible time.

It is the tax-paying public that finances the operation of most veterinary medical laboratories. It is only fair and proper, then, to consider the needs of this broad segment of the population in formulating philosophy, objectives, and policies. At this point it appears appropriate to point out that many persons other than livestock and poultry producers contribute tax-wise to support State and Federal laboratories. For example, recent figures from the United States Department of Agriculture give the estimated dog population as about 25 million and the number of cats as about 27 million. The majority of these are owned by nonfarm people. The pet food industry is quite a large one and utilizes considerable quantities of agricultural products. It appears, therefore, that publicly supported laboratories are obligated to provide good...
service to all animal owners and not to farm livestock and poultry owners alone. Wild game animals, zoo inmates, and pets of all species should be included. Studies on disease problems of all animals can most conveniently be accomplished in one laboratory, and there is no valid reason to separate the work by species.

The fact that the public is paying the expenses of a laboratory does not imply that this group is best qualified to formulate policies for its function. This unfortunate situation has prevailed many times in the past. Many animal owners have a great feeling of propriety and personal importance in being able to present sick or dead animals directly to the laboratory without prior consultation with a veterinarian. The direct scientist-animal owner relationship is often insisted upon, and invariably results in poor utilization of laboratory personnel and of physical facilities. The judicious management of economic assets in the public sector is just as mandatory as in private business. These people also insist on receiving a direct report with recommendations which they cannot possibly understand or apply. When the report does contain recommendations for treatment, it promptly finds its way to the druggist or feed store. This accomplishes nothing more than to further corrupt the community services of these good business people by intimating qualifications that they do not possess. Unnecessary losses may then occur in the diseased herd, and neighboring animals will be endangered. If these same animal owners should require personal medical laboratory assistance, it would not be available even from State institutions, except through the services of a physician. This is as it should be, and the same policy applies equally well in veterinary medicine. For the past seven years, this writer has been associated with a consulting laboratory providing services to graduate veterinarians only, and it has proved to be a most agreeable arrangement. The practicing veterinarian is the logical laboratory contact for many reasons. He has specific scientific training and experience for the interpretation and immediate application of laboratory results. He can also advise on procedures for protecting neighboring animals in the event of a contagious disease. Good accurate reports to the veterinarian will serve as a productive educational medium and benefit the entire community on a long-term basis. If animal owners first consulted a veterinarian, many problems would be solved locally and the needless routine work load of the laboratory would be materially reduced. Personnel and facilities would then be freed for work on problems of greater significance to the animal population.

Research in disease problems appropriate for the local situation should be considered an essential part of the laboratory program. For instance, especially difficult field problems should be pursued until the syndrome has been satisfactorily characterized. Results of thorough and accurate laboratory work maintained in a good record system can serve as a valuable guide in veterinary education, methods of disease prevention, and in regulatory procedures. New techniques must be developed, and there is always a need for refinement and adaptation of existing procedures. However, such projects must not be allowed to interfere with the primary objective of the laboratory.
Now, consideration may be given to a few thoughts on the organization of a veterinary medical laboratory from the standpoint of functional units. Reference to the general objective may assist in deciding on the disciplines to be included in the organization. Here is a skeleton framework that might be used as a baseline for any laboratory.

**Record System**

A system should be designed to insure a smooth and automatic flow of all records. This would include the distribution of work sheets to individual units when cases are entered and the collection of these forms when the work is completed. Good secretarial help can operate the record system, interview for case histories, and receive shipped specimens, with minimal attention from the scientific staff. The pathologist handling the case will give the necessary instructions in unusual situations and maintain close contact with the laboratory staff. Some kind of coded punch card system is needed to make useful case record data readily accessible for future evaluation. There are several simple and economical hand-operated plans available as well as the larger automatic data processing systems.

**Clinical Pathology**

This area should be directed by a trained medical technologist who has fulfilled the requirements for registry by the American Society of Clinical Pathologists. This person, with appropriate modern equipment, will be quite capable of performing all the necessary work in this area, including hematology, blood chemistry, urology, and parasitology.

**Histopathology**

A medical technologist of the American Society Clinical Pathologists Registry level is also desirable here so that special stains and cytologic procedures, including histochemistry, can be utilized. In small laboratories, a registered technologist can adequately train general science majors of the Bachelor Degree level as assistants and supervise the work in both clinical pathology and histopathology. A qualified technologist can be trained to cut and trim most wet fixed tissues and thereby save the pathologists time for more specialized and higher level activities. With proper planning, stained tissue sections can routinely be presented to the pathologist for reading and reporting within 24 hours.

**Microbiology**

It appears logical to consider pathogenic bacteriology, mycology, virology, and serology as a single discipline, and they function best as one coordinated unit. For instance, the techniques of virus serologic work in embryonated eggs and cell culture are basically similar to that used in virus isolation and characterization in these media. Serology in bacterial diseases often utilize the same basic principles as in virology, and many other overlapping techniques could be mentioned. Microbiology majors of the Bachelor or
Master level are entirely adequate and probably desirable for this combined unit. Each person in the unit may be assigned a specific responsibility, but during vacations, illness, or peak loads, personnel can readily assist in areas other than their own. This permits a more flexible and smooth-running organization.

**Experimental Animals**

Isolation units suitable for virus studies in animals as large as cattle should be provided for use by the microbiology section. Diseases, such as canine distemper, infectious canine hepatitis, and hog cholera, require considerable care for successful isolation. Animal technicians with a high level of intelligence, dependability, and an innate ability with animals are mandatory. Other than routine animal care, these men ability with animals are mandatory. Other than routine animal care, these men can render valuable assistance in simple clinical observations, collecting specimens, and keeping records.

**Animal Dissection and Collection of Specimens**

Animal technicians can save the pathologist much valuable time in this area without sacrificing proficiency. Blood and urine samples and tissues may be collected and the time-consuming part of the dissection can be adequately handled by a trained assistant. In many situations the procedure can be standardized so that a brief examination of the exposed organs by the pathologist with specific instructions for collection of tissue specimens is just as productive as a personal laborious dissection ritual. Veterinarians as a whole have had a tendency to insist on performing many minor tasks that should have been delegated to an assistant.

**Clinical Toxicology**

When a case is presented that warrants toxicologic investigation, the services of a scientifically competent and legally qualified toxicologist are essential for satisfactory results. However, two extreme situations are likely to exist in this area. In a few laboratories, a qualified toxicologist may be employed full time to study cases in which poison is suspected. This situation is highly desirable when cases are presented that actually should be investigated. Experience has shown that bona fide poison-suspected cases are relatively rare and in most laboratories the total number probably does not justify a full time, highly trained person. On the other and the more likely extreme, no service in toxicology may be available at all except a few relatively crude qualitative tests that are of little actual assistance. One highly satisfactory arrangement is a consultanship with a legally and professionally competent person for a set fee for specified services. Prior routine laboratory studies for other diseases will eliminate many poison suspect cases and guide the toxicologist in those cases eventually submitted.

**Making the Report and Summarizing the Laboratory Data**

After the technical staff in the individual units have completed their work and returned the work sheets to the secretary, the data must be promptly
reported. The work sheets should be reviewed by the pathologist if possible before the histopathologic slides are evaluated. In some cases, however, a quick review of stained tissue sections can give the microbiologist valuable direction in characterizing pathogenic bacteria. Preliminary telephone reports are sometimes highly desirable.

The laboratory data should be presented in an orderly manner and briefly summarized to conclude the communication. The veterinary pathologist is not locally in contact with the field disease problem, and he should rarely be expected to make a specific diagnosis. The responsibility of the final diagnosis rightfully belongs with the attending veterinarian and it should be left there. Prevention, control, and treatment procedures may logically be discussed if requested by the veterinarian. In this role, the scientific laboratory staff actually serve as consultants to veterinary practitioners. Copies of a report should be sent only to persons authorized by the attending veterinarian.

Laboratory Director

The principal administrator should be a veterinarian experienced in the laboratory investigation of animal disease problems in the field and has demonstrated good management ability. The appropriate person for this position in a given situation may be a veterinary pathologist or a veterinarian with general training and experience.

Naming the Laboratory

After the above discussion of laboratory organization and function, brief consideration might be given the selection of a fitting name. You may have observed that until now "Veterinary Medical Laboratory" has been used. Other possibilities are Veterinary Pathology Laboratory, Animal Disease Laboratory, and Animal Pathology Laboratory. Terminology that emphasizes the veterinary profession most properly places the desired connotation. The term "diagnostic" does not seem compatible with the philosophy expressed above and may best be omitted from the name and vocabulary of the laboratory.

SUMMARY

In summarizing the preceding thoughts on laboratory organization, the following points appear to be logical conclusions.

1. The objectives of the laboratory should be to make the maximum contribution to the current health of all segments of the animal population. The laboratory must be adjusted to the needs of the area served.
2. The organization operates best when divided into coordinated functional units.
3. The staff should consist of carefully selected persons that are appropriately trained for the specific areas. Duties and responsibilities should be well planned and categorized so that assignments make the best possible use of individual training and ability. This is especially important at the professional level.
4. Maximum results are obtained by working only through the graduate veterinarian both from the standpoint of referring cases and in submitting reports.

5. Since the laboratory is usually separated from the field problem by many miles, it is desirable to serve as a consulting institution rather than a final diagnostic authority.

6. The laboratory might be best advised to select a name most compatible with its objective and philosophy with particular emphasis on the veterinary profession.

In conclusion, experience has convincingly demonstrated that when a laboratory meets the needs of the community it serves, many new opportunities for greater service will be presented. The rewards in personal satisfaction alone are most gratifying.
DIAGNOSIS OF CANINE DISEASES

A. Infectious.
B. Blood and Blood Forming Organs.

J. H. SAUTTER, D.V.M., Ph.D. and VICTOR PERMAN, D.V.M.

University of Minnesota, St. Paul, Minn.

The content of this paper is directed toward the diagnosis of canine diseases with emphasis on simple techniques. By these I mean, making a diagnosis with history, necropsy, microscopic examination of tissues and bacteriological techniques.

The ideal diagnostic situation is a simple procedure or test which will yield a positive diagnosis as soon as possible after the presentation of the specimen. I would like to emphasize the importance of a careful necropsy since it may be neglected in favor of a shiny laboratory with its complicated equipment.

CANINE DISTEMPER

This disease will be discussed first because of its frequency, importance and difficulty in diagnosis, due to the various forms of the disease. When a young dog is presented with a history of anorexia, nasal and eye discharge, a logical assumption is the acute form of distemper. However, when a dog is presented with a vague history of illness, then the diagnosis becomes more difficult because of the various manifestations the disease may produce after acute signs have subsided.

FIGURE 1

Distribution of Lesions from 525 Cases of Canine Distemper

<table>
<thead>
<tr>
<th>Type Lesion</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encephalitis</td>
<td>325</td>
<td>62.0</td>
</tr>
<tr>
<td>Interstitial pneumonia</td>
<td>67</td>
<td>12.7</td>
</tr>
<tr>
<td>Bronchopneumonia</td>
<td>50</td>
<td>9.5</td>
</tr>
<tr>
<td>Mixed pneumonia</td>
<td>5</td>
<td>0.95</td>
</tr>
<tr>
<td>Inclusions present</td>
<td>150</td>
<td>28.5</td>
</tr>
<tr>
<td>Hard pad</td>
<td>32</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Total Distemper (cases 525) 18.2.
Total Necropsies (2885).

Necropsy findings. Figure 1 shows the major categories of post-mortem findings encountered at the University of Minnesota during a 10-year period (1950-1960). In 2,885 canine necropsies in a ten-year period, 525 or 18.2
percent were diagnosed as distemper. It will be noted from Figure 1 that the
majority (62 percent) of cases had either acute or chronic encephalitis. The
presence or absence of encephalitis apparently is not always dependent on the
duration of clinical signs, however, the degree of chronicity appears to be
related to the duration of the disease.

The microscopic changes in the brain vary in location, however, the
majority are in the basilar portion. This is very helpful to know for diag-
nostic purposes. The type of encephalitis encountered is usually classified as
nonsuppurative with the major portion showing glial nodules, demyeliniza-
tion and perivascular cuffing. A wide variation however is described ranging
from neuronal degeneration to encephalomalacia.

We routinely make a transverse cut through the cerebellum and medulla
thus ending with two or three sections, depending on the size of the brain.
These are examined for the presence of lesions which can be associated with
distemper. The lesions, if present, together with a history typical of distemper
makes a diagnosis reasonably certain. The presence or absence of inclusions
in the brain is not important since they are present only on the average of
six-seven percent, Figure 1.

Respiratory System. According to our data, 23 percent had pneumonia and
of the 23 percent, 12.7 percent was interstitial, 9.5 percent bronchopneumonia
and approximately one percent was mixed.

At necropsy the presence of interstitial pneumonia is not easily detected
unless quite extensive. Palpation and section are the best ways to detect its
presence. Grossly, the lung is solid, the color is near normal and micro-
scopically there is a mixed population of mononuclear cells, i.e., septal,
macrophages, plasma. In some instances there are cytoplasmic and nuclear
inclusions and varying numbers of neutrophils. The bronchi are usually clear
of exudate.

It is felt by some investigators that interstitial pneumonia is due to the
distemper virus and bronchopneumonia to secondary invaders (1). There is
frequently seen at least in our laboratory a mixed type in which a
bronchopneumonia appears to be superimposed on the interstitial type. This
is easily recognized microscopically. We have noted that when a pneumonia
of either type is well advanced, inclusions bodies are usually absent.

Smears of the tracheal epithelium or tongue stained with Seller’s stain will
clearly show inclusions if present, however, their absence does not exclude
distemper.

Hard pad or hyperkeratosis is a useful diagnostic feature when present.
According to our records, six percent or 32 had the condition which appears
to be another manifestation of distemper, although not diagnostic because
hardening or hyperkeratosis of the pads is reported in other conditions.

Diagnosis. The problem of making a diagnosis of distemper in which one
can be certain is not easy. If inclusions are not present one must depend on
a typical history, necropsy lesions and microscopic findings. The gross
necropsy is frequently completely negative and the diagnosis is then dependent
DIAGNOSIS OF CANINE DISEASES

upon either inclusions or other lesions such as interstitial pneumonia or a typical encephalitis. These latter findings actually are not conclusive, and animal inoculation is not practical for routine diagnosis.

INFECTIOUS CANINE HEPATITIS

Canine hepatitis is with our present knowledge of the disease much easier to diagnose than distemper. This condition was recognized by diagnosticians and described by Green (3, 9) and others as fox encephalitis. It was also known as tonsillitis and various other names. In foxes it affects principally the nervous system whereas in the canine a mild encephalitis may occur but the principal diagnostic lesion is a hepatitis with intranuclear inclusions. The condition has been fully elucidated by Rubarth (10) and others; thus making the diagnosis of the disease much easier for both clinician and pathologist.

Necropsy and Diagnosis. The diagnostician is usually presented with a dead animal having a history of sudden death with no visible lesions or sign of injury—poison is frequently suspected.

In our experience at necropsy the liver is found to be mottled with small light areas of necrosis and the gall bladder wall is edematous. Congestion of intestinal lymph nodes and other hemorrhages or congestion of the stomach is variable and inconsistent.

A history of sudden death, together with gall bladder lesions, are very strong and helpful diagnostic indications of acute hepatitis. At this point a diagnosis can be made by taking a small piece of liver and either crushing or dicing, blotting away the blood and touching the material with a heated slide. The slide is then placed in 10 percent formalin for a few minutes to fix and then stained with a standard hematoxylin and eosin procedure. The resulting slide in our experience is satisfactory to demonstrate inclusions and is much easier and quicker to prepare than a tissue slide.

The microscopic lesions in the liver consist of central lobular necrosis, congestion, pigment and intranuclear inclusions in the liver cells. Inclusions in the reticulo-endothelial cells of the liver, spleen, kidney and endothelial cells of the brain can be found but are much more difficult to locate and utilize for diagnostic purposes.

LEPTOSPIROSIS

Leptospirosis, although known for a long time, is recognized as a serious disease and a public health problem.

The organism commonly causing the disease is Leptospira canicola but L. icterohemorrhagiae does occur in a much smaller percentage and more recently there is seriological evidence that other species can affect the canine (1). Acute leptospirosis is characterized by vomiting, dehydration, fever, bloody diarrhea and frequently icterus.

Some animals survive the acute state, then later die of uremia due to lesions which develop in the kidneys. Other animals do not show the acute phase and are never icteric but develop uremia due to the kidney involvement.
Lesions. Lesions in the acute disease are those of septicemia, usually icterus, dehydration, hemorrhages on serosal surfaces and varying degrees of hemorrhagic enteritis.

Microscopically, the liver cells show dissociation and have a granular appearance with an excess of bile pigment and hemosiderin.

The changes in the kidneys in the acute phase are not helpful in making a diagnosis unless the organism is demonstrated with a silver stain. The tubular cells show a granular cytoplasm but few other definite lesions.

In the subacute form, dehydration, emaciation and a possible uremic odor may be the only lesions noted except the kidneys. The kidneys are enlarged and turgid. The capsule strips easily and on the cut surface in the cortex white or gray areas are noted. Microscopically, large foci of lymphocytes, plasma cells and macrophages involve the tubules and glomeruli. Silver stains, in our experience, are frequently negative and this situation may parallel the condition in cattle as described by Seibold (13) when in the subacute or chronic stage the organism cannot be demonstrated. This may be due to the disappearance of organisms as antibodies appear (12).

Diagnosis. A positive diagnosis of leptospirosis may be difficult. If there is a history of clinical signs indicative of acute leptospirosis and the post-mortem lesions correspond, then, it is reasonable to make a presumptive diagnosis of leptospirosis. Additional measures such as silver stains of tissues and animal inoculation are desirable if time and facilities permit.

If a dead animal is presented having no history of icterus and no serological test, then necropsy findings including microscopic changes in the kidneys are the only immediate aids in making a diagnosis. A negative silver stain and negative animal inoculation are not conclusive evidence that the animal did not have leptospirosis.

GRANULOMATOUS DISEASES

This is a troublesome group of diseases both to the clinician and diagnostician. They are, as the name indicates, a group of diseases characterized by granulomatous inflammation. It is a large group and only the most important will be discussed. This group for the most part does not respond well to treatment, produces vague clinical manifestations and are frequently mistaken for neoplasia (14).

Diagnosis made from histological preparations may be difficult and warrant serial sections due to the paucity of organisms in the tissues. Then should no organisms be found cultures and animal inoculation are necessary to establish a diagnosis.

ACTINOBACILLOSIS, ACTINOMYCOSIS AND NOCARDIOSIS

These three conditions are distinct entities if one considers the characteristics of the organism. However, gross and microscopic examination of tissues and even preliminary bacteriological procedures are insufficient to make a positive identification. Although Topley and Wilson set up definite
a. 800x H. & E. Colony of Actinomyces bovis. Note uneven staining of fringe.

b. 800x H. & E. Colony of Nocardia asteroides. Note even staining.

c. 1000x P.A.S. stain. Budding blastomyctete in lung tissue.

d. 1200x H. & E. Coccidiodomycosis. Lesion in lung showing sperule containing endospores.

e. 1200x H. & E. Toxoplasma in peritoneal exudate.

f. 800x H. & E. Cryptococcus organisms in brain lesion.

Figure 2
identification procedures, in our experience the organisms vary in certain properties.

The literature is confusing on these conditions because the etiological agents are difficult to properly identify and it is evident that many cases frequently diagnosed as actinomycosis were either actinobacillosis or nocardiosis.

The usual type affecting dogs enter the skin apparently through injury and the initial sign may be a lump or discharging lesion in the skin. The discharge may be absent, insignificant or at a distance from the lump. A tentative diagnosis can be made only by microscopic examination of the tissue. Smears of exudates or tissues are also very helpful. In some instances there is an overgrowth of tissue and a paucity of organisms, thus making serial sections necessary if histological studies are employed. Similarly bacteriological techniques require the grinding of large amounts of tissue. The majority of the cases reported show skin or subcutaneous tissue involvement which later becomes systemic, affecting the pulmonary system or other systems (14, 15, 16, 17).

**Diagnosis.** Tumor-like masses in the skin with or without ulceration or discharge with or without internal lesions are suggestive of actinobacillosis or similar condition.

Smears of the pockets of suppuration containing the granules or histological sections are useful in establishing a presumptive diagnosis. *Actinomyces bovis* stains pale pink with hematoxylin and eosin and the colony has course club-like borders, Figure 2a. They are Gram-positive and non-acid fast. Actinobacillosis usually has a smaller colony, finer clubs on the edge, has more of a bluish cast with hematoxylin and eosin and is Gram-negative. The colonies in Nocardiosis show less clubbing, Figure 2b, and are Gram-positive and acid fast. According to Topley and Wilson (19) and other investigators (15, 17) the acid fast qualities are variable.

These criteria are sufficient to make a presumptive diagnosis but to be certain of the type further laboratory work must be undertaken to properly identify them.

**BLASTOMYCOSIS**

This is a chronic infectious disease of animals and man caused by a fungus. The disease is reported mainly as a pulmonary form which may become systemic, or partially so.

**Diagnosis.** Finding pulmonary lesions resembling nodules or abscesses are not diagnostic in view of other members of this group of diseases. Histological examination of the lesions usually show the large double refractile walled organisms surrounded by neutrophiles, macrophages and epitheloid cells and perhaps a few giant cells. The presence of the easily recognized organisms are sufficient to make a diagnosis, Figure 2c.

**HISTOPLASMOSIS**

Histoplasmosis is another member of this group of diseases caused by a fungus and is widely disseminated in our animal population and very difficult
to diagnose in the living animal. There are special means such as skin tests and demonstration of the organism in the blood which are employed but not successful in every instance. A recent publication (18) states that 31 percent of spontaneously infected dogs whose mesenteric lymph nodes were cultured were positive for histoplasmosis.

*Diagnosis and lesions.* Here again necropsy findings are nonspecific. There may be a few nodules or exudative pneumonia in the lungs and proliferation of lymph tissue. The organisms cause a proliferation of reticuloendothelial cells throughout any organ which they invade. The organisms are found both free and in macrophages and can be shown with hematoxylin and eosin stain or better with one of the fungal stains involving the periodic acid Schiff reaction. Demonstration of the organisms in the tissues is sufficient to make a diagnosis.

**TOXOPLASMOSIS**

This condition is caused by a protozoan and research and experience has shown that it is worldwide in distribution and affects practically all animals and man and has been found in practically every organ. The organism may be present in subclinical or carrier states and it is known to be transmitted in utero in both animals and man. There are several seriological tests which have been devised to detect the presence of the disease in both man and animals.

*Diagnosis.* The organism is described as being found both free, Figure 2e, in macrophages and in cyst like structures in the various organs and lymph nodes. They are frequently, but not always, associated with necrosis and various inflammatory cells such as neutrophiles, macrophages and lymphocytes. In smears the organism usually appears as a crescent form. Diagnosis is usually made by demonstrating the organism in the tissues. If this fails, then mouse inoculation intraperitoneally using infected tissue is necessary. After each mouse passage a smear is made of the peritoneal fluid.

**COCCIDIOIDOMYCOSIS**

This condition is caused by a fungus which lives in the soil and inhalation of spores may cause the disease. It is worldwide in distribution and encountered in a very wide range of wild and domestic animals. Southwestern United States has the highest incidence in our country.

*Diagnosis.* The lesions resembles a tuberculous granuloma, grossly and microscopically, and occurs in the lungs, liver, spleen, bone marrow and lymph nodes. There are several phases of the organism and each attracts a certain type cell. The endospore when escaping from the large capsule, or spore case, Figure 2d, attracts neutrophiles and then as the endospores mature into spherules, macrophages and finally epitheloid cells are present. The cycle then repeats. A diagnosis is easily made if the organisms can be found. They are readily demonstrated by ordinary stains and do not show buds as does the blastomycetes or the cryptococcic organisms.
CRYPTOCOCCOSIS

This condition is caused by a yeast-like fungus and it has been reported occurring in both animals and man.

Diagnosis. The organism produces a granulomatous nodule and the organisms are thick-walled spherules and many exhibit a single bud. They are easily recognized by hematoxylin and eosin or fungal stains, Figure 2f.

B. BLOOD AND BLOOD FORMING ORGANS

Diseases of the blood and the blood forming organs are usually difficult diagnostic problems. This is true of those diseases that are functional in nature and others where the morbid anatomy is masked by the confusing heterogeneity of the hematopoietic tissue examined.

COAGULATION DEFECTS

Alterations or deficiencies in blood coagulation are of occasional occurrence. Deficiency in the coagulation of blood may result in fatal hemorrhage or contribute to the demise of the animal. Platelet deficiencies usually are associated with a prolongation of the bleeding time. Bleeding from the natural body openings are common. Petechial hemorrhages of the mucous membranes are usually found particularly early in the course of the disease. Diffuse and massive hemorrhages into the subcutaneous tissue of the body do occur.

Deficiency in thromboplastin factors or the conversion of prothrombin to thrombin leads to the prolongation of the clotting time. The associated bleeding is usually more severe and massive. Hematoma formation is more likely than with a platelet deficiency. Ecchymotic and petechial hemorrhages of mucosal and serosal surfaces may be found.

Platelet deficiencies resulting in purpura do occur. A decrease in production of platelets as in aplastic anemia or hypoplasia of the bone marrow may result from the toxic effects of drugs. The dog is unusually sensitive to estrogens, particularly estradiol (20). The use of this drug in the treatment of anal gland adenomas should be followed with hematologic studies, particularly white blood cell and platelet counts at weekly intervals. Hypoplasia of the marrow may also result from chronic inflammatory processes and neoplasias involving the bone marrow space.

Hemophilia, a deficiency of thromboplastin factors, occurs in certain breeds of dogs (21). The disease is hereditary and associated with the presence of a sex-linked recessive gene. The prolonged coagulation is due to a deficiency in the thromboplastin factors necessary to convert prothrombin to thrombin.

The interference with the conversion of prothrombin to thrombin or hypoprothrombinemia does occur. Dicumarol or warfarin poisoning interferes with the production of prothrombin. The production of an anti-thrombin agent, heparin, in the mast cell sarcoma or basophilic myelogenous leukemia may lead to extensive hemorrhagic lesions.
Coagulation defects are more readily evaluated in the living animal. The diagnostician, if presented with a living animal may obtain valuable information on the nature of the disease which may not be obtained at necropsy. It is imperative to complete the examination of the coagulation system before necropsy of the animal where a hemorrhagic disease is suspected. The simple tests that are extremely helpful are: 1. coagulation time; 2. prothrombin time; 3. platelet count; and 4. a complete blood count. Tissue sections of sternal bone marrow may be used to detect the hypoplastic bone marrow.

**DISEASES OF THE BONE MARROW**

Primary diseases of the bone marrow are not common. Hypoplasia or aplasia of the bone marrow may result from physical or chemical injury. Total body irradiation with gamma, X-rays or neutrons interferes with hematopoiesis and results in hypoplasia to aplasia of the bone marrow. The resultant leucopenia, thrombopenia and anemia contribute to the fatal outcome of the disease. Figure 3 shows the effect of total body irradiation on the peripheral blood elements of a dog receiving a lethal dose of X-irradiation.

![Graph showing effects of X-irradiation](image-url)

**Figure 3.** Shows the peripheral blood cell and body temperature responses in an adult male dog receiving 420r of total body X-irradiation as measured free in air at the proximal skin surface.
Hyperplasia of the bone marrow occurs secondary to many diseases. Erythroblastic hyperplasia of the bone marrow occurs in and is a reaction to most anemic conditions. Myeloid hyperplasia occurs in chronic inflammatory conditions and is commensurate with the type and degree of stimulation.

Neoplasia of the hematopoietic tissue in the bone marrow is not common in the dog. Primary neoplasms or myelogenous leukemia have been studied at the College of Veterinary Medicine, two of which were predominately basophilic, one megakaryocytic and three neutrophilic. Necropsy findings in myelogenous leukemia revealed hepatomegaly and splenomegaly in most cases. The kidneys are usually invaded with myeloid cells. In some cases the lymph nodes are enlarged and the gross findings are indistinguishable from malignant lymphoma. Imprint smears used in conjunction with sectioned material aids in more precise identification of the cell type involved.

Complete replacement of bone marrow by lymphoid tissue in malignant lymphoma occurs. Metastasis of other neoplasms may be found locally and on occasion to widely spread foci.

Osteomyelitis is of common occurrence. The infection is usually pyogenic with spread from a local wound. The process is confined locally and usually to a single bone.

DISEASES OF THE SPLEEN

Primary diseases of the spleen in dogs are relatively uncommon. Acute splenic congestion is commonly seen at necropsy in dogs receiving barbiturates. The spleen is turgid and bluish-black in color with a mottled surface. On section, there is marked congestion and normal splenic elements are scattered. Similar findings are found in passive congestion associated with right heart failure.

Myeloid and erythroid metaplasia of the spleen is found secondary to many diseases. The spleen may be enlarged. On cut surface the red pulp is of the consistency of raspberry jam. The sinuses contain small quantities of blood. The oozing of blood on cut surface is minimum in comparison to the acutely congested spleen. Myeloid metaplasia is secondary to chronic inflammatory diseases and is a frequent microscopic finding. Erythroid metaplasia is frequently seen in chronic regenerative anemias.

Nodular lymphoid hyperplasia is commonly seen in older dogs. The lesions are multiple and raised and consist of masses of lymphoid cells. No significance may be attached other than normal hyperplasia and this lesion may be associated with senile atrophy of lymphoid tissue in general. Splenic atrophy is common in older dogs. The spleen is diminished in size. The white pulp is usually decreased in amount.

Traumatic injury to the spleen and rupture with fatal hemorrhage is quite common. Splenic hematoma of massive size to five kilograms or more are of frequent occurrence. The hematoma may be located in any part of the spleen. Hematoma may be mistaken for neoplastic enlargement.
Primary neoplasms of the spleen do occur. Hemangioendothelioma and fibrosarcomas are the most common. The tumor masses may be multiple and variable in size. Metastasis of the hemangioendothelioma is frequently observed. Occasionally splenomegaly is the principal and only gross lesion of malignant lymphoma in dogs. Myelogenous leukemia usually causes marked splenomegaly. Histologically myeloid metaplasia may be confused with myelogenous leukemia. Diagnosis from section material may be difficult. Smears or tissue imprints stained with Wright’s stain are helpful in cell type identification. Metastatic neoplasia to the spleen is less frequent than that to the lungs, liver and kidney.

Functional disturbances of the spleen may be of clinical importance and extremely difficult to evaluate at necropsy. The disturbance in man is termed by some, hypersplenism. Thrombopenia, neutropenia and a hemolytic anemia singly or in combination as a pancytopenia may occur. Response in man to splenectomy, although variable, is used in treatment along with steroid therapy in recent years. Magrane, Magrane and Ross (23) report on a case of the thrombocytic purpura in a dog. It is possible that some of the unexplained hemolytic anemias commonly seen in dogs may be associated with a hyperactivity of the spleen. Diagnosis at necropsy will usually be given as thrombopenia or hemolytic anemia of unknown etiology.

**LYMPH NODE**

Lymphadenopathy is of common occurrence in the dog. Benign hyperplasia with hypertrophy may be regional or generalized and associated with local or systemic chronic inflammatory processes. Generalized lymph node enlargement associated with chronic dermatoses may be confused with canine lymphoma. Histologically the normal architecture of the lymph node is apparent. The follicular areas may be prominent. Developing plasma cells may be readily found, particularly near the cortical-medullary area. Areas of myeloid metaplasia may be found in such lymph nodes.

Acute lymphadenitis is seen in acute systemic diseases. The lymph nodes are enlarged due to a combination of exudation and proliferation of reticuloendothelial and lymphoid cells. The nodes are enlarged, edematous and bulge on cut surface. Hemorrhages into the subcortical sinuses and medullary are commonly observed. The regional lymph nodes are frequently involved in chronic granulomatous diseases. Diagnosis is best made on histopathological examination.

Primary neoplasia of lymphoid tissue, canine lymphoma, is of frequent occurrence. The disease occurs in all age groups with the greatest incidence in the four year and older age group (24). The typical case is presented with bilateral and symmetrical enlargements of all internal lymph nodes. Emaciation is usually marked. In the younger age groups single or regional nodal enlargement particularly of the internal nodes may be found. The liver, spleen and bone marrow are frequently involved. A review of the surgical file in the Division of Pathology for the past five years reveals 53 cases of canine lymphoma and 12 cases of the transmissible venereal tumor.
The transmissible venereal tumor, histiocytoma or transmissible lymphosarcoma, a readily transmitted tumor, may be mistaken for canine lymphoma, although it fulfills most criteria for a malignant tumor, it frequently disappears spontaneously. Diagnosis is best made from sectioned material and from the location of the primary tumor which is usually associated in close proximity to the genital mucosal surfaces or may be found about the mouth or nose. Imprint smears stained with Wright's stain may be helpful in distinguishing this disease from the mastocytoma.

Mastocytoma of the skin is of frequent occurrence. The tumor is most likely to be located on the posterior part of the animals body. The growth causes thickening of the dermis or is bulging and frequently ulcerated. Metastases are common. The tumor is locally malignant and frequently reoccurs after removal. Nielsen (25), in 100 cases found no metastasis without a primary tumor located in the skin. In diagnosis by tissue section, differentiation from the histocytoma, lymphoma and the less frequent myelocytoma may prove difficult. Tissue imprints made at necropsy and stained with the common blood stains will frequently make the diagnosis of the mastocytoma simple.

Secondary neoplasia to regional lymph nodes is frequent. Metastases by lymph channels, blood stream or extension occurs with many neoplasms.

REFERENCES

SOME ASPECTS OF THE DIAGNOSIS OF DISEASE IN WILDLIFE

CARLTON M. HERMAN*

Laurel, Maryland

The purpose of this presentation is to discuss some of the problems of disease diagnosis in wildlife. In a broad sense, wildlife could mean fish and even the invertebrates, but since in my official capacities I am concerned chiefly with the higher vertebrates, I will confine my remarks to problems involving wild mammals and birds.

There are many individuals and agencies engaged to some extent in studies on wildlife diseases, but diagnostic services are far from adequate. I have recently reviewed the current status of the field of wildlife diseases, in a paper published in the Bulletin of Medical Research (Herman, 1959), and pointed out the challenge it offers. The wide scope of interest in wild animal diseases ranges from pure biology and conservation to the increasingly important zoonoses and public health aspects.

Let us look at the diagnostic services available and the possibilities of improving such services in the future. The Federal conservation agency was aware of so-called western duck sickness (botulism) and lead poisoning early in the present century, but it was not until 1932 that a disease section was established in the Bureau of Biological Survey of the United States Department of Agriculture. Some of the activities of this section were devoted to studies of botulism and some work was done on diagnosis (Quortrup and Shillinger, 1941). However, most effort was applied to studies of captive species such as the domestic rabbit, fox, and mink, which were of particular interest to the fur farm industries. Just prior to World War II, the Biological Survey and the Bureau of Fisheries of the Department of Commerce were transferred to the Department of the Interior and redesignated the Fish and Wildlife Service. In the process, responsibility for studies of disease in captive furbearers was retained by the Department of Agriculture, thus leaving little disease research in the wildlife agency. Since 1950, efforts have been made to increase research on diseases of wild animals. Currently, work in the field is being done at two stations of the Branch of Wildlife Research of the Bureau of Sport Fisheries and Wildlife. One is the Patuxent Wildlife Research Center in Laurel, Maryland; the other is the Bear River National Wildlife Refuge in Utah, under jurisdiction of the Denver Wildlife Research Center. At both of these stations, the chief emphasis is on research, with only limited effort applied to diagnostic needs.

Only a very few of the State conservation agencies support laboratories where diagnostic procedures are performed. California, New York, Wis-

* United States Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland.
Wisconsin, Michigan, and Wyoming have been most active. Several other States have had personnel assigned intermittently to some diagnostic work or to study of specific problems involving disease. A few States have solicited the collaboration of Universities, Veterinary Departments, Agriculture Experiment Stations, and others, when specific losses demanded that something be done. Thus, such institutions as the veterinary section of the University of Minnesota have made extensive contributions. At present, New Jersey is working closely with Dr. Richard Shope of the Rockefeller Institute for Medical Research in a study of a virus disease of deer.

Many of the activities of the above mentioned States, as well as some work in a few additional States, are supported in part by Federal funds. Under authority of the Pittman-Robertson Act of the United States Congress, matching funds are provided for State wildlife activities.

The United States Public Health Service also is engaged in studies of disease of wildlife. These efforts are chiefly under the jurisdiction of the Communicable Disease Center and are studies of specific zoonoses, for example: rabies, leptospirosis, and encephalitis. The various stations and personnel involved in such studies are concerned chiefly with determining the extent to which specific agents occur in wildlife and the relationships of such findings to disease in man or domesticated animals. They have little or no time to consider general diagnosis beyond the specific disease they are studying.

Other agencies, particularly the Department of Defense and various University Schools of Public Health and State Health Departments, as well as some veterinarians, are involved in similar investigations and have the same limitations. Leads to important new information have had to be ignored because of the dictates of a full program in other directions. A good case in point can be cited from my own experience. As you all know, when an animal suspected of having rabies is obtained, the animal or its brain is submitted to specific State or public health laboratories for diagnosis. In Maryland a few years ago, the enforcement personnel of the conservation agency observed many raccoons with clinical signs suggestive of rabies. However, the State and Federal laboratories reported these specimens negative for rabies. What then was the cause? The rabies diagnostic services were not in a position to solve this problem. They had other duties absorbing all their time. When the problem was presented to me, I solicited the collaboration of Drs. Lawrence Kilham and Robert Habermann of the National Institutes of Health in Bethesda, Maryland, and we were able to demonstrate a high incidence of distemper (Kilham, Habermann, and Herman, 1956; Habermann, Herman and Williams, 1958). Since then, many investigators in other parts of the country have made similar diagnoses in both foxes and raccoons. The State Health Department of Florida has suggested that distemper can cause sufficient losses in the fox population to have a bearing on the spread of wildlife rabies.

The United States Fish and Wildlife Service recognizes its responsibility toward diagnosis of disease losses in wildlife. Like other agencies, it must
operate within a given budget. The staff for disease research has increased somewhat in recent years and the growth can be expected to continue in the years ahead. In fact, a new laboratory is under construction at the Patuxent Center and will be ready for occupancy in 1962. It will provide space and facilities for more than twice our present staff. However, this Federal agency can never hope to solve all the diagnostic problems that arise. The problems are too numerous and too extensive to ever dream of such an achievement.

All diagnosticians are aware of the requirements for examination of a specimen that will yield proper classification of an etiologic agent or disease syndrome. The closer a diagnostician is to the source of a carcass, the more adequate will be his specimen for the dissections and tests he must perform. To ship a dead animal half way across our country and expect a laboratory diagnostian to answer all questions is ridiculous. Whether the specimen is shipped frozen or fixed in preservative, complete study is no longer possible. We often still can recognize gross parasites and even some types of tissue damage, but isolation of specific bacterial or viral agents or study of histological tissue changes becomes most difficult if not entirely impossible. An increase in local interest by qualified animal disease diagnosticians backed by adequate laboratory staff and facilities is much needed.

One way to partly combat the difficulty of obtaining fresh specimens is the establishment of regional laboratories. One such laboratory has been established by the cooperation of a number of southeastern State conservation agencies. They have pooled financial resources and established the Southeastern Deer Disease Cooperative Laboratory at the University of Georgia School of Veterinary Medicine, under the direction of Dr. Frank Hayes. With the assistance of both post- and pre-doctoral personnel and a few undergraduate students, this group has been investigating possible disease outbreaks, primarily in deer but also in other species. They also are making surveys to determine the incidence of some of the entities they have recognized. Although local groups in other regions have discussed the development of other such units, none has yet been established. However, a few such regional laboratories cannot hope to begin to meet the needs confronting us.

As a conservation measure we must know the impact of disease losses on wild animal species and ultimately develop control measures where survival is threatened. Further, we must strive to maintain our wildlife in as healthy a condition as possible.

Let us also look at the problem from aspects other than conservation. Wildlife, with its habitat and other relationships, provides an ideal laboratory for study of the role of disease in natural population fluctuations. A classic example already has been demonstrated in the basic studies being conducted on myxomatosis in rabbits in Australia, with attenuation of virulence, development of resistance, and approach of a balance between the virus and its host.

Reduced to its simplest terms, the theoretical population growth can be pictured as an ascending curve that is increasingly steep (Fig. 1). At some point this curve will approach an ascending straight line above X. Since this
is unattainable, at some point the population will crash, level off, or gradually decline. For any animal species, the curve actually would be much more complex than the simple diagram shown here, so that there would be many minor fluctuations or deviations. In essence, this curve repeats itself in cyclic fluctuations of animals, reaching the apex in years of peak population. Sometimes these cyclic peaks can be predicted with surprising accuracy; a seven year cycle may be recognized for one species, perhaps an eleven year cycle for another, and longer or shorten cycles for still others. Many hypotheses have been expounded to explain these cyclic phenomena, ranging from sun spots to disease. Because disease may contribute the biological stress that causes population declines or fluctuations, it is important to understand the ecological factors that affect the occurrence and incidence of disease. We may seek to achieve, through management, a population level that suits our interests. For example, many public health agencies believe the way to control rabies is to keep the population of foxes (or other species involved) at a low enough level that the disease is not transferred to man or his domestic livestock.

![Diagram of theoretical population growth potential.](image-url)
In a recent review of three books on population, Coale (1961) pointed out that before about 1650 A.D. the average time required for the population of the world to double was more than 10,000 years. Subsequently, the world population doubled in less than 200 years, then it doubled again in about 90 years, and at present the population is increasing at a rate that will result in doubling in 35 years. We are approaching the top of the curve in Figure 1. Will man's technology prove adequate to provide a means for leveling the curve before the catastrophe of a crash? It is my belief that the study of wildlife diseases and other ecological factors might contribute to a better understanding of the factors influencing this growth curve and development of possible management mechanisms that could be used for control of our human population.

Hull (1955) has reviewed the diseases of animals transmissible to man. The World Health Organization of the United Nations recognizes over 100 such diseases. Awareness of this fact throughout the world has led to establishment of several laboratories specifically for study of this problem. The most recently established organization of this kind is the Zoonoses Institute in the School of Veterinary Medicine at the University of Illinois. As pointed out earlier, many public health agencies are actively engaged in this field. However, I would like to emphasize again that the study or survey of only the specific zoonotic agents is but a small part of the problem. Other diseases could play an important part. A certain population density of wildlife is necessary for transmission of some diseases to other wildlife, man, or domestic animals. It is necessary for us to determine the density of the population which provides this potential hazard level. This is perhaps most important in relation to both the problems of zoonoses and problems of survival or health in the wildlife species. For example, how many foxes are necessary in a given area before they become a potential hazard in the transmission of rabies? Or to what extent must we reduce a fox population during a rabies outbreak to remove this hazard? Secondary diseases may play an important part in regulating populations by keeping them in balance below the potential hazard density for transmission of a primary disease such as rabies.

The invitation to present this paper was extended to me by your chairman with a view to pointing out problems that could be of mutual interest to your organization, the Conference of Veterinary Laboratory Diagnosticians, and the Wildlife Disease Association. To have devoted this presentation to a review of diseases known to occur in wildlife or to have reported specific losses or special techniques of diagnosis, would not have left me sufficient time to discuss the problems I have outlined. I can assure you however, that the problems of diseases in wildlife are as extensive as those of man or the various laboratory or domestic animals with which you regularly occupy your time; and the diagnostic laboratory techniques are much the same. The main difference to the technician is the additional problem of obtaining adequate specimens or samples under often adverse field conditions; or trying to develop adequate procedures for getting field specimens to the
laboratory in usable condition. I have attempted to picture for you the state of diagnostic services for wildlife disease, emphasizing that there is room for, and need for, much more work in this field. I have very briefly pointed out the justification for work by many biomedical and parabiomedical personnel as well as by personnel specifically concerned with conservation. I hope you will have opportunity to become more deeply involved in some of these problems. I hope I have also inspired some interest among you.

In conclusion I wish to point out that diagnostic services can serve two purposes: 1) they can explain the reason for direct losses; and 2) they can serve to emphasize research priorities. For efficient use of diagnostic survey data, it is necessary to have a mechanism for coordination and dispersal of information. There already is some exchange of information, chiefly through newsletters, particularly on specific zoonotic diseases. Most of these newsletters are developed and circulated by government agencies or specific organizations of personnel involved. A clearing house is needed for dissemination of diagnostic data on all wildlife diseases, both to coordinate the data and to stimulate further interest and study. The Wildlife Disease Association currently issues a newsletter three or four times a year that contains occasional case reports. As yet, however, there has been no attempt to coordinate diagnostic findings. Perhaps the development of a system for exchange of information of mutual interest could be discussed jointly by members of both our organizations.

REFERENCES

The National Animal Disease Laboratory is organized into two main areas of work—the regulatory laboratory and the basic research area. The laboratory is directed by Dr. W. A. Hagan and serviced by a service staff consisting of Central Services, Engineering and Plant Management, Administration, and Animal Quarantine.

The Central Service area is responsible for supplying common service items such as media, tissue culture, chemicals and other non-expendable supplies. Services such as glassware washing and laundry are also furnished by this area.

The Engineering and Plant Management area is responsible for maintenance and repairs of the physical plant.

The Administrative staff handles procurement, fiscal and other administrative matters.

The Animal Quarantine area supplies the entire laboratory with animals for research, biologics evaluation and diagnostic purposes. This area also supplies items such as red blood cells, sera, and animal tissues.

Diagnostic Services and Biologics Control are part of the Regulatory Laboratories, under the direction of Dr. C. D. Van Houweling, Assistant Director for Regulatory Laboratories. The Biologics Control area is responsible for the laboratory evaluation of commercially produced biologics and the maintenance and development of certain standards for their production. Diagnostic Services furnishes the Animal Disease Eradication Division with laboratory and field diagnostic and epidemiologic consultation.

There are approximately 375 people employed at NADL. Of these approximately 43 work directly in Diagnostic Services. Of the 43, 18 are professional employees consisting of bacteriologists, chemists and veterinarians. The remaining 25 are technicians, secretaries, and animal caretakers.

Diagnostic Services receives its money for operation from appropriations made to the Animal Disease Eradication Division in Washington. The primary program is designed to augment and supplement the programs of the Animal Disease Eradication Division. There is no specific appropriation and no funds for assistance to State Diagnostic Laboratories, except in those diseases which are sufficiently related to ADE programs as to warrant the laboratory assistance. In these instances money appropriated for these diseases such as tuberculosis or brucellosis is provided for the operation of the laboratory.

* Doctor Wedman is Chief Veterinarian, Diagnostic Services, Animal Disease Eradication Division, National Animal Disease Laboratory, Ames, Iowa.
Assistance can be given to State Diagnostic Laboratories in the isolation and typing of Brucella spp., Mycobacterium spp. and Bacillus anthracis, the examination of specimens from suspected vesicular diseases, certain pathology reference examinations, such as scrapie and tuberculosis. Diagnostic Services can supply laboratory and field diagnostic assistance when there is need to differentially diagnose a condition from foreign exotic diseases. In addition to these services approximately 1,000,000 ml. of Brucella antigen will be produced this year. The testing and evaluation of Brucella Strain 19 vaccine and tuberculin which are used in official ADE programs are also a responsibility of this area. A limited amount of work in leptospirosis is also conducted. A project to aid in a survey of the incidence of swine brucellosis in certain parts of the country has just been initiated. In addition a hog cholera eradication program is anticipated and it is felt that the laboratory will be offering consultant and laboratory reference assistance.

One of the major laboratory functions of Diagnostic Services at NADL is the training of Federal and State employees in the diagnosis of diseases for which A.D.E. has responsibility. Among these are hog cholera, brucellosis, tuberculosis, and the differential diagnosis of diseases which are similar to foreign exotic diseases.

There may be other reference services which State Diagnostic Laboratories would like to have made available. The laboratory would like to be able to render more of these services where desired. However, your attention is again called to the present basis for the budget in Diagnostic Services. As outlined before, funds are not directly available for assisting State Diagnostic Laboratories. This limits the amount of reference service which can be offered to the State laboratories. If there are other services needed by the State Diagnostic Laboratories, it is suggested that this matter be discussed with State officials such as State veterinarians, directors, and secretaries of agriculture. If it is agreed that such services are necessary, these officials can appeal to the Department and Congress for appropriations specifically for this purpose.

for which ADE has responsibility. Among these are hog cholera, brucellosis, appropriations for assisting State Diagnostic Laboratories, the number of such consultant and reference services to State Diagnostic Laboratories will have to be quite limited and will be second in priority to the other laboratory functions related directly to Division programs.
COMMENTS ON THE LABORATORY DIAGNOSIS OF LEPTOSPIROSIS IN DOMESTIC ANIMALS WITH AN OUTLINE OF SOME PROCEDURES

EARL E. ROTH, W. V. ADAMS, BETTY GREER, G. E. SANFORD, KAY NEWMAN, MARY MOORE.

Baton Rouge, Louisiana

INTRODUCTION

The laboratory methods available for use as aids in the diagnosis of leptospirosis in domestic animals can arbitrarily be divided into methods that simply demonstrate the organism (demonstrable), methods that reveal the presence of antibodies (serological), and methods involving isolation of the organism (bacteriological). There is no ideal diagnostic tool among them that is applicable in all situations. This is not entirely due to inherent inadequacies of the diagnostic procedures, but is more properly due to the basic features of leptospiral infections. These features must be kept in mind in order to properly apply and interpret the various laboratory tests.

Before proceeding further, it appears appropriate to review briefly the pathogenesis of bovine leptospirosis caused by L. pomona. Leptospiremia generally occurs from the fourth through the eighth day after infection and precedes the febrile stage by several days, but they both terminate about the same time. Antibodies normally appear about the 10th day, but may appear as early as the fifth day and as late as the 15th day. They reach their peak during the second and third week and may persist at a moderate level for several months to several years. Some individuals retain a high level of antibodies for several years. Anemia, icterus, and hemoglobinuria when present occur between the seventh and 14th day following infection. Pregnant cows that become infected during the last trimester may abort three to four weeks after infection and, of course, are serologically positive when they abort. Leptospires generally appear in the urine about the 11th day and may persist for periods up to three months. It is just as important to realize that numerous infections of L. pomona are not manifested clinically. Temporary gross and microscopic lesions of the kidney can be demonstrated in some of these animals if they are killed and examined within a short time after infection.

From Department of Veterinary Science, Agricultural Experiment Station, Louisiana State University.

The authors wish to acknowledge the assistance of Miss Kay Ringer and the guidance of Dr. W. T. Oglesby, Head, Department of Veterinary Science. Studies on leptospirosis at this station have been augmented by funds received under Research Grant E2348R-1 from the National Institutes of Health, Division of Allergy and Infectious Diseases.

520
DEMONSTRATION METHODS

Let us begin our discussion of laboratory methods by considering the procedures that merely demonstrate the organism. Leptospires are not easily stained by the usual dyes; and therefore, no simple staining procedure can be recommended.

Leptospires may on occasion be demonstrated in body fluids and tissues by darkfield examination performed at the appropriate time. It is seldom possible to find organisms in the blood of cattle, swine, or dogs. They may sometimes be found in the urine of recovered animals and in the fresh tissue suspensions of fatal cases. Leptospires can be seen regularly by darkfield microscopy in the blood and tissue suspensions of infected laboratory animals. This is particularly true if the leptospiral serotype is relatively pathogenic for the laboratory animal. Although darkfield microscopy is an indispensable tool in leptospiral investigations, it is of little value in the immediate diagnosis of leptospirosis. Leptospires are difficult to detect and are easily confused with “pseudo spirochetes” which are filaments left by degenerative processes. A diagnosis of leptospirosis based on darkfield examination should only be tentative and always supported with additional data.

The demonstration of leptospires in tissues of fatal cases, in aborted fetuses, and in placental tissues can be accomplished by the appropriate application of silver stains. The Warthin-Starry method as modified by Bridges (1), or Levaditi’s method are most commonly employed. Special precautions are necessary to obtain reliable results. It must be emphasized that no differentiation of serotypes can be made in acute fatal cases. Positive serological results of herd associates augments positive microscopic findings. Leptospiral organisms present in renal tissue only may reflect subclinical infection and be unrelated to the cause of death. In fatal leptospirosis, lesions are present in other organs, particularly the liver. The histological demonstration of leptospires in the tissues of an aborted fetus, along with the finding of a high level of antibodies in the dam’s serum, not only warrants a diagnosis of leptospiral abortion, but also gives highly suggestive evidence of the serotype involved. The concurrent application of histopathological and serological methods reveals valuable information upon which to base a diagnosis of leptospirosis if in accord with the clinical observations.

The fluorescent antibody procedure may prove of value in demonstrating leptospires in urine and tissue of infected animals (2, 3, 4, 5). Doctor G. Maestrone* through personal communication, advises that leptospires may be demonstrated in formalin-fixed tissues with the fluorescent antibody technique.

SEROLOGICAL METHODS

A number of serological tests have been developed to detect antibodies incited by leptospires. The complement fixation test (6) and the hemolytic test (7, 8) tend to be genus specific. They are not practical for small

* Margaret Caspary Institute for Veterinary Research, 350 Lafayette St., New York 12, N. Y.
laboratories and hence are not widely used. A modification of the hemolytic test shows some promise in that it may correlate with the carrier condition (9, 10).

Agglutination tests, of which there are microscopic and macroscopic versions, are most commonly employed to detect leptospiral antibodies. The microscopic test can be employed using either living or formalin killed organisms as antigen. The microscopic agglutination test using live antigen is considered to be the reference test and is preferred by the author. Macroscopic tests (11, 12, 13, 14) have been developed and are being used in some laboratories. The most widely used are the Galton and Stoenner tests.

In leptospirosis, as in other diseases, serological tests have certain disadvantages and can be wrongly interpreted. Moreover, in leptospirosis it is not advisable to attach an absolute value to the results obtained. Besides the variables inherent in the serological tests, the antibody response of the host to leptospiral infections is even more variable. The results must be considered case by case or herd by herd and evaluated in relation to all other data, particularly clinical observations.

The absence of antibodies during the first week of disease prevents an early diagnosis of leptospirosis by serological means. In individual animals the antibody titer remains low throughout the disease. Death may occur in calves before antibodies become demonstrable.

Positive serological results do not prove that the subject from which the serum was taken is actually suffering from leptospirosis. Some individuals retain agglutinating antibodies for several months to even years after infection. The author has observed high agglutinin titers in serums from cattle five years after natural infection with \( L. \) pomona.

When serological tests are interpreted in their proper perspective, they constitute a valuable laboratory aid in the diagnosis of leptospirosis. The demonstration of a significant rise in antibody titer affords excellent evidence of recent infection. Most of the time leptospirosis in cattle and swine is a herd problem. The serological results of a representative number of animals, when evaluated in the light of clinical and pathological findings, are most valuable in arriving at a diagnosis. Since the clinical signs of leptospirosis are also caused by other diseases, differential tests should be applied. Negative results for differential tests strengthens positive serological findings for leptospirosis.

**BACTERIOLOGICAL METHODS**

Isolation of leptospires, with their subsequent identification, affords the most conclusive proof of infection. The choice of tissue or body fluid to be cultured is dependent upon the stage of disease. Organisms can be recovered from a variety of tissues during the first week, but normally blood is examined from the living animal. If death ensues and tissues are obtained soon after death, leptospires can be isolated from the parenchymatous organs such as liver and spleen. Should death be delayed, as may be the case in dogs, the kidney would most likely contain leptospires.
Leptospires can be isolated from urine by direct culture into semisolid mediums. Several methods, namely aseptic collection of urine by bladder tap in dogs and extended dilutions can be used to avoid and overcome contamination (15, 16). We have combined the bladder tap technique and dilution of inoculum with excellent success. We have also isolated leptospires from naturally voided bovine urine by the dilution method. Strains of the hebdomadis serogroup \((L. \text{hardjo})\), which are most difficult to isolate, were recovered in pure culture by this method.

The indirect isolation of leptospires from tissue suspensions and body fluids through the use of laboratory animals such as guinea pigs, hamsters, chinchillas, and gerbils can be used with some success. None of the laboratory animals are equally susceptible to all serotypes of leptospires. Therefore, the choice of animal depends upon the serotype suspected. In the author’s opinion, properly executed bacteriological procedures are more sensitive than laboratory animals for the isolation of leptospires.

The direct or indirect isolation of leptospires is not beyond the realms of a diagnostic laboratory. Leptospires are not as difficult to cultivate as is generally believed. One would hesitate to suggest leptospiral procedures as a routine, but in many instances they are indicated and should be applied. If performed diligently, with attention to several details, the results will be most rewarding.

**SUMMARY**

As is evident from the preceding discussion, the diagnosis of leptospirosis in domestic animals cannot be made in the laboratory without the full cooperation of the clinicians. They should be encouraged to submit adequate clinical histories. The close cooperation of diagnostic laboratories and clinicians results in better practice of veterinary medicine.

**BRIEF OUTLINE OF SOME LABORATORY PROCEDURES**

I. **General Remarks:**

Since leptospires are more sensitive to changes in pH than the average bacterium, considerable attention must be given to the glassware used. Most laboratory washing powders are satisfactory, but those recommended for tissue culture work are suggested. All glassware should be thoroughly rinsed in water that does not impart an acid reaction. In some areas distilled water or tap water may be satisfactory whereas in some areas phosphate buffered water may be necessary.

Screw cap test tubes are suggested for cultural procedures because they retard dehydration of prepared mediums for several months; they are efficient in preventing unwanted contamination and are convenient.

Aluminum foil is an almost indispensable item. It can be used to wrap instruments, mortars and pestles, and many other items, in preparation for sterilization.
Pipettes are used in serological and bacteriological procedures. All pipettes should be plugged with cotton and sterilized before use. When handling living organisms, for safety reasons, pipettes should not be filled more than one-half full; nor should small bored pipettes be used. Two ml. or five ml. pipettes are most conveniently utilized.

For safety, Luer-Lok syringes are preferred for leptospiral procedures. A convenient way to sterilize them is to place them with needle attached in a plain test tube and wrap the top with brown paper. Scissors and thumb forceps are prepared similarly, except foil is used. It is highly desirable to have a laboratory equipped with ultraviolet germicidal lights. Such a room increases the efficiency of leptospiral procedures.

Sterile technique is essential in laboratory procedures, and strict attention must be given to even the smallest details. The success of leptospiral procedures begins in the washroom.

II. Sterilization of rabbit serum:

Mediums used to cultivate leptospires are normally enriched with rabbit serum.* Heat destroys the major growth promoting qualities and hence the serum must be sterilized by other means. Although other methods of sterilization are available, Seitz filtration is most widely used. Of the various Seitz filters† available the 100 ml. and 2,000 ml. pressure types are most useful. Compressed air is more convenient, but hand pumps may be used to affect filtration. The pH of the serum should be adjusted to 7.5 before filtering through an ST-3 asbestos pad. The smaller filter is fitted to a Salvarsan tube equipped with a tubing bell. The larger filter is attached with rubber tubing to a four-liter aspirator bottle with a tubing bell attached to the lower outlet. An air vent consisting of a cotton-plugged glass tube should be placed in the rubber stopper of either set up. The assembled apparatus is autoclaved before use. The serum is filtered with a minimum pressure of five to seven pounds. If the ST-3 pad stops flowing, it may be necessary to prefilter through an ST-1 pad. Usually one to two liters can be sterilized at one time. After filtration, the serum is tubed aseptically to sterile screwcap tubes of other suitable containers in convenient quantities depending upon the batch size of mediums prepared. In the procedures to follow, semisolid medium will be prepared in two-liter quantities and liquid medium will be prepared in three-liter amounts. Consequently 100 ml. aliquots are conveniently utilized. Pyrex milk dilution bottles are convenient for storing sterile rabbit serum at 4°C until needed. If the unfiltered rabbit serum contains a lot of precipitate, it may be advisable to prefilter through an ST-1 asbestos pad before final sterilization.

* Available from H. F. Pelphrey and Sons, Rogers, Arkansas.
† Republic Seitz Filter Corporation.
III. Preparation of mediums:

A. Liquid Stuart’s medium used in the production of antigen and as diluent in various procedures is easily prepared.

(1.) Dissolve 10.2 grams of Stuart’s Concentrate (Difco) in three liters of demineralized distilled water and add 300 ml. of prefiltered rabbit serum and 15 ml. of glycerin. After mixing thoroughly, adjust the pH to 7.5. Sterilize by filtering the completed medium through an ST-3 asbestos pad in the two-liter filter attached to a four-liter aspirator bottle with tubing bell attached. One pad will handle three liters of medium. In our laboratory the medium is prepared in 10-liter amounts and sterilized with a semi-commercial (model 200) Seitz filter. This method is preferred over method 2 which follows.

(2.) Dissolve 10.2 grams of Stuart’s Concentrate (Difco) in three liters of demineralized distilled water in a four-liter aspirator bottle fitted with a tubing attachment. Adjust pH to 7.5 and autoclave for 15 minutes at 121°C. After cooling below 50°C., add 300 ml. of sterilized rabbit serum.

In either method, the medium is dispensed in the same manner. In method 1, the tubing between the aspirator bottle and filter is clamped in two places and detached from the filter. Medium may be dispensed in 10-12 ml. amounts into sterile 20 x 125 mm. screw cap tubes, or in two-25 ml. amounts into sterile 25 x 150 mm. screw cap tubes. It can be tubed in different amounts as needed. Tighten caps well and determine sterility by incubation at 37°C. for 36 hours. Until routine is established, determine growth promoting qualities. An antigen strain such as L. pomona strain Pomona should produce a visible powdery growth in four days after three to four transfers made at 72-hour intervals using 10 percent inoculum.

B. Fletcher’s semisolid medium is used for direct isolation, maintenance of stock strains, and to produce inoculum for antiserum production. It is prepared by dissolving 5.5 grams of Fletcher’s base (Difco) in 2,000 ml. of demineralized distilled water by gentle boiling and constant stirring. Pour this basal solution into a four-liter aspirator bottle fitted with a tubing bell and autoclave at 121°C. for 15 minutes. Cool to 56°C. in a water bath and add 200 ml. of sterile rabbit serum (pH 7.5) that has been warmed to 56°C. Tube aseptically into 16 x 125 mm. screw cap tubes in nine-10 ml. amounts. For antiserum production, tube 15 ml. into 25 x 150 mm. screw cap tubes. Incubate at 36°C. for 36 hours to ascertain sterility. Until routine is established, determine growth-promoting qualities by inoculating three to four tubes with one to two drops of an antigen strain. Leptospiral growth should appear
as a ring about five to 10 mm. below the surface in 10-14 days. This is known as Dinger's phenomenon.

C. Semisolid Stuart's medium is used for the same purposes as Fletcher's medium. Some strains upon original isolation grow better in Stuart's semisolid medium, but later adapt to and grow well in Fletcher's medium. Both are used in our laboratory. To prepare, dissolve 6.8 grams of Stuart's basal concentrate (Difco) and six grams of nutrient agar (Difco) in two liters of demineralized distilled water by gentle boiling and frequent agitation. Follow the remaining steps as described under the preparation of Fletcher's medium.

D. The solid medium of Cox is prepared according to the published report (17). It can be used as an ancillary method for primary isolation of leptospires and for purification of contaminated strains.

E. The mediums described above should be stored in a cool place with the caps well secured and preferably in the dark if a long storage period is anticipated. The semisolid mediums will keep at least four months and probably longer. The liquid medium also keeps well, but the serum may precipitate after prolonged storage and cause inferior antigen for the microscopic agglutination test. The solid medium of Cox should be stored in the ice box and used before dehydration is apparent.

IV. Direct isolation of leptospires:

Numerous techniques for the isolation of leptospires have been advanced. In our laboratory we have used different mediums and procedures, but have adopted the procedures that follow. They may appear somewhat laborious, but more than 600 primary isolations have been made with these procedures. Each detail is included for a reason which will be stated.

(A.) Direct isolation from the blood of calves, swine, and dogs during the leptospiremia stage is accomplished with regularity in experimental infections. The laboratory diagnostician does not have as ample an opportunity to isolate leptospires because antibiotic treatment so frequently administered, not necessarily prevents, but certainly reduces the chance of isolation. Furthermore the laboratory diagnostician frequently does not see the animal whose malady he is to diagnose.

Blood for culture must be collected and handled aseptically. After shaving, cleansing, and disinfecting the appropriate site, two to three ml. of blood are removed and two to three drops should be inoculated into two tubes of Fletcher's and two tubes of Stuart's semisolid mediums. Reapply the disinfectant and with another syringe remove additional blood and inoculate
four tubes of medium as before with one drop of blood. Chances of isolation seem to be enhanced when small inoculums are used because inhibition is circumvented. The blood may be treated with anticoagulants (sodium oxalate 0.5 ml. of a one percent solution to five ml. of blood) and taken to the laboratory where mediums are inoculated. The duplicate sampling is advised to increase chances of obtaining a culture free of contamination. In either procedure a whole blood sample for serological studies should also be collected.

Incubate the cultures at 28-30 C. or at room temperature in a dark place. Examine at seven- to 14-day intervals by high power darkfield microscopy for evidence of leptospires. Some strains are detectable as early as five to seven days, whereas, the more fastidious leptospires may not be detectable until after 75 days. Generally they are present within six weeks.

(B.) Leptospires can also be isolated from tissue suspensions. The choice of tissue depends upon the stage of disease and the species involved. In acute cases a septicemia exists, but the tissues normally chosen for culture are liver and spleen. Heart blood, as well as pericardial fluid, may also reveal leptospires. If death is anticipated, as may be the case in laboratory animals, it is better to kill the animal and prepare cultures. Death in dogs may occur after a sub-acute to chronic course of disease and in this case leptospires may be present only in renal tissue and urine. In our example let us assume that we are examining liver or kidney. Perform the necropsy with regard for asepsis. Shave the abdomen of laboratory animals and in larger animals shave the line of incision. Reflect the skin back and with sterile instruments open the abdomen. With a new set of sterile instruments (forceps and scissors) remove a section of the liver or kidney in larger animals and the entire organ in smaller animals. Place the tissue in a sterile container (Petri dish) and immediately transport to the laboratory for cultural examination. The cultures may be prepared with or without extended dilution of the inoculum. In our laboratory, we have found the extended dilution method the more sensitive, however it is rather laborious and requires more effort. Most strains can be isolated by the simpler method using limited dilution of inoculum.

In the limited dilution method triturate the tissue with mortar and pestle and add Stuart’s liquid medium to give an approximate 10 percent suspension. With sterile cotton-plugged pipette (five ml.) or syringe with 20-gauge needle, inoculate two tubes of Fletcher’s and one tube of Stuart’s semisolid with five drops; repeat procedure using three drops of inoculum; repeat again using one drop of inoculum.
Thus six tubes of Fletcher’s and three tubes of Stuart’s medium have been inoculated. The three attempts are employed to increase chances of obtaining sterile cultures and the amount of inoculum is varied to reduce inhibition apparently caused by tissue, pH, or antibodies. Secure caps tightly and shake the tube gently to distribute inoculum. Incubate and examine as previously described.

In the extended dilution method as employed in our laboratory, the 10 percent tissue suspension is diluted serially through an additional 4 tubes thus resulting in dilutions of 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000. With separate pipettes, two tubes of Fletcher’s and two tubes of Stuart’s semisolid are inoculated with three drops of inoculum from each dilution.

(C.) Direct isolation from urine can be accomplished if contamination is overcome. Urine may be collected by bladder tap (15) and at necropsy using aseptic technique. The bladder tap method has been used successfully in dogs (16) and the author has used it successfully in several species of wild animals. Chances of isolation are lessened, but leptospires can be isolated from normally voided urine in the case of cattle. Regardless of collection, the cultural procedures are the same. Immediately after collection, dilute the urine 1:10 in Stuart’s liquid medium. If extremely acid, the Stuart’s medium will turn yellow due to the indicator (phenol red) present. If it does turn yellow, immediately dilute further to 1:100 by transposition of one ml. to another tube of Stuart’s medium. Although not proven, the author is of the opinion that the rabbit serum in the diluent protects the leptospires and enhances the chance of isolation. Transport the diluted urine to the laboratory without delay, and prepare additional serial 10-fold dilutions through 1:1,000,000 using a separate pipette or syringe for each dilution. As the dilutions are being prepared, inoculate two tubes of Fletcher’s and two tubes of Stuart’s medium with three drops from each dilution. Label, incubate, and examine as described. We have successfully isolated leptospires from naturally voided bovine urine by this method. On a herd basis 5-10 animals should be cultured.

(D.) The solid medium of Cox may be used as an ancillary method for the isolation of leptospires from tissues, particularly kidney suspensions (18, 19). As used in our laboratory, three plates are inoculated with 0.1 ml. of 1:100, 1:1,000, and 1:10,000 dilutions of kidney suspensions prepared in liquid Stuart’s medium. The inoculum is distributed with a glass or wire spreader. The Petri dish is sealed with waterproof adhesive tape and incubated at 30 C. in an atmosphere of high humidity. Leptospire growth
may be colonial in nature or it may cover the plate imparting a hazy appearance. Growth may be evident as early as the seventh day and as late as the 30th day; it is best detected grossly by directing light from a microscope lamp through the plate obliquely. Suspected growth should be confirmed microscopically. We have occasionally isolated strains by this method where contamination prevented isolation in semisolid methods. Gross contamination cannot be overcome.

V. The indirect isolation of leptospires:

The indirect isolation of leptospires involves the use of laboratory animals such as guinea pigs, hamsters, gerbils, and chinchillas. Their efficiency varies according to their susceptibility and depends upon the leptospiral serotype and its relative pathogenicity. Young guinea pigs develop illness accompanied by icterus following inoculation with field strains of *L. icterohaemorrhagiae*. This serotype can easily be isolated from cardiac blood using methods already described. Guinea pigs are more refractory to other serotypes; however, young guinea pigs may be used successfully for the isolation of *L. pomona*. They may or may not show a temperature rise. Regardless, perform blood cultures on days four, six, and eight, inoculating two tubes of Fletcher's and two tubes of Stuart's with two to three drops of blood. On about the 21st day, kill the animals, collect a serum sample for serological tests, and also collect the kidney aseptically and prepare cultures as previously described. If indicated, inoculate another set of animals with 0.5 to one ml. of pooled 10 percent kidney suspension from the original set of animals. Two to three guinea pigs should be employed for each isolation attempt.

Weanling hamsters may be used successfully to isolate most strains of *L. canicola* and *L. icterohaemorrhagiae*, but they are not equally successful for isolating *L. pomona*. Each of the four animals should be inoculated with 0.25-0.75 ml. of tissue suspension or body fluid suspected of containing leptospires. Collect blood aseptically from the heart on post inoculation days four, six, and eight, and inoculate two tubes of Fletcher's and two tubes of Stuart's semisolid mediums with two to three drops of this blood. If the leptospiral serotype involved is highly pathogenic, organisms may be demonstrated by darkfield microscopy using high power. Some strains of *L. canicola* and *L. icterohaemorrhagiae* kill hamsters in seven to 10 days while most strains of *L. pomona* do not cause visible illness. If the animals survive, kill them 21 days later and prepare cultures from the kidney, and also inoculate additional animals with pooled 10 percent kidney suspension.

In our laboratory, field strains of *L. canicola*, *L. icterohaemorrhagiae*, and *L. ballum* have been isolated using the Mongolian gerbil (*Meriones unguiculatus*) as the laboratory animal. These serotypes
are easily isolated from the blood as described for hamsters. If any survive, kill, culture kidney, and inoculate new animals. The gerbil is not satisfactory for the isolation of *L. pomona*. Van der Hoeden has used another species of gerbil for the isolation of leptospires (20). Chinchillas have recently been recommended for the isolation of *L. pomona*, and *L. canicola* (21, 22). Most field strains of *L. pomona* cause illness characterized by jaundice and hemorrhage. *Leptospira pomona* is easily isolated from their blood from post inoculation days four to seven. Many chinchillas die following inoculation with field strains of *L. pomona*. The authors have found them highly satisfactory for the isolation of *L. pomona*.

VI. The microscopic agglutination test:

(A.) There are many versions of the microscopic agglutination test employing live antigen. Standardization is desirable, and consequently several reports of the Leptospirosis Committee of the United States Livestock Sanitary Association have dealt with this problem. The procedure suggested by this Committee for use in animal disease diagnostic laboratories is essentially as follows:

1. Using one ml. serological pipettes, prepare serum dilutions of 1:50, 1:500, 1:5,000, and 1:50,000 in phosphate-buffered saline (pH 7.4).
2. Mix an equal amount (0.1 ml. or 0.2 ml.) of each serum dilution with living antigen. The final dilutions now are 1:100, 1:1,000, 1:10,000, and 1:100,000.
3. An antigen control (equal amount of antigen and diluent) and positive control (antiserum diluted same as diagnostic serums) should be included.
4. Incubate for two hours at 37 C.
5. A darkfield microscope system providing a magnification of approximately X150 should be used to determine the results.
6. A drop from each dilution is examined and the reaction (agglutination and or clearing "lysis") judged percentage wise in regards to the antigen control. A 50 percent reaction should be regarded as positive.
7. Use type strains as antigens (See section VI C).

Several other details appear worthy of mention:

1. Dilutions are conveniently prepared in 13 x 100 mm. test tubes.
2. As the dilutions are being made, the desired amount to be mixed with antigen can be dispersed to tubes immediately behind the dilution tube.
3. Cornwall pipettes are convenient for dispensing diluent and antigen where a large number of tests are being performed.

4. A bacteriological loop may be used to place a drop from each dilution on a microscope slide in preparation for darkfield examination. No cover slips are used. Begin at the highest dilution (1:100,000); blot the loop between dilutions and wash in buffered saline between samples.

(B.) Leptospiral cultures when four to six days of age serve as antigens in the microscopic agglutination test. The desired serotypes are maintained by transfer in liquid Stuart's medium three times a week. Ten percent by volume of a three-day-old culture should be used to initiate cultures to be used as antigens. By regularly maintaining frequent transfers, suitable antigen and inoculum are always available. The small or large tube of medium as previously described, depending on requirements, is satisfactory. This procedure becomes routine in a very short while. Always examine for purity before use. Oblique light from a microscope lamp shows up leptospires as a powdery growth distributed throughout the medium. Most contaminants change the color of the medium from red to yellow because of acid formation. If leptospiral growth is more dense than desired, dilute with uninoculated Stuart's medium.

(C.) The serotypes recommended for use in the microscopic agglutination test depend upon the purpose of examination and the species being examined. For routine diagnostic laboratories where funds and personnel are limited, the following are suggested:

- Cattle  *L. pomona* strain Pomona.
- *L. hardjo* strain Hardjoprajitno.
- Swine  *L. pomona* strain Pomona.
- Dogs  *L. canicola*, strain Hond Utrecht.
- *L. icterohaemorrhagiae*, strain M-20.
- Horses  *L. pomona*, strain Pomona.

Other serotypes may be involved and if facilities, funds, and personnel are available, they may be included in the tests. Generally, it is advisable for the average laboratory to keep the test simple, and if necessary refer serums to laboratories conducting a broader spectrum of tests.

The authors (not engaged in diagnostic work) employ the microscopic agglutination test differently. A fourfold-dilution scheme is used for all serological studies except when doing the agglutinin-absorption test where the tenfold interlocking system of Wolff (25) is used. Separate pipettes are used to prepare
each dilution. In the fourfold scheme, 75 percent reaction is taken as the end point, whereas in the scheme of Wolff, a 25 percent reaction is taken as the end titer.

VII. Preparation of Antiserums:

Antileptospire serums are used as controls in serological tests and for identification of leptospiral isolates. High titered antiserums can be prepared by inoculating young mature rabbits with 10-day-old Fletcher's cultures. The Fletcher's cultures (15 ml. in 25 x 150 mm. tubes) are initiated by inoculating them with one ml. of an actively growing Stuart's liquid culture. The large tube allows a larger ring (Dinger's) of growth to form. One tube furnishes two ml. of inoculum which is removed from the top including the ring of growth. Inoculations of two, two, four, and six ml. are given via the ear vein at seven-day intervals. The rabbit is killed and the blood harvested seven days after the last inoculation. The serum, after separation from the blood cells, is mixed with an equal amount of glycerin and sterilized by Seitz filtration (ST-3 pad). Tube into sterile screw cap tubes and store at —20C. until needed.

VIII. Miscellaneous procedures:

(A.) A simple method for the preparation of phosphate-buffered saline is to dissolve with the aid of a magnetic stirrer 23.91 grams of sodium phosphate (dibasic anhydrous), 4.32 grams of potassium phosphate (primary monobasic) and 25.5 grams of sodium chloride in three liters of distilled water. Determine the pH, and if not near 7.4 adjust the amount of acid or basic phosphate accordingly. Bottle as desired and autoclave at 121 C. for 15 minutes.

(B.) Several methods may be employed to purify contaminated cultures of leptospires. These methods include: (1) The use of laboratory animals, (2) The use of solid medium where several plates are inoculated with diluted inoculum ($10^{-3}$ to $10^{-7}$) and (3) The use of Swinney Filters equipped with an ST-3 sterilizing pad.

(C.) The identification of newly isolated leptospires is based upon serological tests. Antigen is prepared for the unknown and is tested against known antiserums. The final identity is confirmed by agglutinin-absorption tests which should be performed in laboratories experienced with the test. The details of these procedures have been omitted from this paper.
COMMENTS ON DIAGNOSIS OF LEPTOSPIROSIS

GENERAL REFERENCES


SPECIFIC REFERENCES

HARD TISSUE LESIONS ASSOCIATED WITH MALNUTRITION

WILLIAM S. MONLUX, D.V.M., Ph.D.*

Ames, Iowa

The hard tissues (bone, cartilage, and teeth) are very sensitive to improper nutrition and react very quickly to even minor variations in the ration. Not only are these reactions distinct but often they appear early in the course of an illness. Frequently they are an indelible imprint of the experience and persist for the entire life of the animal.

There is a general tendency to regard hard tissue diseases as a manifestation of a deficiency of some substance in the ration. On the contrary, the concept of nutritional balance is the key to diagnosis and rational treatment of hard tissue diseases. The point which is usually overlooked is that an excess of any ingredient in the ration is just as important as a deficiency in the production of a nutritional disease. The same lesion may be produced by an excessive amount of a certain substance in the ration as will be produced by an inadequate amount.

Nutritional balance can best be illustrated with the teeter totter. If an excess of one substance appears in the ration then there must also be a corresponding increase in other ingredients if nutritional balance is to be maintained. The teeter totter moves up and down during the lives of our animals but as long as it continues to fluctuate constantly a nutritional balance will be maintained.

To this first factor, nutritional balance, let us add the second important factor in nutritional disease and that is rate of growth. This factor is especially important in the diseases of hard tissue, and is the key to success or failure in the production of lesions in hard tissue research. The faster the rate of growth the greater is the probability that a bone disease will appear if the nutritional balance is not correct. In contrast, a slow rate of growth decreases the likelihood that a hard tissue disease will develop. Therefore, it is the rate of growth which determines why a group of animals in one experiment or on one farm develops a bone disease while another group of animals in another experiment or on another farm does not develop the disease even though the ration, management, and environment are similar.

The second factor, rate of growth, is best illustrated by the height of the fulcrum in the diagrammatic teeter totter. If the fulcrum is in the center of the plane of balance and of the proper height, then normal fluctuation can occur. However, if the rate of growth is increased or rather the height of the fulcrum is greater than normal, then any variation in balance produces a greater excursion of the plane of balance and the teeter totter tips excessively to the side of the greater ration imbalance and a hard tissue disease is the

* Professor of Pathology, Iowa Veterinary Diagnostic Laboratory, Iowa State University.
result. A slow rate of growth can be depicted by lowering the height of the fulcrum which in turn reduces the possible magnitude of the excursion of the plane of balance. If the rate of growth is greatly reduced the fulcrum becomes so low that extensive excursions of the plane of balance are limited or impossible and therefore a hard tissue disease will probably not occur.

The third factor which exerts an influence on the hard tissues is the intrinsic causes of disease (genus, breed, race, family, color, sex, age, and idiosyncrasy) over which the individual himself has no control. Large breeds of dogs (Saint Bernard and Great Dane) have a greater incidence of bone disease than do the smaller breeds. The Filipino pony is less likely to develop bone disease as the result of a deficiency of phosphorus than is the Thoroughbred. Rickets in the human being occurs in the young and is more frequently observed in males than in females.

The importance of the intrinsic causes of disease in the production of hard tissue lesions can be illustrated by the position of the fulcrum along the plane of balance. If the intrinsic causes are compatible with normal growth then the diagrammatic fulcrum can be placed in the center of the plane of balance. However, if an intrinsic cause of disease exerts an incompatible influence on growth then the fulcrum is shifted to some position other than the center of balance. Then, unless a compensating adjustment is made in the ration, the probability exists that a hard tissue disease will result.

It is a fallacy to believe that each nutritional defect in the ration will produce a pathognomonic lesion. The entire basis of pathology is that tissue reacts in a limited number of ways and a large number of etiologic agents will produce an identical tissue reaction. As a result, instead of trying to attach pathognomonic importance to each hard tissue alteration, the causes of hard tissue disease should be arranged into groups which have similar lesions. Once the basic lesions of the group are recognized, it is then comparatively easy to decide the probable cause or causes of the existing hard tissue disease.

Lesion A. Excessive amounts of osteoid tissue produced by osteoblasts. The presence of excessive amounts of osteoid tissue indicates there is a normal or increased rate of osteoblastic activity together with an inadequate mineralization of the newly formed tissue. It is the type of reaction observed when inadequate amounts of vitamins D and minerals, especially calcium and phosphorus, are present in the ration. It represents the failure of the individual to mineralize the osteoid tissue thereby converting it into osseous tissue. This is the type of reaction observed in classical rickets in the human being, where failure of mineralization is attributed primarily to a deficiency of vitamins D.

Classical rickets occurs only in primates whose bone development is slow. In other species of animals, bone growth, at the time of birth, has already passed that period of development in which classical rickets can occur.

A form of rickets can be produced in the rat and chick. However, to accomplish this the vitamins D, calcium, phosphorus, and nutritive constituents of the ration must be present in very definite amounts, and a rapid
rate of growth must be obtained if excessive osteoid tissue is to be produced. The only reason a disease simulating classical rickets can be produced in the rat and chick is that the young of these animals have a comparatively poorly developed skeleton at birth and on an experimental ration, if they can be made to maintain a rapid rate of growth, they may develop an osteodystrophy.

The appearance of excessive amounts of osteoid tissue in other species of animals is seldom observed, and when it does appear it does so under very unusual conditions and involves only certain species of animals. A deficiency of vitamins D alone will probably not produce classical rickets in domesticated animals. To produce excessive amounts of osteoid tissue in domesticated animals there must also be a mineral imbalance and the animals during the experimental procedure must maintain a rapid rate of growth. If a rapid rate of growth cannot be maintained, osteoblastic activity is also suppressed and osteoid tissue is not produced in excess. Without excessive amounts of osteoid tissue a diagnosis of rickets cannot be made.

Lesion B. Excessive amounts of osteoid tissue produced by metaplasia of cartilage and connective tissue. Metaplasia of cartilage into osteoid tissue is frequently observed in bone disease and is the attempt of the tissues to compensate for the impaired osteoblastic endochondral ossification. It is the most frequent cause of excessive osteoid tissue in turkeys and chickens and is the lesion observed in the so-called rickets in poultry. It is not a diagnostic lesion for a deficiency of vitamins D in the ration. Instead, it is an indication of an osteopathy which may be due to a variety of causes. It is usually due to mineral imbalances (calcium, phosphorus, magnesium, and iron) in the ration and seldom, if ever, is due to a deficiency of vitamins D alone.

The white fibrous connective tissue in the bone marrow and periosteum, especially in the vicinity of epiphyseal plate, may undergo metaplasia and change into osteoid tissue. The connective tissue in this region becomes excessive when an osteopathy is present. This hyperplasia of connective tissue is the reaction of the bone to the stresses and strains placed upon it when it lacks the strength and rigidity required for normal body support and movement. It is especially prominent in the proximal ends of the ribs and is the lesion which is erroneously used as an indication that a deficiency of vitamins D is present.

Lesion C. Inadequate amounts of osteoid tissue. In the normal development of bone, osteoid tissue must be present if osseous tissue is to be formed. If osteoid tissue is not produced bone growth does not occur. Osteoid tissue is produced by the osteoblast. This cell is extremely sensitive to even minor changes in the nutrition and health of the animal. Inadequacies in fat, carbohydrate, or protein, as occurs in starvation, are immediately reflected by a suppression or cessation of osteoblastic activity. Stunting of the animal is the result.

The osteoblast is also very sensitive to the presence of vitamin A. Apparently vitamin A is very closely associated with the metabolic activity of this cell. A deficiency of vitamin A results in hypoplasia or atrophy of the osteo-
blast. When this occurs bone ceases to grow. A similar reaction occurs when too much vitamin A is present. An excessive amount is toxic to the osteoblast. As a result, osteoid tissue is not produced and normal bone growth does not occur. This is one of the dangers of adding excessive amounts of vitamin A to the ration, especially when animals are feeding on luxuriant pasture. Again the biological teeter totter of balance is exerting its influence and neither an excess nor a deficiency of vitamin A can exist if normal bone growth is to be obtained.

The osteoblast is also very sensitive to the action of bacteria and viruses. Hog cholera, either the vaccination for hog cholera or the natural disease, is an excellent example of this. The hog cholera virus causes hypoplasia, atrophy, and even death of the osteoblast. The swine influenza, canine dis-temper, avian encephalomyelitis virus, as well as other viruses, will produce a similar reaction in the osteoblast. When this occurs bone growth ceases. The effects of these infectious agents on the osteoblast and the resulting changes in bone have led to many erroneous diagnoses of rickets in both swine and poultry.

Lesion D. Excessive amounts of cartilage as manifested by excessively broad and wide epiphyseal plates. This is an indication that the cartilage is not being invaded by the capillaries from the bone marrow and that endochondral ossification is not occurring. The zones of cartilage columns and vesicles are excessively broad. The epiphyseal line becomes very undulating and irregular, and long tongues of cartilage persist in the diaphysis. The excessive amount of cartilage is due to the inability of the capillaries to invade the cell walls of the apparently mature cartilage cells of the vesicular zone of cartilage.

The inability of the capillaries to invade the cartilage cells is, at times, due to a deficiency or an excess of vitamin A. The endothelial cells do not show the active hyperplasia they normally do. If invasion of the cartilage lacunae does occur, metaplasia of the endothelial cells into osteoblasts does not occur or is suppressed and osteoid tissue is not formed.

The endothelial cell is also very sensitive to various viral diseases. The hog cholera virus is particularly injurious to endothelial tissue and when the endothelial cells are injured by either the attenuated virus of the vaccine or the virulent virus of the natural disease, the endothelial bud of the invading capillary is no longer able to penetrate the walls of the cartilage lacunae and produce osteoblasts.

Faulty nutrition or chronic debilitating diseases also prevent capillary invasion of cartilage. The endothelial cells of the capillary bud are very sensitive to malnutrition, soon lose their normal vigor, and then are no longer able to invade the mature vesicular cartilage cells. Cartilage is not as easily injured by faulty nutrition, infectious diseases, or chronic debilitating diseases as is the osteoblast and the endothelial cells of the capillaries. As a result, cartilage continues to grow at a normal or near normal rate even though there is a suppression of the rate of conversion of cartilage into bone. Because of this discrepancy between cartilage formation and con-
version, excessive amounts of cartilage are present. This causes the epiphyseal region of the bone to be unusually large in proportion to the underdeveloped diaphysis. The enlargement of the ends of the bones as the result of the excessive amounts of cartilage, which is mistaken for osteoid tissue, often leads to an erroneous diagnosis of rickets.

A deficiency of vitamin C may produce a similar reaction. When a deficiency of vitamin C is present there is, in addition to the suppressed activity of the endothelial cells and the osteoblasts, an increased fragility of the endothelial cells of the capillaries which results in hemorrhage. If hemorrhage occurs along the epiphyseal line, large lakes of blood appear in the area and the capillaries which are present in this region are obliterated. Since the capillaries are no longer present, no invasion of cartilage occurs in this area, a long tongue of mature cartilage may then persist in the diaphysis, and no bone is produced in this area. Hemorrhage from traumatic injury or other diseases, such as hemorrhagic disease in the chicken, will cause a similar interference with bone growth.

Lesion E. Twisted, compressed, distorted, and fractured cartilagenous, osseous, and osteoid trabeculae. This is an indication that faulty mineralization of cartilagenous, osteoid, and osseous tissue is present and that these structures do not have sufficient rigidity to support the weight of the animal and the stresses and strains placed upon it.

This lesion may be due to a deficiency of vitamins D which are necessary for the most efficient use of mineral by the body. In most of our domesticated animals a deficiency of vitamins D alone will not produce the bone lesion. It is associated with a deficiency or an excess of some mineral (calcium, phosphorus, iron, magnesium, sulfur, and others) in the ration.

Because of the distortion which results at the ends of the bones, this lesion is frequently called rickets. In the midwest, particularly in horses, feeder cattle, swine, and poultry, it is most frequently caused by a deficiency of calcium in the ration. In those areas of the country, such as the southwest, where a phosphorus deficiency in the soil exists, the same lesion will occur because of the imbalance of the calcium-phosphorus ratio.

Lesion E'. A decrease in the number and size of the osseous trabeculae. This lesion is due to two factors. First, it is associated with a suppressed activity of the osteoblasts in which adequate amounts of osteoid tissue are not produced. Without the osteoid tissue, osseous trabeculae are not formed. The causes of inadequate amounts of osteoid tissue have been discussed under Lesion B.

Secondly, it is associated with an imbalance of mineral in the ration in which the mineral stores in the bone are removed to supply the needs of metabolism and as a result of this withdrawal, atrophy of the osseous trabeculae occurs. This is commonly observed in the bones of the laying hen since progressive osteomalacia occurs during the egg-laying period unless adequate amounts of mineral are added to the ration.

Lesion G. Hypoplasia of the bone marrow. Hypoplasia and even aplasia of the bone marrow occurs during a deficiency of vitamin A. It may also
occur during gross malnutrition, chronic debilitating diseases, and in some toxicoses. It is also a manifestation of some viral diseases such as hog cholera.

Lesion H. Hemorrhage in the bone marrow and periosseous structures. Hemorrhage is observed in scurvy and is best demonstrated in the guinea pig. It is found to be present in hemorrhagic disease in the chicken, sulphide and sulphite poisoning, septicemic diseases such as hog cholera, and when traumatic injury to bone occurs. The hemorrhage may seriously interfere with the growth of bone and is particularly injurious if it occurs along the epiphyseal plate where active bone growth is taking place.

Lesion I. Periostitis. The periostitis associated with ration imbalances occurs at those points where tendons and ligaments are inserted into bone. If the bone is not of normal strength it is not able to withstand the stresses and strains placed upon it by the ligaments and tendons. Irritation and even minute fractures occur in the areas of attachment of these structures and periostitis is the result. It is particularly common when calcium, phosphorus, magnesium, iron, copper, and vitamins A, C, and D imbalances are present.

Lesion J. Thrombosis of blood vessels. This lesion is observed when an osteopathy is present and is most frequently observed in the epiphyseal plate. It is a common lesion when adequate mineralization has not taken place. This lesion is seen more frequently in the turkey than in other species. The character and location of the lesion suggests that the inadequately mineralized cartilagenous and osseous structures are compressed by the weight and movement of the animal and crush the soft structures, such as blood vessels, which they contain. The injury to the endothelium of the blood vessel results in thrombosis.

Thrombosis of the blood vessels is very frequently observed when septicemic diseases (hog cholera, staphylococcosis, and salmonellosis) are present. It is also a common finding when chronic respiratory disease is present in poultry and probably explains part of the lameness observed in flocks affected with this disease.

Lesion K. Degeneration and necrosis of cartilage. This lesion is most frequently observed when thrombosis of the nutrient vessels, as described in Lesion J, has occurred. The cartilage in the immediate vicinity of the thrombosed vessel shows degenerative alterations. At times, focal areas of liquefactive necrosis will be observed in the zones of columns and resting cells. These appear as small cyst-like structures. The degeneration and necrosis is a manifestation of the infarction which occurs when the blood supply is destroyed.

Lesion L. Hypoplasia of enamel and dentine. Since teeth originate from epithelium it is logical that dental defects will occur in those nutritional diseases which affect the skin. The enamel organ is very sensitive to nutritional imbalances, especially those involving magnesium and vitamins A and C. When these deficiencies are present there is an incomplete differentiation of the enamel organ into ameloblasts and odontoblasts. As a consequence
these cells may fail to produce enamel and dentine, produce it in inadequate amounts, or produce it in an irregular manner.

General malnutrition (starvation) also causes a suppression in the rate of the production of enamel and dentine. Various viral and bacterial diseases, such as canine distemper, cause hypoplasia of enamel and dentine. Imbalances of fluorine and magnesium result in the formation of enamel and dentine which is abnormal in structure and mineral content.

Lesion M. Peridental osteoid hyperplasia. When examining teeth the periodontal bone may show excessive amounts of osteoid tissue. This lesion is due to a deficiency of vitamins D and an imbalance of minerals. Excessive amounts of osteoid tissue has been previously discussed under lesion A.

Lesion N. Disproportionate rates of body growth as compared with osseous growth. By selection, diet, and environment an animal can be produced which has an amazingly rapid rate of growth. The most critical tissue in this rapidly growing animal is the skeleton. Apparently the osseous tissue has considerable difficulty in developing rapidly enough to support the ever-increasing weight of the body. This is especially true in the pig which normally has a very small skeleton. It is also well known that the more rapidly bone grows the more critical becomes its nutritional requirements if the biological teeter totter is to be kept in balance. As a result bone disease is very common in rapidly growing animals.
A definitive diagnosis of ornithosis in turkeys is dependent upon isolation and identification of the causative agent (1). Frequently the diagnostician is confronted with making a diagnosis within a flock in which typical lesions are not observed or with making a differential diagnosis in flocks presenting a similar or a mixed pathological syndrome. The indirect complement fixation (ICF) test has been found to be of significant importance as an aid under these and other circumstances; however titers of 1:2 to as high as 1:256 were obtained in turkey flocks exhibiting vague clinical signs and no lesions on autopsy and without recovering the agent in mice. Similar experiences were encountered with pigeon flocks exhibiting high complement fixation (CF) titers. It is axiomatic that pigeons or turkeys may have been exposed to ornithosis which resulted in a persistent complement fixing antibody titer; however a sufficient number of failures to isolate the virus from birds having a significant antibody titer prompted this study of the specificity of the complement fixation reaction.

It is the purpose of this study to report further on the nature of the reactions obtained with pigeon and turkey serums with various antigens and using different testing procedures. Some of these tests, because of their simplicity or in the event that all ingredients for the ICF test are not available, may be very useful in the diagnostic laboratory.

MATERIALS AND METHODS

The comparative tests for the detection of antibodies in serums of turkeys infected experimentally were conducted with a combination of test procedures and antigens.

Complement fixing antigens. Two types of antigens prepared from ornithosis agents were used in the CF test. The Texas (Lot 16) antigen was prepared from chicken embryo yolk sacs infected with the 6 BC strain of *Miyagawanella psittaci* by Dr. R. W. Moore, Texas Agricultural Experi-
NATURE OF SEROLOGICAL REACTIONS IN AVIAN SERUMS 543

The Nature Station by the procedure originally described by Karrer, et al. (2) and modified by Meyer and Eddie (3).

The commercially prepared antigen was a psittacosis antigen produced by Lederle Laboratories. This antigen was a purified phosphatide-like antigen prepared according to the method described by Volkert and Christensen (4).

A third antigen used in the comparative studies was prepared from the Herellea-like bacterium. The bacterium was described as a gram-negative pleomorphic coccoid-rod often retaining partial gram-positiveness and was grown on tryptose blood agar. The organisms were described by De Bord in 1942 (5). Antigen was prepared as a saline suspension of bacterial colonies grown on agar which were then either boiled, or extracted with acetone and ether, as described in another report (6).

Test Serums: The turkey serums used in the test were obtained from birds experimentally infected with strains C1, C2, and CP3 ornithosis virus which were originally isolated from natural infections in turkeys (C1 and C2) and pigeons (CP3) in California (7, 8). The serums had been used in previously reported serological studies and were stored at —20 C until the present time.

The 24 pigeon serums for the comparative studies were selected from a number of feral pigeons captured in various parts of the State of California to insure of having a wide range in titers.

Two known positive serums from human patients with psittacosis (Lot RE and VZ) were employed in the direct complement fixation test and also for titrating the ornithosis and bacterial antigens for the ICF test. Lot RE serum was used as the indicator serum for the ICF test throughout the experiments. As controls, 11 serums were collected from apparently healthy humans working in the laboratory.

The indirect complement fixation (ICF) test: The procedure was conducted as recommended by Karrer et al., as modified by Meyer and Eddie and is the official testing procedure adopted by the Agricultural Research Service (ARS) and the Agricultural Experiment Stations as a diagnostic tool for detecting ornithosis in turkeys (2, 3). A slight modification was made in determining the optimum dilutions of antigen and indicator antibody in the test and was described in a previous report (9).

The direct complement fixation (DCF) test: Three methods of conducting the direct (DCF) tests were employed.

a) Lederle DCF test. The test described by Lederle Laboratories, using their prepared psittacosis antigen, is a micro-modification of the Kolmer-Boerner method (10). Briefly, the test was conducted with 0.1 ml. of antigen containing two units as determined by previous titration* and 0.1 ml. of

*In this study the antigen was titrated in the presence of known positive human anti-psittacosis serum to determine the antigenic unit instead of the prescribed dilution of antigen recommended on the package.
inactivated serum prepared in two-fold dilutions. Two units of complement in a volume of 0.2 ml. of guinea pig serum were added to each tube and held at six to eight degrees centigrade for 18 hours. To each tube was added 0.2 ml. of sensitized sheep red blood cells composed of equal volumes of haemolysin (two units in 0.1 ml.) and two percent sheep cells.

b) Brumfield's modified DCF test. Brumfield and Pomeroy (11) found that antibody in chicken and turkey serum fixed guinea pig complement providing normal turkey or chicken serum respectively, was added to the immune system. Turkey serums were tested in the presence of undiluted normal chicken serum. The amount of chicken serum necessary for each 0.2 ml. volume of two-fold dilution of the inactivated test serum was previously determined by titration. The latter was conducted by using increasing amounts of undiluted normal chicken serum which was added to a set of a serially diluted known positive turkey antiserum. The amount of normal chicken serum giving the maximum titer without producing an anti-complementary reaction in the antigen control was considered optimal. The amount usually employed was 0.006 to 0.01 ml. per 0.2 ml. volume of antigen. To the mixture of serums was added 0.2 ml. of antigen containing two units and 0.2 ml. of complement containing four exact units, each of which was determined by previous titration. Fixation of complement was allowed to take place at 37 C for one hour. Two-tenths of a ml. of the sensitized sheep red blood cells, which consisted of equal parts of haemolysin containing two units per 0.2 ml., and a four percent sheep red blood cell suspension was added to each tube. Incubation was continued for 50 minutes at 37 C in a water bath before reading.

c) Karrer's et al. DCF test. With this procedure, which was adopted by the ARS and the Experiment Stations, four units of antigen and two full units of complement were employed in each instance.

Suitable controls including known positive and negative serums were included in all tests. Titers of serums were expressed as the reciprocal of the highest dilution of serum causing complete fixation, or inhibition of fixation, of complement.

RESULTS

A. Antigens Prepared from Ornithosis Agents

Comparison of Lederle's DCF test and the Karrer's et al. DCF test with pigeon serum. Serum from 24 pigeons was tested for the ornithosis reaction using two units of Lederle antigen with their recommended test procedure and Karrer's et al. DCF test, which employs four units of antigen. As shown in Table I, it is evident that the Lederle test was more sensitive in reacting with pigeon serum than the Karrer's et al. DCF test. In 11 instances titers of 1:2 to 1:32 by Karrer's et al. procedure gave corresponding titers by the Lederle method which varied from 1:8 to 1:256. Of seven non-reacting serums with the Karrer's et al. test, titers of 1:4 to 1:64 were obtained with the Lederle procedure. Three samples were non-reactive to either test. The titers obtained by the Lederle test were consistently about two to four times (statistical mean = 3.89) higher than titers obtained by Karrer's et al. DCF test procedure.
TABLE I

Correlation of Titers in Pigeon Serums with the Lederle and Karrer's et al. 
DCF Test Procedures

<table>
<thead>
<tr>
<th>Titer—Karrer's et al. DCF test</th>
<th>1/256</th>
<th>1/128</th>
<th>1/64</th>
<th>1/32</th>
<th>1/16</th>
<th>1/8</th>
<th>1/4</th>
<th>1/2</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lederle antigen and test</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Comparison of the ICF test and the Brumfield's DCF test using the Texas and Lederle antigens with turkey serums. A total of 73 known serums from turkeys infected with C1, C2, and CP3 strains of ornithosis were tested by the ICF test using Texas antigen and by Brumfield's DCF test using Texas and Lederle antigens. Two units of antigen were used in each test.

In general good agreement was observed with all three tests, but the ICF test proved to be more sensitive than the Brumfield's test using either antigen. A better correlation was obtained between the ICF test and the Brumfield's procedure with the same antigen (Texas) than between the ICF test and Brumfield's procedure using the Lederle antigen. Statistical analysis of 73 turkey antiserums showed a correlation coefficient between the ICF test and Brumfield's procedure using the Texas antigen as +0.756 and with the ICF test and Brumfield's procedure using the Lederle antigen as +0.701.

As shown in Table II, 33 (or 45.2 percent) of the 73 serums were negative by all three tests. Serums which did not react with the ICF test in no instance reacted with Brumfield's procedure using either antigen.

Of the 40 remaining serums, 13 samples presented similar reactions by the three testing procedures (Group I). Therefore, a 63 percent agreement was obtained in the 73 serum samples by all three tests. Twenty-seven serums which reacted to the ICF test (Groups II, III, and IV) in all but one instance (Group II) presented titers which were equal or less by Brumfield's test using either antigen. However, 13 serums having titers of 1:4 to 1:32 by the ICF test (Group IV) did not react to either antigen using the Brumfield's procedure. In one instance a serum with a titer of 1:128 by the ICF test was negative with Brumfield's procedure using either antigen (Group IV).
TABLE II
Percent Agreement of the ICF Test and Brumfield's Procedure Using the Texas and Lederle Antigens

<table>
<thead>
<tr>
<th>Group*</th>
<th>ICF</th>
<th>BL</th>
<th>BT</th>
<th>No. of Serum</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>146</td>
<td>63.0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>3</td>
<td>4.1</td>
</tr>
<tr>
<td>IV</td>
<td>±†</td>
<td>-</td>
<td>-</td>
<td>14</td>
<td>19.2</td>
</tr>
</tbody>
</table>

+ Serums with titers of 1:64 or greater; ± serums with titers of 1:4 to 1:32; — no reaction at 1:4.
* Grouped for convenience of description.
† ICF titer 1:128.
BL Brumfield's test using Lederle antigen.
BT Brumfield's test using Texas antigen.
ICF Texas antigen.

B. Bacterial antigen in the CF tests.

To determine the antigenic activity of the bacterial antigen the Lederle antigen and test procedure was compared with Karrer's et al. DCF test with two known positive and 11 human serums obtained from apparently healthy humans. Two units of antigen were used in each test. As shown in Table III, the two positive serums presented identical reactions with either

TABLE III
Human Serums Tested with the Boiled Bacterial Antigen

<table>
<thead>
<tr>
<th>No. Sera</th>
<th>Bacterial Antigen Karrer's DCF Test</th>
<th>Lederle Antigen Lederle Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human psittacosis known positive serums</td>
<td>1</td>
<td>128</td>
</tr>
<tr>
<td>Serums of apparently normal humans</td>
<td>11</td>
<td>16</td>
</tr>
</tbody>
</table>

* Two units of antigen in each test procedure.
antigen and test procedure and the eleven serums from the apparently healthy humans did not react in the tests. These results showed some relationship between the bacterial extract and the agent of the Miyagawanella group.

Comparison of bacterial and ornithosis antigen (Texas) in the Brumfield’s DCF test. The study was conducted on 81 serums of turkeys previously infected with ornithosis virus. Two units of antigen were used in each test.

The results summarized in Table IV show that 16 or 19.8 percent of the samples showed a titer of 1:64 or greater with either the bacterial antigen or Texas antigen. Twenty-seven or 33 percent of the serums were negative with the bacterial antigen whereas 32 serums or 39 percent were negative with the Texas antigen. Serums (28) showing titers between 1:4 and 1:32 presented a satisfactory correlation in which the bacterial antigen appeared to be slightly but not significantly more sensitive than the Texas antigen.

Comparison of the bacterial and ornithosis (Texas) antigens in the ICF test. One unit of each of the antigens which were previously titrated was used in the testing procedure.

The results obtained with 100 turkey serums (Table V) showed that 25 serums showed a titer of one to 64 or greater with either the Texas or bacterial antigens. Each of the 38 serums that were negative to one antigen was also negative to the other antigen. Eighteen serums showing titers between 1:4 and 1:32 showed similar titers with either antigen.

Eighty-one of the 100 serums tested showed parallel titers with both antigens. A few serums which reacted with one of the antigens, did not react with the other. It was noticed that 10 serums that showed negative reactions with the bacterial antigen gave positive reactions with ornithosis
antigen; in two instances, the titer with the Texas antigen was 1:64 or greater. In general, there was a close relationship of titers with the ICF test using either antigen. Statistically the correlation coefficient was found to be +0.842.

**DISCUSSION**

The results observed in these studies showed that the ICF and Brumfield’s DCF test in which two units of 6·BC ornithosis antigen (Texas) were employed, were found to be in high agreement (correlation coefficient = +0.756) with serums of turkeys infected experimentally with ornithosis virus. These results confirm the data obtained by Boulanger and Bannister although they had used an ICF test originally developed by Rice (12, 13). The titers obtained with Brumfield’s procedure using a commercially available psittacosis antigen (Lederle) was also in good agreement with the two other tests with turkey serums, but the titers were slightly lower than with the ICF or Brumfield’s DCF test using two units of the 6·BC ornithosis antigen. No serious discrepancies between serum samples which did not react by either test or titers which were highly or of little or no diagnostic significance were observed. These observations would indicate that the differences in titers were due to the test procedure and not wholly dependent upon the antigen employed. A comparative study of these procedures indicated that the ICF test was the most sensitive to react with serums of turkeys artificially infected with ornithosis. No false positive reactions were observed with 73 turkey serums using the Brumfield’s procedure with either the commercially available psittacosis or the antigen prepared from the 6·BC strain of ornithosis virus. It is evident from this comparative study that the Brumfield’s procedure may be employed as an aid in the diagnosis of ornithosis with either antigen.
Pigeon serums tested with a commercially prepared psittacosis antigen using their recommended procedure gave titers two to four times higher than the Karrer’s et al. DCF test. This may be explained by the difference in the test procedure or the amounts of antigen employed in each of these tests.

One of the difficulties in making a diagnosis of the Miyagawanella infections in man has been the non-specificity of the CF test for identifying an agent within the group. The aspecificity with serums of turkeys was demonstrated by the high correlation of CF titers between antigens prepared from the 6 BC ornithosis agent and a water soluble extract prepared from a bacterium of the genus Herellea. A serologic relationship between ornithosis virus and a bacterium was first observed by Volkert and Matthiesen (14) of Denmark. They employed a gram-negative coccoid bacterium which was closely related to the Bacterium anitratum. In this study prolonged boiling (four hours or more) of a saline suspension of the Herellea-like bacteria also showed a high correlation in CF titers with serums of turkeys experimentally infected with three strains of ornithosis virus. This high correlation was observed with the ornithosis antigens using the Brumfield’s DCF test and the ICF tests. In each instance the same amounts (units) of antigen were used in each of the prescribed tests. It was also interesting to observe that two known human positive antiserums and serums from 11 apparently normal humans gave identical reactions with a commercially available antigen and test procedure and the bacterial antigen using Karrer’s et al. DCF test.

The results of this investigation of comparing the testing procedures and antigens definitely indicate that in an emergency the commercially available psittacosis antigen and testing procedure can be employed with pigeon serums; however, the titers were two to four times greater when compared with Karrer’s et al. DCF test. It is to be noted, however, that the amount of antigen in each of the tests differ, which may influence the sensitivity of the testing procedures.

In view of the fact that Brumfield’s modification of the CF test for avian serums appears to be considerably simpler than the ICF test and in spite of the lesser sensitivity, the Brumfield’s test can be readily employed as a diagnostic tool.

The maintenance of sufficient quantities and quality of antigen for the CF test prepared from virulent strains of ornithosis constitutes a danger to laboratory personnel. To decrease the potential danger of infection, it is feasible that an antigen can be prepared from a relatively non-pathogenic, easily cultivatable, rapidly growing bacterium. Such an antigen can be quickly prepared from stock cultures in laboratories situated in areas where the disease is not enzootic or frequently encountered. The high correlation of reactions between the bacterial antigen and an antigen prepared from Miyagawanella warrants the suggestion of using the bacterial antigen in the Brumfield’s DCF test to establish a presumptive diagnosis of the disease in turkey flocks.
A commercially available psittacosis antigen and test procedure resulted in titers two to four times greater than the Karrer’s et al. DCF test with serums of feral pigeons.

Comparative studies using the ICF test and the Brumfield’s DCF test procedures using a commercially available antigen and an ornithosis antigen (6 BC strain) on 73 known turkey serums presented a 63 percent agreement by the three tests (correlation coefficient of +0.762).

A water-soluble, heat-stable, gram-variable coccoid bacterium of the genus Herellea appears to be antigenically related to the ornithosis virus. The antigen was found to give identical reactions with two known positive human and 11 negative serums to ornithosis when compared with the commercially available psittacosis antigen and testing procedures.

A comparison of the bacterial and ornithosis antigens (6BC) in the Brumfield’s DCF test or the use of these antigens in the ICF test with turkey serums showed a high degree of correlation (+0.899 and +0.842 respectively).

The Brumfield’s DCF procedure showed a high correlation with the reactions obtained with the ICF test; however the latter presented higher titers and appeared to be more sensitive than the former.

The use of the simpler and more rapid Brumfield’s DCF test with a readily available, safe, and prepared bacterial antigen which can be available to most laboratories for presumptive diagnosis of ornithosis in turkey flocks was discussed.

REFERENCES

11. Brumfield, H. P., and Pomeroy, B. S.: Direct Complement-Fixation by Turkey
    146-149.
    Direct Complement-Fixation Test in the Diagnosis of Turkey Ornithosis. Canad.
MASTITIS CONTROL IN DEMONSTRATION HERDS

W. H. THOMPSON,* C. W. BURCH, C. OLSON, and H. Blobel

Madison, Wisconsin

Bovine mastitis is recognized as one of the most costly diseases of dairy cattle. It has been estimated that the annual loss in the United States amounts to over a quarter of a billion dollars. The cost to the average dairyman has been estimated as over a dollar per cow per month. This is the accumulated result of the disease through reduced milk production of infected cows, lowered milk quality, reduced productive life of dairy cows, costs of treatments, loss of value and occasionally death of affected cows.

The public health concern of residues of antibiotics in milk as a result of treatments has been another stimulus for better control of mastitis in dairy cattle. Along with this has come the realization that treatment alone is not the answer to the mastitis problem.

As a part of mastitis control activities in Wisconsin, 20 herds for demonstration of mastitis control were selected in 14 counties during the fall and winter of 1958-59.

The program with the demonstration herds involved the cooperation of two kinds of groups. A single advisory group at the University consisted of representatives of the departments of Dairy Husbandry, Dairy and Food Industries, Agricultural Engineering, and Veterinary Science. Twenty local groups, each intimately associated with the individual herds, consisted of the herd operator, the local veterinarian, the county agent and the dairyplant fieldman. The program was coordinated by the representative of the Veterinary Science Department.

A series of meetings to discuss the proposed program were held in the dairying areas of the State and at the annual meeting of the Wisconsin State Veterinary Medical Association. Following this the individual demonstration herds were selected on the basis that the herd operators would be willing, anxious, and able to do something about mastitis. Willing in that they would be cooperative, anxious by realizing that they were having trouble with mastitis in their herds, and able in that they would have reasonable facilities to carry out recommended management procedures.

At the first visit to each herd, the details of the program were discussed with the dairyman and the physical facilities, management and general mastitis picture in the herd were surveyed. Details were recorded on a form designed for that purpose.

During the second visit to the herd, the local group made recommendations for mastitis control based on study of the previous survey and these recommendations were noted on a check list. These were based largely on im-

* Department of Veterinary Science, University of Wisconsin.
proved management, especially those including proper milking procedures. The dairyman arranged with his veterinarian for routine mastitis examinations and treatments in the herd. Subsequent visits were made to each farm by the local groups at intervals of four to six weeks to help the dairyman adopt the recommended practices and to observe the effect of these changes in the herd. These visits were usually made during evening milking time in order to actually observe the milking procedure which was considered an important part of the program.

Particular attention was given to proper operation of the milking equipment considering such factors as adequacy of vacuum pump and motor, vacuum line, gauges and vacuum controller. Clean, properly operating milking machine units were emphasized with attention to the teat cup liners. Milking procedures included proper preparation of the udder using warm approved sanitizing solution, individual towels and strip cup, application of the units at milk let-down, machine stripping and removal of the machines as soon as the milk was out of the quarters. It was found that the milking units were often left on the cows far too long and recording of the milking times with a stop watch not only gave data on the milking time of individual cows, but impressed on the dairyman the need for prompt removal of the units.

Recommendations made for prevention of injury to the teats and udders included a close check for such hazards as loose fence wires, barnyard obstacles, high door sills, and slippery floors. Inadequate stall sizes in some of the stanchion barns proved to be a problem hard to correct without considerable capital expense. Several owners who replaced small-sized stalls found that reduced teat injuries and increased cow comfort were well worth the expense.

Recommendations for sanitation included proper cleaning and storage of the milking machine, individual towels for preparing udders, routine use of a strip cup, and proper rinsing of teat cups between cows. Some of the dairymen used an extra head for the milking machine, allowing each set of teat cups to soak in the sanitizer for 40 to 50 seconds in rotation between cows, thus increasing the effectiveness of the sanitizer. Some of the dairymen applied sanitizing solution to the ends of the teats following removal of the machine.

The suggested milking order consisted of milking clean heifers and cows first and known infected cows last, however the ideal milking order was difficult to establish and maintain. Routine testing for mastitis by the veterinarian was encouraged not only to aid in proper treatment, but to help determine infected cows which should be milked last. The importance of not bringing infected cows into the herd was emphasized and the advantages of raising replacements was stressed.

The adoption of the suggested milking practices by the dairymen seemed at first to be complex, but it was found that when the milking operation was properly organized the recommendations could be followed efficiently. The extra time taken by such practices as routinely using a strip cup and rinsing
of teat cups between cows was sometimes balanced by time saved through proper preparation of cows and closer attention to milking time. Occasionally time was actually saved by reducing the number of milker units handled per man. A general rule of two units per man in a stanchion barn was followed, when his duties also included preparation of cows and carrying milk to the cooler.

An important step in the adoption of these recommendations in a stanchion barn herd was the introduction of a milking cart. This was equipped with a paper towel dispenser, strip cup and four pails (one with sanitizing solution for washing udders, a second for discarding used towels, one with lukewarm water for rinsing the milk off of the teat cups following removal from the cow, and the fourth pail with warm approved solution for sanitizing teat cups before placing them on the next cow). At the start of the program there was only one herd with a milking cart, but most of the herds later adopted their use. A variety of homemade carts were used. Proper working height, stability and durability of construction were especially important. Use of the milking cart made routine milking more efficient and allowed time for attention to individual cows during the milking operation.

Individual cow records were kept on mastitis, treatments, production, age and reasons for sales or deaths. The incidence of mastitis and treatments in the second year of the program was less than half of that reported during the first year.

Comparisons were made of average production per cow in the 13 herds which had complete Dairy Herd Improvement Association records during two years before and for two years after the start of the program.

The average number of pounds of butterfat produced per cow for 1957 was 413, 413 in 1958, 453 in 1959 (the first year of the program) and 457 in 1960. This was an increase of 40 lbs. of butterfat per cow during the first year and a further slight increase during the second. This is a significant increase when compared with the average production of all cows in the Wisconsin D.H.I.A., which was 389, 401, 420, and 415 for the same respective four years.

The cows in the herd with the largest increase in production had over a hundred pounds of butterfat production increase per cow, with yearly averages of 402 lbs. in 1957, 402 in 1958, 510 in 1959 and 518 in 1960. Three-fourths of the herds made increases larger than the average for Wisconsin D.H.I.A. cows. The herds making the greatest increase were usually those which had most completely followed the recommendations.

The herds in the program had higher production on the average than other herds in the State. Those with the lowest production at the start of the program showed the greatest capacity for increase. With an average of over 40 cows in each demonstration herd, and considering the average production increase of 40 pounds of butterfat per cow, at current milk prices this increase represents an average of more than 1,600 dollars additional income during the first year for each dairyman in the program. This higher level of income remained during the following year.
Further data are being analyzed including individual cow production graphs, mastitis histories, results of bacteriological examinations and catalase tests of the bulk milk in the herds in order to determine more about how these benefits have come about.

There was a corresponding increase in milk quality as indicated by routine tests of dairy plants purchasing the milk from these herds. A longer productive life of cows is expected to be of considerable long-range benefit.

The key man in mastitis control is the dairyman. He not only needs advice and help in reducing mastitis in his herd, but also the encouragement that his efforts will be worthwhile.

The results with these herds convinced the dairymen and those working with them that the application of these recommendations has been profitable. The benefits include lower costs of production and better quality milk.

REFERENCES

STUDIES ON THE NATURAL AND EXPERIMENTAL INFECTIONS OF ANIMALS IN FLORIDA WITH THE ENCEPHALOMYOCARDITIS VIRUS

J. H. Gainer*

Kissimmee, Florida

Introduction:

The author (1) in a preliminary report in April, 1961, described the first isolation in North America of the encephalomyocarditis (EMC) virus from the hearts and other tissues of swine. The virus was recovered and identified in mice and has been designated the Trenton strain since it was isolated from pigs near Trenton, Florida. The swine had acute focal myocarditis accompanied by calcification. Murnane et al. (2) in 1960 reported the first isolations of the virus from swine affected with myocarditis. Pope (3) in 1959 refers to serologic evidence gathered in 1955 that dogs, cows and pigs were natural hosts for the virus in Surinam. Pope and Scott (4), 1960, reported one horse out of 44 tested in Australia having neutralizing antibodies against their Innisfail strain. Prior to these reports of the infections of domesticated animals by the EMC virus, the isolation of the virus had been restricted to rodents, primates, and man; in man most infections have been mild. Warren (5) reviews the infection thoroughly.

This paper deals with studies in progress at the laboratory in Kissimmee in: (1) Making further virus isolations, (2) performing neutralization tests for antibodies in serums from various species against the Trenton strain, (3) describing the initial studies on the Trenton strain and experimental inoculations in swine, rabbits, guinea pigs, dogs, embryonating eggs and young chickens, (4) reporting clinical data, mortality figures, epizootiology, gross and histologic studies of the heart and brain of natural infections as diagnosed by virus isolation or neutralizing antibodies, and (5) reporting E.K.G. changes of experimental studies.

I Materials and Methods:

A. Virus Isolation Procedure: Four week old white mice of both sexes were used for initial virus isolation studies. Swine hearts revealing gross

*Veterinary Virologist, Florida Department of Agriculture.

Acknowledgment for technical assistance is gratefully given Mr. G. W. Stroup and thanks to Drs. T. E. Murchison, J. W. Needham, E. M. Ellis, D. E. Cooperrider and W. L. Sippel for pathologic studies, chemical determinations, bacteriologic studies, parasitologic studies and encouragement and advice respectively. Especial thanks are due Dr. W. L. Jackson, Lakeland, Fla., for performing the E.K.G.'s and to Dr. D. Patterson (Univ. of Pennsylvania) for interpreting the E.K.G. tracings.
lesions suggestive of EMC infection and other tissues, namely, spleen, brain, lungs, and colon and contents were stored in a Revco* deepfreeze at \(-70^\circ C\) until they could be worked up. The tissues were trituration in a mortar and pestle with alundum and made into 20 percent suspensions with heart infusion broth (HBPSS) containing 10 percent heat inactivated (56\(^{\circ}\)C for 30 minutes) normal rabbit serum, 1,000 units penicillin and one mg. dihydro-streptomycin per ml. Suspensions were centrifuged for 30 minutes at 3,000-3,500 RPM in a 10 inch diameter angle head. The supernatant was pipetted off and stored in plastic or glass tubes in the Revco or immediately inoculated intracerebrally (IC) into each of six mice, 0.03 ml. per mouse, while the mouse was under light ether anesthesia. The mice were observed daily for 14 days for any sign of paralysis or death. If all six mice survived the 14 days, the original inoculum was considered free of virus. If one or more of the six mice developed paralysis and died within the two-week period, the brain of the mouse was removed aseptically and a 20 percent suspension of it was made in HBPSS. At least four new mice were inoculated intracerebrally with 0.03 ml.; stock material was stored in the deepfreeze. Were the EMC virus present in the inoculum, second passage mice evidenced posterior paralysis and death within two to five days after inoculation. Usually after only one intracerebral passage, mouse brain suspension would infect new mice almost equally by whatever parenteral route was used—intracerebral (IC), intraperitoneal (IP), or intramuscular (IM). The IC route was most sensitive for primary isolation; specimens that seemingly contained no virus upon IP inoculation of 0.1 ml. revealed virus when inoculated in 0.03 ml. quantity IC.

B. Neutralization Test Procedure:

Identity that the original Trenton isolate was the EMC virus was proved by neutralization test as suggested by Hilleman (6) with specific EMC rabbit antiserum†, using constant serum and variable virus dilutions. In this instance IC inoculations were used to conserve serum. LD\(_{50}\)'s were determined by the method of Reed and Muench (7). Mice were observed for 14 days at which time the neutralization test was terminated.

C. Antiserum Production:

Antiserum to the Trenton strain was prepared in rabbits by three weekly subcutaneous (SC) inoculations of 0.5 ml. 20 percent suspension of fifth passage mouse brains—the suspension in heart infusion broth without the addition of normal rabbit serum. The two rabbits used showed no signs of illness following inoculation. Blood was collected by intracardiac puncture 10 days following the third inoculation. Serum was pipetted off following centrifugation of the clot, inactivated at 56\(^{\circ}\)C for 30 minutes, and stored at


† Dr. C. J. Gibbs, Jr., N.I.A.I.D., N.I.H., Bethesda, Md., and Dr. W. L. Pond, Chief, L.T.V., O.D., N.I.A.I.D., Jackson Memorial Hospital, Miami, Fla., kindly supplied the E.M.C. immune serum.
Neutralization tests in mice by IP or IC routes of inoculation revealed neutralizing antibodies of greater than 10^4.0 logs of protection. No antibodies were detected in pre-inoculation serum samples. Antiserums obtained from experimentally infected pigs were used later on in the study.

D. Stock Virus Preparations:

Stock virus suspensions (Trenton strain) for neutralization tests were prepared from the brains of four week old mice inoculated IC or IP and dying three to four days later. Twenty percent suspensions of brain in
HPBSS were centrifuged at 3,000 RPM for 30 minutes; the supernatant was pipetted off and stored as a stock virus suspension in the Revco. The virus has been passed four to seven times in mice; repeated freezing and thawing of the material over several months has not diminished its LD\textsubscript{50} which has been of the order of $10^{-7.0}$ whether tested IC or IP.

E. Screen Test for Antibodies:

For the screen test for neutralizing antibodies, a single virus dilution estimated to be about 100LD\textsubscript{50} was prepared in HPBSS. Three-tenths ml. quantity of serum plus 0.3 ml. virus dilution were mixed in a tube and incubated at 37°C for one hour. Four, four-week-old mice were inoculated intraperitoneally with 0.1 ml. serum-virus mixture. Mice were observed for 14 days and deaths and day of death recorded. Stock virus was also titrated either with normal rabbit or swine serum or without the addition of normal serum. The addition of normal serum above that already present in HPBSS did not significantly alter the LD\textsubscript{50} of the virus. Survival of two mice in a group was accepted as indicative of the presence of neutralizing antibodies in the serum tested, and these sera were further studied to determine their neutralizing indices.

F. Case Reports:

History, clinical findings, clinical pathology, bacteriology, chemistry, parasitology, and gross and microscopic findings, some or all when indicated, were made on animals submitted to the laboratory for examination. For histologic examinations, tissues were fixed in 10 percent buffered formalin-saline. Bacteriological examinations were made by culturing tissues on blood agar, in heart infusion broth, and in tetrathionate broth for Salmonella isolation.

No evidence has been found for the presence of the EMC virus in the mouse colony at this laboratory, even though a number of routine mouse passages have been made by various routes of inoculation during the course of other work.

II. Clinical and Epidemiological:

Following the initial discovery of the presence of the EMC virus in two groups of swine in Florida, an effort has been made to look carefully and thoroughly for the virus or antibodies in other groups of swine as well as in other species of animals. To date 35 cases of swine have been studied for the presence of the virus, 12 cases of cattle, one cat, one domestic chicken, two horses, one hamster, one goat, one raccoon, three dogs, and one squirrel. A case may have represented one individual animal, e.g., the raccoon, or a group of three or four animals from a large herd. Of the 58 cases examined, the EMC virus has been isolated and specifically identified with immune serum from six swine cases, one bovine, one squirrel and one raccoon; the virus, in addition, was recovered from rat feces and sow feces on the premise where the Trenton strain originated.
The striking myocarditis present in the Trenton swine and group No. 6 led to more careful observations of hearts in subsequent necropsies. Table I lists various findings in these accessions proved positive for EMC virus isolation or positive for EMC neutralizing antibody. Of 29 other swine cases not reported in the table where virus was looked for but was not found and where serums were negative or were not tested for neutralizing antibodies to EMC virus, five additional cases had varying degrees of myocarditis suggestive of EMC infection. The brains of these five cases presented no lesions suggestive of hog cholera.

In examining Table I it is noticed that where white blood counts were available, they were all low. Hog cholera was diagnosed in \( \frac{3}{4} \) cases where counts were made. Diagnosis of hog cholera was based upon history and signs, leukocyte count, and CNS lesions. The leukopenia present in case No. 1 was probably due to pox and EMC infection. The losses are not accurate; they are undoubtedly low for most of these figures were obtained at the time the animals were submitted to the laboratory. In at least four porcine cases marked myocarditis was observed; in one of these four a lymphocytic-monocytic meningo-encephalitis was noticed. A scar was present in the medulla of the hog in accession No. 5 not associated with hog cholera. In none of the porcine cases was encephalomyocarditis the single and only entity. The heart from the calf (No. 7) presented a mild lymphocytic and histiocytic myocarditis and the raccoon a lymphocytic and histiocytic myocarditis; interestingly enough, serum from this calf neutralized bovine virus diarrhea virus in 1:100 dilution.*

The myocardial calcified lesions described in the original paper (1) in the first two cases (Nos. one and six) have not been seen since; still the virus has been recovered on several occasions.

The typical history in these EMC infections in swine was rapid onset of illness, high fever (106.3 to 108.1), lack of inappetence and sudden death in one to three days after signs of illness were noticed. Much stunting occurred in survivors in Case No. 1.

III. Experimental:

A. Study of the Trenton Strain and other Strains in Laboratory Animals, Dogs, and Swine.

1. Mice: On Oct. 24, 1960, three pigs, two alive and one dead, about six weeks of age, were brought to the laboratory from near Trenton, Fla. These animals at necropsy had unusual white streaks one to two x six to eight mm. in their myocardiums just beneath the epicardium. Other pertinent information is listed in Case No. 1 of Table I, the Trenton strain. Bacteriologic, chemical, and parasitologic examinations were negative even though there was considerable concern that the pigs may have died from china berry poisoning. Intracerebral inoculation into four, six week old mice of a 20 percent sus-

* Bovine virus diarrhea neutralization test was kindly performed by Dr. A. J. Kniazeff, Univ. of Florida, Gainesville, Fla.
### TABLE 1

**Information on Natural Occurrences of E.M.C. Infections**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Species</th>
<th>Tissues Exam.</th>
<th>WBC</th>
<th>Losses</th>
<th>Lesions</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3500</td>
<td></td>
<td>LmME</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>S</td>
<td>Br</td>
<td>NA</td>
<td>1</td>
<td>Autolysis</td>
<td>EMC</td>
</tr>
<tr>
<td>3.</td>
<td>P*</td>
<td>H</td>
<td>NA</td>
<td>8/60</td>
<td>NA</td>
<td>NA EMC, Salt Poison</td>
</tr>
<tr>
<td>4.</td>
<td>P*</td>
<td>Int, Lu, Br, S, H</td>
<td>9000</td>
<td>7/55</td>
<td>LM</td>
<td>HC HC, EMC, Salmonellosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10800</td>
<td></td>
<td>HC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>P*</td>
<td>Int, Lu, Br, H</td>
<td>8250</td>
<td>23/150</td>
<td>Neg</td>
<td>HC, Scar in Med not HC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7850</td>
<td>25% ill</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>more ill</td>
<td>LHM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10850</td>
<td></td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>R</td>
<td>S, Br, Lu, H</td>
<td>NA</td>
<td>1</td>
<td>LHM</td>
<td>Hem EMC</td>
</tr>
<tr>
<td>11.</td>
<td>P</td>
<td>Lu, H, S</td>
<td>NA</td>
<td>9/30</td>
<td>Neg</td>
<td>NA EMC, Pneumonia</td>
</tr>
<tr>
<td>13.</td>
<td>E*</td>
<td>None</td>
<td>10800</td>
<td>0</td>
<td>0</td>
<td>Normal</td>
</tr>
</tbody>
</table>
Species Legend  | Tissue Legend  | Lesion Legend
---|---|---
B—bovine  | Br—brain  | C—calcification
R—raccoon  | H—heart  | EMM—eosinophilic monocy. myocarditis
S—squirrel  | Int—intestine  | Hem—hemorrhage
E—equine  | K—kidney  | LHME—lymphocytic histiocytic myocarditis
  | L—liver  | LM—lymphocytic myocarditis
  | Lu—lung  | LmME—lymphocytic monocytic meningo-encephalitis
  | Mes N—mesenteric ly. node  | ?—unfinished
  | Med—medulla  |  
  | S—spleen  |  

October 25, 1961

* No virus recovered; serum contained neutralizing antibodies to Trenton strain.
† New virus type; it was not neutralized by serum against Trenton strain; infected mice have encephalitis but not typical posterior paralysis; identified as Eastern Encephalitis virus.
§ Italic letters—tissues from which virus was recovered.
NA—not available.
pension from one pig heart resulted in posterior paralysis in one mouse six days later, a second mouse at seven days; on the seventh day a third mouse appeared sick with rapid respirations and ruffled hair. The two paralyzed mice were killed on the seventh day after inoculation; their brains and spinal cords were subpassaged. Subsequent repeated attempts at isolation of the virus from the original pig heart suspension by IC inoculation into mice resulted in this classic posterior paralysis and death in 50 percent of the mice. Mice were observed for 14 days and then were discarded. The mice were quite alert even with the flaccid paralysis of the hind limbs; they would drag their paralyzed legs behind them. Intramuscular inoculation of this same heart suspension 0.25 ml., two mice, 0.125 ml., two mice, failed to induce disease or deaths; these were observed for two months. Inoculation of 0.5 ml. IP with the pig heart or pig spleen suspension, two mice each, failed to induce disease over a 14 day period. The same splenic suspension inoculated IC, 0.03 ml., induced, as did the heart suspension, a 50 percent mortality in four week old mice held for 14 days; the same signs developed. Suspensions of kidney, liver, brain, lung, from the same pig inoculated IC failed to induce disease or death in four week old mice.

Brain and spinal cord suspension of the first mouse passage inoculated IM killed \( \frac{7}{4} \) mice and IC \( \frac{5}{6} \) by the time of termination at 21 days. Second mouse passage titered about \( 10^{-2.0} \) by IC route. Third IC mouse passage titered less than \( 10^{-4.0} \). Two week old mice, 0.02 ml. IC died in 20 hours at \( 10^{-1} \) dilution, two days at \( 10^{-2} \) dilution. The fourth IC mouse passage titered \( 10^{-7.5} \) using four mice per dilution, four week old mice, 0.03 ml. IC Survivors in this instance were killed 18 days post inoculation. Harmonic mean of survival time was 2.7 days at \( 10^{-2} \), 3.2 at \( 10^{-3} \), 3.4 at \( 10^{-4} \), 4.0 at \( 10^{-5} \), 5.3 at \( 10^{-6} \), 5.6 at \( 10^{-7} \), and infinity at \( 10^{-8} \) and \( 10^{-9} \). Within the limits of lethal doses, survival time was inversely related to the inoculum. An experiment comparing routes of inoculation versus LD\(_{50}\) gave a figure of about \( 10^{-8.3} \) for 0.1 ml. IM into right thigh, about \( 10^{-7.5} \) for 0.1 ml. IP, and less than \( 10^{-4.0} \) for 0.3 ml. orally using sixth mouse passage virus. Deaths occurring prior to the fourth day were usually not accompanied by the flaccid posterior paralysis. Subsequent strains isolated and identified as EMC virus behaved similar to the Trenton strain except for isolation No. 12 (Table No. 1). Intracerebral inoculation is used on primary isolation and for first and second passages, IP thereafter.

2. Guinea Pigs: Two adult guinea pigs were inoculated with 0.1 ml. I.M. of sixth mouse passage brain suspension, Trenton strain. No evidence of disease was seen in these animals. They were killed about 10 weeks after inoculation and no lesions were noticed at necropsy. Two other adult guinea pigs were inoculated with third mouse passage brain suspension of Case No. 3; these were held for 10 weeks without the development of signs and without lesions at necropsy. Four young guinea pigs, about three months old, weighing about 250 grams, were inoculated IP with 0.5 ml. sixth mouse passage, Trenton strain. One animal was comatose four days later and was
killed in extremis; its heart was pale. The other three animals killed 10-21 days later had no lesions. Virus titer in this animal's brain, heart, and spleen as examined by inoculating 0.03 ml. 20 percent suspension IC into six mice each revealed most virus in brain, next in heart, least in spleen. One of six mice died in 14 days from splenic suspension, % from brain, % from heart; harmonic mean survival time in "brain" mice was three days, in "heart" mice was 3.8 days. Inoculation of 0.5 ml. guinea pig lung suspension and oxalated blood IP into each of two adult guinea pigs induced no signs or lesions over an eight week period. No further work has been done with guinea pigs.

3. Rabbits: Two rabbits, as discussed under Materials and Methods, were used for the production of antibodies to the Trenton strain. No illness developed in these two rabbits while undergoing immunization with live vaccine. They lived for eight months, were reimmunized and then exsanguinated. They were not studied further.

4. Chickens and Embryonating Eggs: Several attempts were made to induce infection in embryonating eggs. Original tissue suspensions from the Trenton swine killed in two to three days part of the 10-12 day embryos inoculated chorioallantoically or allantoically. Second mouse passage virus, Trenton strain, also induced deaths in part of the embryos inoculated. Passage of allantoic fluid from the dead embryos failed to induce death in new embryos. Similar results were obtained with Case No. 5. No further studies were made with embryonating eggs.

A fourth mouse passage virus of the Trenton strain was inoculated IC, 0.1 ml., 10⁻¹ through 10⁻⁷, five day old chicks, six or seven chicks per dose. Considerable excitement attended this dosage for a few hours following inoculation. No deaths occurred and birds were killed four weeks later. Another group of chicks, two to four weeks old, were inoculated IC with sixth mouse passage Trenton strain, seven birds; first mouse passage strain No. five to six birds; third mouse passage strain No. five to six birds; and third mouse passage strain No. three to 12 birds. Seven birds from these groups developed ataxia within one to four days and either died or were killed in extremis. Remainder of birds were killed in 16 days and discarded. Passage of chick brain suspension into seven chickens with Trenton strain, eight chickens with strain No. five, induced deaths in 7/8 with strain No. five, splenic suspension, on first and sixth days thereafter; remaining chicks were killed in 12 days and discarded. Passage of chick brains to mouse brains back to chick brains for one or two inter-species passage failed to adapt the virus to chick brains even though lethal doses for mice still remained in the chick brain tissues. No further work was done with chickens.

5. Dogs: Doctor Jackson (8) observed transient EKG changes in young dogs. It was theorized that dogs might be infected as pups with the EMC virus, and that they might undergo transient heart disease. Such theory was put to test. Unfortunately only one dog was young—a six month terrier;
the remaining three dogs were mature; two of them were two year old pointers, one male, one female; the fourth dog a 10 year old male hound. EKG's were obtained on all four dogs prior to inoculation; each received one ml. 20 percent brain suspension fifth mouse passage Trenton strain I.M. into the posterior aspect of the left thigh. Daily temperatures were recorded, weekly blood samples were drawn, and EKG's were done 10 days after inoculation in all except the female pointer that had had her second EKG 17 days following inoculation.

All four EKG's following inoculation were not essentially different from preinoculation tracings. Temperatures were within normal limits on the three adult dogs; the young dog had 104.6°F two days after inoculation. Total leukocyte counts were high on all pre-inoculation blood samples, and post inoculation counts did not change appreciably in the two pointers. The leukocyte count on the young dog was 20,150 two days pre-inoculation, 11,150 four days after inoculation, and 9,150 six days later; on the 10 year old dog the white count was 16,300 the day of inoculation, 13,700 four days later, 7,500 nine days later. Hematocrits done on all blood samples revealed no change. The dogs were killed by electrocution 10 to 17 days after inoculation. The gross lesions were edema of the mesenteric lymph nodes in the old dog and in the female pointer and many heart worms in the male pointer. Mesenteric lymph nodes of the old dog did not contain virus as tested intracerebrally in mice. Neutralization tests conducted on young dog pre-inoculation serum versus serum collected 10 days after inoculation revealed a neutralizing index of greater than $10^{3.21}$.

6. Swine: Three trials were conducted in inoculating twelve 40 to 60 pound pigs with the EMC virus. The first trial was most extensive and is described in considerable detail. No pigs died in the course of the experiments.

Trial No. 1 consisted of six hogs; Table No. II lists pertinent information. Temperatures were taken daily and were begun one day before the day of inoculation. Blood samples were drawn the day of infection and twice weekly thereafter for the first two weeks and weekly thereafter until the animals were disposed of. Total leukocyte counts, hematocrits, and viremic studies were performed on the bloods. Fecal samples were collected the same days as the bloods were drawn and were retained in the deepfreeze until they could be processed for virus recovery. Three pigs designated I, II, III were taken to Doctor Jackson's hospital for EKG tracings two days before inoculations were made. Five animals, I-IV and VI were kept in one large isolation room. Pig V, a non-infected control, was kept in a separate isolation room. Weights were taken on all pigs at the beginning of the experiments and again when the animals were killed. EKG's were done again Feb. 1 on all inoculated animals. On Jan. 13, 1961, the five animals were inoculated. The IC inoculation was performed under chloroform anesthesia. Pig III vomited immediately after inoculation. Oral infections were done by putting the inoculum on the feed. Pig No. III was killed by electrocution 2/6/61, Nos. IV and VI, 2/9/61, Nos. I, II, V, 2/10/61.
### TABLE II

Data on Pigs Experimentally Infected With E.M.C. Virus

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route-Dose</td>
<td>Intrac.</td>
<td>Oral</td>
<td>Intraven.</td>
<td>Intramus.</td>
<td>Control</td>
<td>Oral</td>
</tr>
<tr>
<td>Original</td>
<td>36</td>
<td>40</td>
<td>48</td>
<td>41</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>Final</td>
<td>63</td>
<td>58</td>
<td>60</td>
<td>57</td>
<td>90</td>
<td>71</td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus in Tissues?</td>
<td>Days p.i.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
<td>-</td>
<td>+†</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>-</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Neutr. Index</td>
<td></td>
<td>&gt;103.0</td>
<td>&gt;102.8</td>
<td>&gt;103.0</td>
<td>&gt;104.0</td>
<td>Neg.</td>
</tr>
<tr>
<td>Virus in Tissues?</td>
<td>Days p.i.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EKG</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>Not Done</td>
<td>—</td>
</tr>
<tr>
<td>Virus from Tissues</td>
<td>Lumbar cord</td>
<td>S.,* Int.</td>
<td>Mes. N.*</td>
<td>Lu, S, Br*</td>
<td>Not Done</td>
<td>S, Br</td>
</tr>
<tr>
<td>S, Br, Int, &amp; Cnts</td>
<td>Colon,</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mes, N.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Inoculum—fourth mouse brain passage, Trenton strain.
Neutralization Index—preinoculation serum vs. serum collected just prior to electrocution, Trenton strain used.
Lesion Legend—P is pericarditis; M. is myocarditis; R.V. is right ventricle.
Scar. M.—scar in myocardium and healing.
* Suspensions from italic words caused deaths in mice; it was not confirmed that such deaths were due to the E.M.C. virus.
† Specifically identified as the E.M.C. virus by neutralization test; Other +'s only tentative.
Days p.i.—days after inoculation.
Hematocrits, leukocyte counts, and temperatures were not essentially different from normal in the course of the experiments. Transient inappetence occurred the third day following inoculation; all inoculated animals were having loose bowel movements four days after infection and especially No. II which had a pronounced diarrhea that day. Average weights in the five animals at the beginning of the study was 44.2 pounds, at the end 62.6 pounds. The noninoculated control weighed 56 pounds at the start and 90 pounds at the termination of the experiment.

Feces were collected for virus recovery and stored in the Revco and later removed from the Revco and made into 20 percent suspensions in HBPSS. High speed centrifugation, 15,000 for 30 minutes, followed the low speed sedimentation of 3000 for 30 minutes. One-tenth ml. quantities were inoculated IP into four mice each. Defibrinated blood was held in the Revco; when time permitted, 0.5 ml. was inoculated IP into each of two mice with the three days p.i. bleeding. Three mice each were inoculated with 0.03 ml. defibrinated blood collected three, five, 10 and 14 days following inoculation. Mice were observed for 14 days.

At the time of necropsy, portions of tissue were removed and frozen; others were fixed. When convenient frozen specimens were made into 20 percent suspensions and tested IC in four mice each.

EKG’s taken 19 days after infection to quote Doctor Patterson (9) “I would say that the EKG’s are not too revealing, there being definitive evidence of myocardial damage only in pig No. three (III), and suggestive evidence in pig No. two (II). It might be that tracings taken at an earlier stage of the disease might show more.” Figure No. two gives the tracings taken of pig No. three (III).

Two other groups of pigs were infected in an attempt to induce more severe myocardial disease and deaths. Two pigs were isolated and inoculated intravenously with second mouse passage of Trenton strain, one animal receiving 0.6 ml. (No. VII) and the other receiving 0.2 ml. (No. VIII). These animals were killed six days later; no gross lesions were observed. Tissues studied for virus were from No. VII, lung, spleen, heart; from No. VIII, pancreas, kidney, spleen, and heart. Suspension from this heart killed 4/4 mice; confirmation of the presence of the EMC virus was not made.

A third group of pigs, two each, No. two and four, were infected orally with 1 1/2 ml. original heart, spleen, lung suspension from Case No. six (Table I) and one cc. 40 hr. washed broth culture of Salmonella organisms. Two other pigs, Nos. five and six, received same quantity of virus only. Pigs were temperatured daily and examined for signs of illness. The two animals receiving the mixed infections were killed six days later, those receiving virus only were killed 12 days later. No gross lesions were observed at necropsy. Tissues examined for virus were as follows: No. two—heart, colon and contents; No. four—heart, spleen, heart (2nd try), colon; No. five—heart; No. six—heart, colon and contents; italicized words as suspensions caused deaths in mice. Salmonellae were recovered from No. 2 colon and contents; these Salmonellae killed mice. Identification of EMC virus was not made.
FIGURE 1. Hearts from pigs experimentally infected with E.M.C. virus. Top row—left, No. IV, right, No. VI; bottom row, left to right, I, II, V.

Notice the atrophy, pitting, and apparent disruption of vascular supply to the right myocardium near the left longitudinal groove in all hearts except the normal heart (No. V)—particularly at the points of the arrows. The normal heart has a well-rounded right ventricle that the infected hearts do not have. The extensive pericarditis in VI may not be associated with EMC; neutralizing antibodies in this animal were equivocal (Table No. 2).

Salmonella were recovered from No. two colon and contents. Serums of pigs No. two and four (collected six days following inoculation) contained no neutralizing antibodies to sixth mouse passage Trenton strain. Serum No. five, 12 days after infection reacted similarly.

B. Gross and Microscopic Studies—1. Swine—(a) Natural Cases:

Gross lesions of numbers one and six, Table I, consisted of white streaks one-two x eight-10 mm. appearing in the myocardium beneath the epicardium. Lesions were confined primarily to the right heart; the spleen was shrunken in Case No. one. Hydropericardium was also present in group No. six. Microscopically the white areas were calcified as determined unequivocally by the Von Kossa (10) stain for calcium; in addition there was lymphocytic myocarditis and monocytic interstitial pneumonia. Moderate lymphocytic, monocytic meningoencephalitis was present in Case No. one. The kidneys,
spleen, and pons were normal; the liver had undergone fatty degeneration. Group No. six pigs had eosinophilic monocytic calcifying myocarditis in both animals examined. Congestion of the liver indicative of heart failure existed. The brain was negative.

Lymphocytic myocarditis was present in at least one other porcine case, No. four, in the bovine No. seven, and the raccoon No. nine. Many other conditions as seen on Table I existed in these natural cases.

(b) Experimental Studies:

Grossly the right hearts in trial No. one appeared most diseased with scarring, atrophy of the myocardium and apparent disruption of the vascular supply to the myocardium. Figure No. one illustrates these hearts. The outer wall of the heart of No. IV appeared much thinner than normal. No white streaks as seen in the hearts of natural cases were seen in the experimental animals. Microscopic examination of these hearts revealed a healing stage of lymphocytic myocarditis.
2. Pathology of Experimental Infections in Mice and Guinea Pigs:

Hearts, spinal cords and brains from mice dying or being killed with the Trenton strain four to eight days following either IC or IP inoculation were examined. Severe lymphocytic myocarditis and encephalitis were present. Scattered areas of perivascular lymphocytic infiltration occurred in the brain and spinal cord accompanied by demyelinization. Grossly the hearts of some mice that died from the infection possessed grayish discoloration of the myocardium and pinpoint white foci characteristic of the lesions in the natural swine cases. Calcification was noticed in some of the mouse hearts; it has previously been described by Schmidt (11). The urinary bladder of dead mice was frequently fully distended and the spleen shrunken.

One guinea pig dying in the course of attempts to infect said species possessed paleness of the ventricular myocardium. Microscopic examination of this heart and the brain revealed extensive lymphocytic myocarditis and encephalitis.

IV. Discussion:

The significance of the presence of this virus or its neutralizing antibodies in several species of animals in Florida is perhaps not yet clearly defined or understood; in the squirrel and raccoon the deaths may be attributed solely to the virus. In the porcine and bovine natural cases, the myocarditis certainly indicates cause and effect by the virus; the complicating conditions such as hog cholera, salmonellosis, phosphorus poisoning, etc., cloud the picture and suggest that at least in swine a secondary stress factor may have to be present to induce full blown disease. The stunting of the survivors in Case No. 1 is significant; a trend toward diminished growth rate appeared experimentally although, admittedly, the experiment was not well controlled. The experimental study in rats by Kilham and Olivier (12) may hold true for swine; they showed that rats previously infected with Trichinella when given the encephalomyocarditis virus sickened and died whereas either parasite inoculation or virus inoculation alone induced little or no disease. Our experimental study in swine pictures the virus as not especially lethal for this species yet still capable of inducing myocarditis; the Panama workers, however, did cause deaths in their studies (2, 13) either by parenteral or by oral administration. Where this virus fits into the scheme of the porcine or bovine enteroviruses is yet to be studied. It has been classified as an enterovirus (14). The significance of finding neutralizing antibodies in an equine serum is not known.

The seeming predilection of the virus for the porcine right myocardium is interesting. Although Murnane et al. (2) and Craighead et al. (13) did not notice such a predilection grossly, they reported the highest virus titer in the right myocardium of all porcine tissues examined. One might speculate as to its cause: (1) Is the vascular arrangement of the right ventricle different from the left; (2) As is true with poliomyelitis in man where only certain neurons become diseased, are certain myocardial fibers more sus-
ceptible to infection than others; (3) Are there virus inhibitors in one area of the heart that do not exist in other areas?

Porcine encephalitis does not appear to be a consistent finding with this infection. The calcified myocardial lesions in the pig hearts are most fascinating. The pathogenesis of their occurrence must be that the myocardial fibers die very suddenly; following necrosis the pH rises causing precipitation of the calcium salts. As blood elements move in, the pH again lowers and the calcium is removed. The calcified lesions are probably of short duration in time, and in fact we have had difficulty in retaining the calcium in museum specimens. Although to date we have found no evidence to refute the findings that the calcified myocardial lesions in swine are pathognomonic for EMC infection, in one dog with distemper and in a pheasant with eastern encephalitis, Gainer and Murchison (15) have seen myocardial calcification.

The distribution of the virus in swine is apparently widespread as the map indicates. Perhaps the rat is the definitive host of the virus and swine become infected by ingesting rat feces. Warren et al. (16), demonstrated widespread occurrence of neutralizing antibodies in rats in various States over 12 years ago and the 87th Congress report (17) lists the virus as worldwide in distribution.

The pathogenicity of the virus for mice, guinea pigs, and embryonating eggs is similar to the findings of Pope (3). Although no gross heart disease was induced in the experimental dogs, the drop in leukocyte count suggested viral infection.

Summary:

1. The encephalomyocarditis virus or neutralizing antibodies thereto have been found in seven counties in Florida in eight cases of swine, a calf, a hinny, a raccoon, and a squirrel. The natural cases are discussed briefly.

2. The prominent lesion associated with the isolation of this agent is a lymphocytic myocarditis sometimes accompanied by calcification; encephalitis is apparently not a common finding in swine.

3. Experimental myocarditis was readily reproduced in swine following oral or parenteral inoculation. Myocarditis and encephalitis were readily reproduced in mice and occasionally in guinea pigs. Rabbits, embryonating eggs, young chicks, and dogs appeared refractory to experimental inoculation. An EKG of a diseased pig is presented.

BIBLIOGRAPHY


9. Patterson, Donald F.: Personal communication, Spring, 1961, Comparative Cardiovascular Studies Unit, The School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Penna.
17. Veterinary Medical Science and Human Health: 87th Congress, 1st Session; Committee Print, Veterinary Activities of Agencies of the United States Government in Relationship to Functions Performed by State and Local Governments, Private Groups, and Intergovernmental Organizations, Aug. 10, 1961, pp. 58.
ROSTER OF MEMBERS BY STATES

ALABAMA
Official member
J. Milligan
Individual members
J. B. Armstrong
E. T. Bray
G. W. Cooper
G. D. Ingram
B. N. Lauderdale
J. Milligan
C. H. Poitevint
C. S. Roberts
J. B. Taylor

CALIFORNIA
Official member
J. E. Stuart
Individual members
D. Addis
R. A. Bankowski
L. E. Bartelt
J. T. Bell
A. G. Boyd
V. C. Bunker
California Cattlemens' Ass'n
Cal. Farm Bureau

ARIZONA
Official member
W. M. Thompson
Individual members
Circle One Livestock Co.
L. H. Fuller
John Jacobs Farms
E. R. Mackery
N. W. Rokey

ARKANSAS
Official member
D. Ibsen
Individual member
T. O. McMillan
E. H. Peterson

ALASKA
Official member
J. Honsinger

Calif. Veterinary Med. Association
H. S. Cameron
J. Carriacaburu
N. H. Casselberry
J. Chapman
J. F. Christensen
C. J. Claire
D. S. Clark
J. C. Davidson
J. M. Davison
G. L. Dayman
Delta Laboratories
R. S. Dickson
A. C. Emminger
C. J. Ferreira
V. E. Fisher
R. W. Fogleman
J. N. Fulmor
T. J. Hage
R. H. Haight
P. Haims
J. Hart
R. J. H. Holte
J A. Howarth
W. T. Hubbert
W. F. Hughes
W. P. Humphrey
R. H. Hurt
L. M. Hurt
T. W. Jackson
S. L. Jamison
D. E. Jasper
H. N. Johnson
K. L. Johnson
F. P. Jones
J. King
C. H. Kinaley
L. H. Krum
W. F. Lamoreux
Brennan & Laskey
L. R. Libby
S. H. Madin
R. C. Maris
B. McGowan
K. G. McKay
D. G. McKercher
M. E. McElroy
MEMBERSHIP ROSTER

K. F. Meyer
L. D. Meyers
W. S. Murphy
J. Nehay
Nicholas Turkey Farms
W. Ofenheim
C. H. Ozanian
J. C. Pace
R. H. Packard
H. H. Page
H. H. Parrell
J. L. Pellissier
F. B. Pulling
L. G. Raggi
G. A. Railsback
R. D. Richards
W. H. Rockey
A. S. Rosenwald
W. L. Rottman
W. W. Sadler
San Diego County
F. F. Saint
K. Schaal
O. W. Schalm
H. E. Schaulis
R. C. Schock
C. R. Schroeder
F. Scott
F. S. Scott
E. F. Sheffield
J. L. Sorensen
A. T. Spencer
D. E. Suther
L. Tietze
G. N. Tucker
W. D. Urban
J. S. Watson Dairy
G. B. E. West
R. W. Wichmann
H. F. Wilkins
W. P. Wing
W. W. Worcester

R. W. McIntyre
L. F. Meier
J. Pecaro
R. L. Phillips
R. Robusto
W. L. Rottman
R. C. Schock
R. J. Schroeder
G. N. Tucker
H. Van Dam
G. K. Van Vleck
W. A. Young

COLORADO

Official member
W. C. Tobin

Individual members
W. W. Brown
T. L. Chow
W. A. Clark, Jr.
E. J. Cole
J. R. Collier
E. S. Cox
C. L. Davis
N. Frank
R. M. Gou
L. A. Griner
A. B. Hoerlein
M. Huff
W. O. Kester
B. Shambaugh
W. C. Tobin

CONNECTICUT

Official member
J. V. Smith

Individual members
J. W. Beck
F. Ferrigno
B. Lipman
J. V. Smith

LOS ANGELES COUNTY

Official member
R. J. Schroeder

Individual members
C. A. Delli Quadri
A. F. Eckert
R. C. Hubbard

DELWARE

Official member
H. J. Ricker, Jr.

Individual members
D. F. Fogg
H. N. Lasher
R. L. Ricker
H. J. White
C. A. Woodhouse

DISTRICT OF COLUMBIA
Official member
R. J. Anderson
Individual members
American Stockyards Ass’n
R. J. Anderson
J. Hourrigan
K. F. Johnson
R. N. Konnerup
J. J. Martin
J. A. McCallam
N. L. Meyer
C. H. Pals
A. F. Ranney
R. C. Reisinger
C. E. Robinson
E. E. Saulmon
H. W. Schoening
J. Splitter
A. L. Tellejohn
C. Wise

GEORGIA
Official member
J. W. Mann
Individual members
O. M. Bateman
Y. J. Jones
J. A. Kimsey
R. J. Lee
J. Lieberman
C. J. Mikkel
L. A. Mosher
W. C. Patterson
T. Russell, Jr.
S. C. Schmittle
L. E. Starr
W. F. Schroeder
J. H. Steele
F. T. Sutton
J. M. Sutton
E. S. Tierkel

HAWAII
Official member
E. H. Willers
Individual members
Kualoa Ranch, Ltd.
G. H. Murphy
E. H. Willers

IDAHO
Official member
A. P. Schneider
Individual members
H. H. Hoyt
A. K. Kuttler
H. L. McEwan
A. P. Schneider

ILLINOIS
Official member
R. A. Thompson
Individual members
R. F. Baker
D. E. Bartlett
C. E. Blye
W. J. Boddington
L. E. Boley
MEMBERSHIP ROSTER

A. C. Bolle
A. E. Bott
A. R. Bott
C. L. Clark
A. J. Coale
J. W. Cunkelman
H. C. Curtis
L. R. Davenport
L. A. Dykstra
A. Freeman
J. O. Gwin
J. Harvey
J. G. Hardenbergh
J. R. Hay
J. G. Heck
H. E. Held
C. D. Hertich
C. B. Hostetler
Institute Amer. Poultry Ind.
M. L. Johnson
R. C. Kamm
E. C. Khuen
E. M. Kiggins
C. H. Koonz
B. L. Lake
A. J. Legner
N. D. Levine
F. H. Madden
F. C. Mau
A. C. Merrick
A. K. Merriman
C. A. Metz
A. G. Misener
R. L. Morin
G. Novotny
J. F. Palmer
D. A. Price
J. D. Ray
H. C. Rinehart
C. M. Rodgers
M. D. Schneider
D. Segre
O. W. Seher
J. Simon
H. L. Sparks & Co.
S. S. Swift
J. R. E. Taylor
W. L. Wake
R. A. Thompson
E. E. Tuttle

L. M. Webb
H. E. Wilson
P. D. Wittlinger

INDIANA

Official member
D. L. Smith

Individual members
Arnold Laboratories
L. R. Barnes
Belt Railroad & Stock Yards Co.
J. S. Bower
F. H. Brown
J. F. Bullard
T. Burnstein
Conner Prairie Farm
C. R. Cutler
A. L. Delez
J. M. Drogé
G. W. Gillie
G. D. Goetsch
F. O. Gossett
D. P. Gustafson
F. A. Hall
P. J. Hirt
R. L. Hogue
Indianapolis Stockyards Co.
R. V. Johnston
R. C. Klussendorf
B. La Salle
McMahah-McClead
R. L. Morter
National Swine Growers Council
J. W. Newberne
O. H. Patrick
V. B. Robinson
J. T. Tumlin
O. Wesson
E. A. White
B. V. Whiteman
J. Williams
C. J. York

IOWA

Official member
A. L. Sundberg

Individual members
P. C. Bennett
G. E. Blake
I. H. Borts
MEMBERSHIP ROSTER

W. C. Brantley  
D. A. Buchanan  
E. A. Carbrey  
F. L. Clark  
J. H. Clements  
Diamond Laboratories  
G. T. Edds  
P. W. Elam  
E. M. Ellis  
A. H. Frank  
W. A. Hagan  
S. L. Hendricks  
J. B. Herrick  
W. A. Higgins  
R. H. Hoyt  
E. D. Hubbard  
T. B. Huff  
D. E. Hughes  
W. H. Hurst  
Iowa Farm Service Co.  
Iowa Farm Bureau  
J. R. Ipson  
G. O. Johnson  
N. K. Jungk  
A. H. Killinger  
G. Lambert  
A. B. Larsen  
C. D. Lee  
C. A. Manthei  
P. J. McAndrew  
W. F. McCulloch  
I. A. Merchant  
B. A. Moore  
L. O. Mott  
G. B. Munger  
H. S. Nicol  
T. F. Oeth  
C. Peterson  
W. R. Pritchard  
A. N. Richey  
M. H. Roepke  
J. E. Salsbury  
J. G. Salsbury  
H. P. Sandberg  
I. M. Saturen  
E. Schneckloth  
G. O. Schubert  
L. H. Schwarte  
J. F. Setler  
R. H. Singer  
G. B. Smith  
M. L. Spear  
A. L. Sundberg  
W. P. Switzer  
J. P. Torrey  
C. D. Van Houwelling  
G. B. Van Ness  
E. E. Wedman  
J. P. Woodbridge  
W. D. Yoder

KANSAS

Official member  
A. G. Pickett

Individual members  
Armour & Co.  
E. L. Boley  
K. M. Curts  
R. R. Dykstra  
V. D. Foltz  
E. J. Frick  
N. D. Harwood  
J. F. Hudelson  
A. Kushner  
E. E. Leasure  
D. O. Manley  
R. B. Meeks  
R. W. Menges  
R. S. Pyles  
L. T. Railsback  
M. J. Twiehaus

KENTUCKY

Official member  
R. W. Hammermeister

Individual members  
E. Beauchamp  
H. N. Davis  
E. R. Doll  
C. E. Eastin  
R. T. Gross  
R. W. Hammermeister  
J. C. Luckett  
F. D. Maurer  
C. H. Myers

LOUISIANA

Official member  
F. B. Wheeler
MEMBERSHIP ROSTER

Individual members
H. B. Elliott  
W. T. Oglesby  
A. G. Pass  
E. E. Roth  
F. B. Wheeler  
L. P. Williams, Jr.

MAINE

Official member  
F. G. Buzzell

Individual members
F. G. Buzzell  
A. R. Corey  
A. Kalavaitis  
T. L. Roundtree  
J. F. Witter

MARYLAND

Official member  
A. L. Brueckner

Individual members
J. S. Andrews  
G. S. Appleton  
F. R. Bang  
P. Brandly  
A. L. Brueckner  
R. J. Byrne  
C. Cotton  
D. E. De Tray  
C. Durbin  
F. Enzie  
J. W. Hastings, Sr.  
L. C. Heemstra  
W. R. Hinshaw  
S. B. Hitchner  
A. A. Holbrook  
O. J. Hummon  
H. W. Johnson  
A. M. Lee  
J. C. Lotze  
L. Manlove  
B. R. McCrory  
G. Migaki  
C. K. Mingle  
J. M. Moultshrop  
L. C. Murphy  
O. L. Osteen  
A. H. Peck  
C. E. Robinson  
T. O. Roby  
H. E. Schaden  
A. S. Schlingman  
W. T. Shalkop  
C. A. Smith  
R. E. Willie  
M. Willis, Jr.

MASSACHUSETTS

Official member  
E. M. Dwyer

Individual members
A. A. Barry  
W. E. Brielman  
E. R. Coon  
A. L. Kelly  
D. Peck  
W. L. Rehkemper  
C. Thibealt  
E. F. Trainor  
H. Van Roekel

MICHIGAN

Official member  
J. Quinn

Individual members
R. D. Barner  
H. S. Bryan  
C. H. Cunningham  
C. C. Ellis  
Hamilton Farm Bureau Coop., Inc.  
R. F. Huffaker  
W. L. Mallman  
J. P. Newman  
G. W. Reed  
H. Rothenbacker  
R. M. Scott  
C. B. Smith  
O. J. Sorenson, Jr.  
G. G. Stocking  
E. J. Van Tilborg  
A. Winter

MINNESOTA

Official member  
J. C. Flint

Individual members
A. M. Anderson
MEMBERSHIP ROSTER

MISSOURI

Official member
L. A. Rosner

Individual members
Allied Laboratories, Inc.
American Hereford Association
C. H. Beckman
F. W. Binkley
E. L. Cary
A. A. Case
T. M. Christison
City of St. Louis
C. M. Cooper
W. Cozad
M. L. Crutcher
R. L. Cuff
J. M. Cullison
C. W. Darby
F. C. Davis
T. Davis, Jr.
G. L. Dunlap
J. K. Farrell
H. E. Gouge
E. F. Huffman
R. M. Keith
R. E. Lubbehusen
J. C. McCrea
H. C. McDougle
F. M. Murdock
C. R. Omer
I. Paton
L. A. Rosner
C. F. Rossow
C. T. Sanders
F. N. Schlaegel
W. C. Schofield
R. Seiden
J. B. Simpson
D. K. Spalding
B. Strickler
M. L. Sutton
B. Weiner
J. A. Whiting
H. W. Young

MISSISSIPPI

Official member
V. D. Chadwick

Individual members
L. J. Pate
B. T. Simms, Jr.
J. E. Williams

MONTANA

Official member
J. W. Safford

Individual members
J. S. Brenner
B. O. Fisher
H. E. Furgerson
S. D. Gates
W. J. Hadlow
O. J. Halverson
W. W. Hawkins
P. Itcaina
H. Marsh
J. D. Melcher
R. J. Miser
Montana Stockgrowers Assn.
B. Orcutt
G. Prescott
John W. Safford
G. A. M. Sharman
R. C. Timmons
E. A. Tunnicliff
I. W. Vinsel
A. Wilson

**NEBRASKA**

*Official member*

F. E. Zegenbein

*Individual members*

Amer. Shorthorn Breed Assn.
E. M. Baldwin
D. L. Berry
A. L. Brown
Central City Veterinary Clinic
Corn States Laboratories, Inc.
C. O. Emrich
O. D. Grace
Grain Belt Supply Company
Grand Island Livestock Auction Market
E. C. Howe
Hundley & Landholm
R. W. Smith
E. C. Jones
W. F. Klusmire
J. J. Lewis
W. T. London
V. Marshall
P. C. Molgard
C. J. Norden, Jr.
E. H. Nordstrom
S. F. Rosner
K. J. Trout
T. R. Weatherly
Webb Livestock Comm.
L. A. Wilcox
F. E. Zegenbein

**NEVADA**

*Official member*

J. L. O'Harra

*Individual member*

W. F. Fisher
E. M. Joneschild
K. L. Kuttler
J. L. O'Harra

**NEW HAMPSHIRE**

*Official member*

R. W. Smith

*Individual members*

F. E. Allen
G. C. Cilley
F. L. Clark
Great Elm Farm, Inc.
R. L. Hill
E. W. Simmons
R. W. Smith

**NEW JERSEY**

*Official member*

E. L. Brower

*Individual members*

R. L. Alkire
W. C. Alpaugh
R. S. Armstrong
C. O. Baughn
G. B. Belloff
J. A. Bivins
W. A. Bonev. Jr.
E. L. Brower
P. R. Cebulka
W. P. Doherty
T. Goldhaft
D. F. Green
R. A. Hendershott
C. P. Huff
C. K. Jewell
R. E. Kerlin
F. W. Kingsbury
J. S. Kiser
W. R. Laslocky
N. N. Lichtman & Bros.
H. K. Mohr
F. Olbrich
D. H. Parella
W. B. Platt
MEMBERSHIP ROSTER

NEW MEXICO

Official member
F. H. Sharp

Individual members
J. Bielfeldt
L. E. Bodenweiser
D. W. Francis
H. E. Kemper
A. K. Mitchell
H. O. Peterson
F. L. Schneider
F. H. Sharp

NEW YORK

Official member
G. S. Kaley

Individual members
Amer. Dairy Science Ass'n
H. P. Aronson
D. W. Baker
J. A. Baker
T. L. Barber
D. O. Bixby
R. Boese
C. G. Bradt
E. R. Braun
N. Bruce
G. E. Burch
J. J. Callis
L. E. Carnichael
R. M. Clark
L. Coggins
E. C. & H. J. Cleveland
H. R. Cox
H. R. Cunliffe
S. P. Dansky
A. H. Dardiri
P. D. Delay
R. T. Ellison
W. M. Evans
M. G. Fincher
R. C. Fish
H. G. Foote
E. J. Froelich
P. Galbiunas
R. E. Geisser
J. H. Gillespie
J. A. Gourlay
J. H. Graves
J. V. Hills
Holstein-Friesian World
G. H. Hopson
J. Hwang
W. E. Jennings
H. L. Jones
E. L. Jungherr
G. S. Kaley
S. J. Kallish
Ketcham Mfg. Co., Inc.
J. S. Kiser
P. Langer
P. P. Levine
E. S. Markham
F. S. Markham
G. W. Mather
J. L. McAuliff
R. T. McCarty
G. E. McConnell
R. W. Metzger
J. C. Minster
W. V. Phillips
G. Poppensiek
C. W. Potter
R. J. Price
F. I. Reed
S. J. Roberts
E. L. Robinson
D. S. Robson
B. Sann
W. P. Sargent, Jr.
J. H. Scruggs
M. S. Shahan
W. H. Shaw
B. E. Sheffy
R. E. Shope
L. R. Sinclair
Sir William Farm
L. Rubin
J. B. Smith
L. L. Smith
G. W. Snook
MEMBERSHIP ROSTER

NORTH CAROLINA

Official member
H. J. Rollins

Individual member
M. P. Hines
J. Rich
T. Zweigert

NORTH DAKOTA

Official member
D. E. Flagg

Individual members
F. M. Bolin
D. F. Eveleth
M. T. Lund
T. R. Myers
R. Schnell

OHIO

Official member
H. G. Geyer

Individual members
E. L. Akins
J. R. Allgyer
T. L. Bibb
E. H. Bohl
C. E. Boyd
J. R. Bracelin
J. K. Bratton
Paul Brown
J. F. Cavanaugh
Ceros Supply Company
G. D. Cloyd
M. S. Coates
W. M. Dillard
J. H. Drayer
J. R. Eggleston
G. L. Ely
H. A. Etling
R. L. Gerber
H. E. Goldstein
J. E. Graber
P. D. Harwood
J. H. Helwig
D. A. Hill
G. L. Hiller
C. F. Jones
E. J. Kersting
F. R. Koutz
W. R. Krill
W. F. Leese
Leipsic Veterinary Service
Linn & Taylor
A. G. Madden, Jr.
W. F. Nape
S. W. Pawlowski
A. C. Peters
R. R. Rainier
L. E. Shawhan
G. L. Shook
E. L. Shroyer
J. L. Stansbury
H. A. Stuchell
J. Van Aken
K. D. Weide
S. T. Wilson, Jr.

OKLAHOMA

Official member
M. N. Reimenschneider

Individual members
P. B. Barto
W. E. Brock
J. B. Corcoran
G. T. Easley
F. W. Hansen, Jr.
G. C. Holm
L. H. Moe
A. W. Monlux
E. G. Ongert
R. J. Panciera
C. C. Pearson
M. N. Riemenschneider

OREGON

Official member
G. B. Rae

Individual members
A. G. Beagle
J. F. Bone
D. H. De Jong
E. M. Dickinson
J. Minor
MEMBERSHIP ROSTER

O. H. Muth
Nyssa Veterinary Clinic
Oregon Dairymans Association
K. J. Peterson
Portland Union Unionstockyds Co.
Rose City Veterinary Hosp.
L. A. Septon
D. H. Smith
J. W. Southworth

Pennsylvania

Official member
R. L. Elsea

Individual members
R. H. Alexander
Mark W. Allam
B. P. Anderson
A. Balshi
F. L. Barshinger
R. W. Boone
C. P. Bishop
C. A. Bottorff
L. G. Clark
L. S. Cushing
M. R. Derk
J. A. Deubler
K. E. Diehl
H. W. Dunne
H. L. Easterbrooks
R. L. Elsea
R. Fagan
R. F. Gentry
C. W. Gifford
Samuel B. Guss
R. A. Huebner
R. B. Jackson
R. A. Kern
W. F. Kline
E. S. Kutish
L. Leibovitz
I. Live
M. P. Lose
R. R. Marshak
H. M. Martin
P. Mayer
J. T. McGrath
R. E. McKinley
C. H. McLaughlin
R. W. McMullen
G. L. Mueller
C. F. Oppenlander
J. E. Prier
E. Power
H. L. Ragsdale
C. Raker
J. A. Rohlf
W. G. C. Savage
W. F. Schaefer, Jr.
J. H. Schall
S. F. Scheidy
P. H. Seitz
R. F. Shigley
H. B. Smith
P. C. Snoke
D. L. Snyder
E. L. Stealry
J. E. Stefanick
J. J. Strickler
E. L. Stubbs
R. E. Swope
L. F. Van Gorder
J. W. Walker
J. R. Wiley

Rhode Island

Official member
T. J. Grennan

South Carolina

Official member
R. W. Carter

Individual members
B. W. Bierer
J. B. Guess, Jr.
C. L. Vickers

South Dakota

Official member
M. D. Mitchell

Individual members
C. Addison
F. C. Driver
G. S. Harshfield
L. D. Jones
R. M. Scott
Sioux Falls Stockyards Co.
S. D. Federation of Dairy Farmers
S. D. Stockyards Ass'n
MEMBERSHIP ROSTER

TENNESSEE

Official member
C. E. Kord

Individual members
J. B. Champlin
L. E. Fredrickson
H. L. Fry
O. E. Harrison
R. W. Lawrence
W. F. Sims
L. W. Turner

TEXAS

Official member
R. G. Garrett

Individual members
D. J. Anderson
G. W. Apple
D. M. Bandy
J. S. Bridwell
M. R. Callaghan
H. D. Carpenter
E. M. Christopherson
R. A. Crandell
R. C. Dunn
J. B. Finley
T. E. Franklin
L. C. Grumbles
H. L. Hurst
F. P. Jaggi
R. J. Kleberg, Jr.
C. F. Layton
J. N. McCamish
R. W. Moore
C. O. Morgan
J. K. Northway
A. A. Price
T. Rea
I. W. Rupel
J. W. Sartwelle
V. H. Scroggs
L. E. Seay
R. D. Turk
B. Wedemeyer
J. L. Wilbur, Jr.
E. R. Willmann

INDIVIDUAL MEMBERS
O. J. Eggers, Jr.
J. E. Manning
L. Miner
J. E. Rasmussen
J. L. Shupe

VERMONT

Official member
A. E. Janawicz

Individual members
J. W. Armstrong
Holstein-Friesian Ass'n
D. Gibson
H. B. Stetson

VIRGINIA

Official member
W. L. Bendix

Individual members
R. J. Anderson
J. W. Atkinson
M. Bay, Jr.
W. L. Bendix
W. W. Brainard, Jr.
M. R. Clarkson
J. W. Davis
D. De Camp
I. Erickson
G. B. Estes
O. F. Foley
F. B. Gluckstein
J. M. Hejl
R. P. Jones
R. L. Knudson
W. W. McMichael
A. R. Miller
D. Miller
F. J. Mulhern
R. E. Omohundro
A. F. Ranney
E. C. Roukema
E. A. Schilf
R. K. Somers
W. R. Strieber
F. A. Todd
J. W. Walker
R. D. Wenger
C. W. Wilder
E. J. Wilson

UTAH

Official member
J. W. Whitely
### MEMBERSHIP ROSTER

#### WASHINGTON
- **Official member**
  - D. McGill
- **Individual members**
  - D. Duby
  - R. K. Farrell
  - R. I. Hostetler
  - Johnsen's Veterinary Clinic
  - D. A. McGill
  - T. R. Phelps
  - F. M. Shigley
  - W. H. Patton
  - H. B. Piper
  - J. D. Russell
  - R. O. Rydell
  - W. H. Thompson
  - M. Usdin
  - E. A. Woelffer
  - W. R. Winner
  - Wis. Feeder Pig Marketing Co.
  - W. Wisnicky

#### WEST VIRGINIA
- **Official member**
  - T. P. Siburt
- **Individual members**
  - J. S. Dailey
  - T. C. Green
  - J. O. Heishman
  - W. B. Homan
  - L. J. Meyer
  - V. H. Miller
  - C. McClintic
  - Morlunda Farms
  - O. Nelson
  - N. O. Olson
  - W. E. Sellards
  - T. P. Siburt

#### WISCONSIN
- **Official member**
  - A. A. Erdmann
- **Individual members**
  - M. Agee
  - W. W. Arzberger
  - D. T. Berman
  - R. F. Bristol
  - C. R. Curtis
  - J. R. Curtis
  - W. Dreher
  - R. P. Hanson
  - W. M. Johnson
  - W. D. Knox
  - L. L. Larson
  - S. H. McNutt
  - I. W. Moyle
  - J. C. Nowlen
  - Carl Olson
  - J. A. Patton
  - W. H. Patton
  - H. B. Piper
  - J. D. Russell
  - R. O. Rydell
  - W. H. Thompson
  - M. Usdin
  - E. A. Woelffer
  - W. R. Winner
  - Wis. Feeder Pig Marketing Co.
  - W. Wisnicky

#### WYOMING
- **Official member**
  - E. D. Barrows
- **Individual members**
  - G. H. Good
  - L. H. Smith
  - J. O. Tucker
  - Wyoming Stock Growers Ass'n.
  - Wyoming Woolgrowers Ass'n

#### ARGENTINA
- B. D. Blood
- F. Rosenbusch
- C. Rosenbusch
- B. Szyfres

#### AUSTRALIA
- G. W. Ward

#### BRAZIL
- P. R. Ellis
- W. M. Henderson

#### CANADA
- **Official member**
  - K. F. Wells
- **Individual members**
  - Alberta Stockyards Co., Ltd.
  - J. G. Anderson
  - E. C. Carlson
  - G. H. Collacutt
  - J. F. Frank
  - J. A. Henderson
  - Holstein-Friesian Ass'n. of Canada
  - R. J. Julian
  - L. H. Karstad
  - H. Konst
  - G. T. Labelle
MEMBERSHIP ROSTER

A. E. Lewis
E. B. Meads
D. C. Maplesden
R. J. McClanaghan
E. L. Nundal
M. Savan

CHILE
R. Rodriguez

COLUMBIA
H. J. Keane

ENGLAND
M. Crawford

HOLLAND
C. N. Dale

ISRAEL
Y. S. Goor

ITALY
M. Petek

MEXICO
F. Camargo N.

NEW SOUTH WALES
A. Webster

NEW ZEALAND
S. Jamieson

PERU
M. Moro

PUERTO RICO
Official member
L. R. Santos
Individual Members
F. E. Henderson

SCOTLAND
A. Robertson

SWITZERLAND
E. Hess
M. M. Kaplan
H. Keller
W. Leeman

TURKEY
H. Baskaya
W. M. Moulton

URUGUAY
H. Trenchi

VENEZUELA
M. Villegas D.

VIRGIN ISLANDS
Official member
L. A. Fahlund

WEST GERMANY
H. C. Roots
D. Strauch

YUGOSLAVIA
T. Angelovski
P. Vladimir
C. Franc
SIXTY-SIXTH
ANNUAL MEETING
Oct. 29, 30, 31, Nov. 1, 2, 1962
MAYFLOWER HOTEL
Washington, D. C.