PROCEEDINGS

SEVENTY-FOURTH

ANNUAL MEETING

of the

UNITED STATES ANIMAL
HEALTH ASSOCIATION

and

THIRTEENTH ANNUAL CONFERENCE
OF AMERICAN ASSOCIATION OF
VETERINARY LABORATORY
DIAGNOSTICIANS

WARWICK HOTEL
Philadelphia, Pennsylvania
October 8, 19, 20, 21, 22, 23, 1970
PROCEEDINGS

SEVENTY - FOURTH
ANNUAL MEETING

of the
UNITED STATES
ANIMAL HEALTH
ASSOCIATION

Warwick Hotel
Philadelphia, Pennsylvania

October 18, 19, 20, 21, 22, 23, 1970
# CONTENTS

| Report of the Committee on Animal Virus Characterization — S. McConnell, <i>et al.</i> | ................................................................. | 1 |
| Report of the Committee on Livestock Commerce — G. B. Rea, <i>et al.</i> | ................................................................. | 4 |
| Report of the Committee on Animal Welfare — G. S. Kaley, <i>et al.</i> | ................................................................. | 7 |
| Field and Laboratory Aspects of Bluetongue in Sheep, Cattle, and Possibly Wild Ruminants — A. J. Luedke | ................................................................. | 9 |
| Report of the Committee on Evaluation and Development of State-Federal Program — J. G. Milligan, <i>et al.</i> | ................................................................. | 22 |
| Report of the Committee on Infectious Diseases of Sheep and Goats — J. L. Hourigan, <i>et al.</i> | ................................................................. | 26 |
| Report of the Committee on Import-Export — J. F. Quinn, <i>et al.</i> | ................................................................. | 31 |
| Report of the Committee on State-Federal Relations — M. D. Mitchell, <i>et al.</i> | ................................................................. | 34 |
| Report of the Committee on Mastitis — J. S. McDonald, <i>et al.</i> | ................................................................. | 48 |
| Extending Regulatory Work Cooperatively — Robert C. Hammond | ................................................................. | 50 |
| Report of the Committee on Professional Education and Extension — R. C. Hammond, <i>et al.</i> | ................................................................. | 54 |
| Report of the Committee on Parasitic Diseases and Parasiticides — W. C. Tobin, <i>et al.</i> | ................................................................. | 55 |
| Trends in Veterinary Biological Production — A. L. Brown | ................................................................. | 60 |
| Report of the Committee on Veterinary Biologics — Carl J. Norden, <i>et al.</i> | ................................................................. | 66 |
| Regulatory Action to Control Violation of Toxic Residues — H. M. Trabosh | ................................................................. | 68 |
| A Look at the Position of Public Health and Preventive Medicine in Current and Future Veterinary College Curricula — W. E. Jennings | ................................................................. | 73 |
| Auto-Tutorial Meat Inspection Training for Contract Veterinarians — James A. Libby | ................................................................. | 79 |
| Report of the Committee on Meat and Poultry Hygiene — W. E. Jennings, <i>et al.</i> | ................................................................. | 85 |

## CATTLE

<i>Infectious Diseases</i>

The <i>Haemophilus somnus</i> Complex — L. N. Brown, R. C. Dillman, and R. E. Dierks | ................................................................. | 94 |

Report of the Committee on Infectious Diseases of Cattle — J. B. Herrick, <i>et al.</i> | ................................................................. | 109 |
Anaplasmosis
Neonatal Isoerythrolysis of the Bovine — J. S. Wilson, and J. C. Trace 115
Progress in the Control of Bovine Anaplasmosis — T. O. Roby, T. E. Amerault, and B. R. McCallon 122
Control of Anaplasmosis Under Wyoming Conditions — G. M. Thomas 129

Brucellosis
Brucellosis Eradication Program — E. A. Schilf, and H. C. King 136
45/20 Vaccine — W. C. Ray and Joe Hendricks 147
Brucella Ovis in Ram — R. E. Simmons, et al. 157

Leptospirosis
Vaccination of Cattle with Leptospiral Bacterins. 1. Serologic and Cultural Results of Leptospiral Challenge — A. H. Killinger, L. E. Hanson, M. E. Mansfield, and H. A. Reynolds 165
Vaccination of Cattle with Leptosporosis Bacterins. II. Potency Assays Using Hamster Passive Protection — Ronald Huhn, Kenneth Claus, and M. E. Machek 178
Report of the Committee on Leptospirosis — R. L. Morter, et al. 197

FOREIGN ANIMAL DISEASES
Spectrum of Clinical Foot-and-Mouth Disease in Steers — J. H. Graves 199
Bovine Herpes Mammillitis-Like Disease Diagnosed in the United States — R. J. Yedloutsching 208
Growth of Foot-and-Mouth Disease Virus in Bovine Pharynx — J. W. McVicar 225
Joint Campaign Against Rinderpest in Africa — D. DeTray 230
Influence of Enterovirus in Foot-and-Mouth Disease Virus Infection — P. Sutmoller 235
Research on Vesicular Diseases 255

DISEASES OF HORSES
VEE, a Disease on the Move — Richard O. Spertzel and R. W. McKinney 268
Report of the Committee on Infectious Diseases of Horses — C. L. Campbell, et al. 276

PHARMACEUTICALS
"FDA" and Antibiotics Used in Animal Feeds — C. D. Van Houweling 282
Animal Industry can Produce Protein for Seven Billion by 2000 A.D. — Robert White-Stevens 288

DISEASES OF POULTRY

Two Aspects of Duck Enteritis: Virus Excretion and Parental Immunity — Thomas E. Toth ................................................................. 304
The Status of the Control of Marek’s Disease by Vaccination — H. G. Purchase, B. R. Burmester, and W. Okazaki ............................... 315
Committee Report on Transmissible Diseases of Poultry — H. E. Goldsein, et al. ................................................................. 323

PUBLIC HEALTH

The Surveillance of Foodborne Diseases in Man and Animals — L. Paul Williams ................................................................. 336

RABIES


SWINE

Studies on Streptococcal Lymphadenitis. I. The Clinic, Hematologic and Pathologic Responses Following Oral Exposure with Various Numbers of Streptococcus, sp. p., Group E — J. A. Schmitz, and L. D. Olson ........ 357
Studies on Streptococcal Lymphadenitis. II. The Influence of Age on the Experimental Induction of Swine Abcesses — J. A. Schmitz and L. D. Olson ................................................................. 368
Response of Swine to Virulent and Modified Pseudorabies Viruses — J. A. Howarth ................................................................. 371
Incidence Studies of Overt Transmissible Gastroenteritis (TGE) of Swine from 1968 to 1970 — D. H. Ferris ................................. 385
Report of the Committee on Transmissible Diseases of Swine — D. P. Gustafson, et al ................................................................. 401

HOG CHOLERA

Status of the State-Federal Hog Cholera Eradication Program — M. J. Tillery . 425
SALMONELLOSIS

A Rendering Plant Manager’s Experience in Salmonella Control — Howard Norton ........................................ 449
Report of the Committee on Salmonellosis — A. A. Erdmann, et al ........................................ 452

TUBERCULOSIS

Epidemiologic Studies on Swine Tuberculosis — Charles O. Thoen and Alfred G. Karlson ........................................ 459
The Status of the State-Federal Tuberculosis Eradication Program — A. F. Ranney and J. D. Roswurm ........................................ 465
Colorado’s Bovine Tuberculosis Trail — M. A. Essey ........................................ 481
Report of the Committee on Tuberculosis — D. S. Ingraham, et al ........................................ 484
OFFICERS AND COMMITTEES FOR 1971

PRESIDENT
M. D. Mitchell ................................ Pierre, South Dakota

PRESIDENT-ELECT
John C. Shook .............................. Mechanicsburg, Pennsylvania

FIRST VICE-PRESIDENT
W. C. Tobin .................................. Denver, Colorado

SECOND VICE-PRESIDENT
Olin H. Timm ................................... Dixon, California

SECRETARY-TREASURER
W. L. Bendix .................................... Richmond, Virginia

COMMITTEE

Committee on Anaplasmosis
Dr. T. F. Zweigart, Chairman, Raleigh, North Carolina
Dr. J. Lee Alley, Co-Chairman, Baton Rouge, Louisiana

Research
W. E. Brock, Stillwater, Oklahoma J. A. Howarth, Davis, California
F. C. Neal, Gainesville, Florida T. O. Roby, Clarksville, Maryland
M. Ristic, Urbana, Illinois

State-Federal Regulatory
J. L. O'Harra, Reno, Nevada F. E. Henderson, Baton Rouge, Louisiana
G. S. Kaley, Albany, New York J. B. Roberts, Little Rock, Arkansas
J. W. Safford, Helena, Montana

Biologics and Pharmaceuticals
J. C. Trace, Fort Dodge, Iowa Charles Phizer, Terre Haute, Indiana
Dreyfus Froe, Terre Haute, Indiana

Livestock Industry
Bob Laramore, Gillette, Wyoming Rodney Larson, Friutdale, S. Dakota
Bert Hawkins, Ontario, Oregon

Sub-Committee
Dr. J. Lee Alley, Chairman J. O. Pearce, Okeechobee, Florida
V. D. Chadwick, Jackson, Mississippi B. R. McCallon, Washington, D.C.

Committee on Animal Virus Characterization
S. McConnel, Chairman, College Station, Texas
C. J. York, Executive Secretary, San Diego, California

R. A. Bankowski, Davis, California H. W. Dunne, University Park, Pennsylvania
B. R. Burmester, East Lansing, Michigan L. E. Hanson, Urbana, Illinois
V. J. Cabasso, Berkeley, California W. R. Hinshaw, Frederick, Maryland
E. A. Carbrey, Ames, Iowa A. J. Kniazeff, San Diego, California
G. E. Cottral, Greenport, L.I., New York R. A. Crandell, Brooks AFB, Texas
OFFICERS AND COMMITTEES

Committee on Animal Welfare — 1971

Dr. Grant S. Kaley, Chairman, Albany, New York
Dr. C. O. Finch, Co-Chairman, Hyattsville, Maryland

R. L. Alkire, Trenton, New Jersey  S. T. Rich, Los Angeles, California
P. B. Doby, Springfield, Illinois  J. C. Shook, Mechanicsburg, Pennsylvania
C. McPherson, Bethesda, Maryland  W. T. Thorpe, St. Paul, Minnesota
R. T. Phillips, Denver, Colorado  J. C. MacFarlane, Pembroke, Massachusetts
Frank Kingsbury, New Brunswick, New Jersey  H. A. Schneider, Chapel Hill, N.C.

Committee on Biologics — 1971

Dr. N. H. Casselberry, Chairman, Berkeley, California
Dr. M. J. Twiehaus, Co-Chairman, Lincoln, Nebraska

M. Bairey, James Wheaton  Carl J. Norden, Lincoln, Nebraska
Harold Chute, Orono, Maine  J. M. Hejl, Hyattsville, Maryland
T. J. Grennan, Providence, Rhode Island  R. L. Sweat, Caldwell, Idaho
A. E. Lewis, Ottawa, Ontario, Canada  E. H. Nordstrom, Lincoln, Nebraska

Committee on Brucellosis — 1971

Dr. Dean E. Flagg, Chairman, Bismarck, North Dakota
Dr. H. G. Wixom, Co-Chairman, Sacramento, California

J. R. Bishop, Tipton, Indiana  C. A. Manthei, Ames, Iowa
G. E. Burch, Albany, New York  R. J. McClanahan, Ottawa, Ont. Canada
Billy L. Deyoe, Ames, Iowa  S. H. McNutt, Madison, Wisconsin
J. B. Finley, Encinal, Texas  J. O. Pearce, Okeechobee, Florida
A. E. Janawicz, Montpelier, Vt.  Fred Phillips, Keating, Oregon
W. D. Knox, Fort Atkinson, Wis.  W. C. Tobin, Denver, Colorado
R. L. Laramore, Gillette, Wyoming  A. O. Wilson, Hysham, Montana
Forrest Lee, Brownlee, Nebraska  H. C. King, Hyattsville, Maryland
Vernon D. Chadwick, Jackson, Miss.  C. Dancer, Dewey, Oklahoma

Committee on Infectious Diseases of Cattle — 1971

Dr. John F. Hudelson, Chairman, Topeka, Kansas
Dr. E. E. Wedman, Co-Chairman, Ames, Iowa

H. D. Anthony, Manhattan, Kansas  E. L. Henkel, Salem, Oregon
R. P. Azeltin, St. Joseph, Missouri  C. H. Huddleston, Uvalde, Texas
D. E. Bartlett, Madison, Wisconsin  J. W. Kendrick, Davis, California
W. D. Bolton, Burlington, Vermont  George Lambert, Ames, Iowa
E. A. Butler, Des Moines, Iowa  Norman Libby, Bueyeros, N. Mex.
Joe Finley, Austin, Texas  Rolland McClymont, Holdrege, Neb.
F. W. Hansen, Jr., Hyattsville, Maryland  R. C. Searl, Ft. Dodge, Iowa
Matt Sutton, Onida, S. Dakota  D. E. Williams, Garden City, Kansas
**Committee on Evaluation and Development of State-Federal Programs - 1971**

Dr. John L. O’Harra, Chairman, Reno, Nevada
* Dr. John G. Milligan, Co-Chairman, Montgomery, Alabama

**Ex-officio Members**

President - M. D. Mitchell, Pierre, South Dakota
President-Elect - J. C. Shook, Mechanicsburg, Pennsylvania
First Vice-President - W. C. Tobin, Denver, Colorado
Second Vice-President - O. H. Timm, Dixon, California
Secretary-Treasurer - W. L. Bendix, Richmond, Virginia

**USAHA Regional Representatives**

Northern - E. L. Brower, Trenton, New Jersey
Southern - J. G. Milligan, Montgomery, Alabama
Midwest - Dean E. Flagg, Bismarck, North Dakota
West - John L. O’Harra, Chairman

**Federal**

E. E. Saulmon, Hyattsville, Maryland (ANH)
Donald Miller, ANH, Hyattsville, Maryland
E. A. Schilf, ANH, Cattle Dis., Hyattsville, Maryland
M. J. Tillery, ANH, Swine Dis., Hyattsville, Maryland
J. L. Hourrigan, ANH, Misc. Dis., Hyattsville, Maryland
N. L. Meyer, ANH, Foreign Dis., Hyattsville, Maryland
C. D. Van Houweling, FDA, Washington, D.C.

**Livestock Industry**

Burton Eller, Cattlemen Assoc., Denver, Colorado
J. Ralph Bishop, Swine, Tipton, Indiana
Paul Zillman - Livestock Cons. Hinsdale, Ill.

**Committee on Foreign Animal Diseases - 1971**

Colonel T. G. Murnane, Chairman, Washington, D.C.
Dr. N. L. Meyer, Co-Chairman, Hyattsville, Md.

Ahmed Dardiri, Southold, L.I., N.Y.
L. Griner, San Diego, Calif.
Fred. D. Maurer, College Station, Tex.
S. McConnell, College Station, Tex.
Peter McKercher, Greenport, L.I., N.Y.
John Mare, Ames, Iowa
J. E. Christy, Charleston, West Va.
Keith Sharan, Ames, Iowa
O. H. Timm, Dixon, California
Burton Eller, Denver, Colorado
D. F. Werring, St. Paul, Minnesota
Nels Konnerup, Washington, D.C.
George Cottral, Greenport, L.I., N.Y.
E. C. Sharman, Washington, D.C.
Donald Johnson, Frederick, Md.
R. C. Reisinger, Hyattsville, Md.
R. O. Spertzel, Frederick, Md.
J. T. Williams, Silver Spring, Md.
H. G. Wixom, Sacramento, California

**Sub-Committee on Vesicular Diseases**

J. J. Callis, Greenport, L.I., N.Y.
J. H. Graves, Greenport, L.I., N.Y.
R. P. Hanson, Madison, Wisconsin
E. W. Jenny, Ames, Iowa
L. O. Mott, Ames, Iowa
R. J. Yedloutschnig, Greenport, L.I., N.Y.
OFFICERS AND COMMITTEES

Committee on Infectious Diseases of Horses – 1971

Dr. C. L. Campbell, Chairman, Tallahassee, Florida
Dr. W. O. Kester, Co-Chairman, Golden, California

O. R. Adams, Ft. Collins, Colorado
J. H. Baldwin, Greene, New York
C. B. Dearborn, Concord, N. H.
P. B. Doby, Springfield, Ill.
J. B. Healy, Jacksonville, Fla.
E. Honnen, Aurora, Colorado
R. S. Jackson, Chino, Calif.
R. C. Knowles, Hyattsville, Md.
F. D. Maurer, College Station, Tex.
R. W. Moore, College Station, Tex.
L. G. Northington, Frankfort, Ky.
S. R. Nusbaum, Herkimer, N. Y.
Wilson Powell, Tallahassee, Fla.
D. L. Proctor, Lexington, Ky.
Earl Rath, Baton Rouge, La.
D. Sherrick, Urbana, Ill.
D. L. Smith, Indianapolis, Ind.
W. R. McGee, Lexington, Ky.

Committee on Import-Export – 1971

Dr. J. F. Quinn, Chairman, Lansing, Michigan
Dr. C. L. Campbell, Co-Chairman, Tallahassee, Florida

Sub-Committee on Domestic Animals and Birds:

C. L. Campbell, Chairman, Tallahassee, Florida

Paul C. Delay, Beltsville, Maryland
James R. Hay, Chicago, Ill.
Frank Harding, Geneva, Ill.
Robert Rumler, Brattleboro, Vt.

Sub-Committee on Animal Products and Byproducts:

J. C. Shook, Mechanicsburg, Pa., Chairman

E. L. Brower, Trenton, N. J.
P. D. Cazier, Lanham, Md.
J. J. Callis, Greenport, L. I., N. Y.
M. G. Hynes, Dublin, Ireland
A. R. Miller, Falls Church, Va.
W. L. Sulzbacher, Beltsville, Md.
M. L. Crandall, Detroit, Mich.
W. W. McMichael, Hyattsville, Md.

Sub-Committee on Wild and Endangered Species of Animals and Birds:

Dr. T. H. Reed, Chairman, Washington, D.C.

R. A. Bankowski, Davis, California
G. E. Cottral, Greenport, L. I., N. Y.
L. J. Goss, Cleveland, Ohio
Dean Price, Juneau, Alaska
John Richardson, Atlanta, Ga.
C. C. Schroeder, San Diego, Calif.
C. L. Smith, Hyattsville, Md.
Robert Willson, Royal Oak, Mich.
R. E. Omohundro, Hyattsville, Md.

Committee on Leptospirosis – 1971

Dr. R. L. Morter, Chairman, Lafayette, Indiana
Dr. Lyle E. Hanson, Co-Chairman, Lafayette, Indiana

S. Diesch, St. Paul, Minn.
W. E. Lyle, Madison, Wis.
C. S. Roberts, Auburn, Ala.
O. H. Stalheim, Ames, Iowa
H. B. Stoenner, Hamilton, Mont.
J. Ralph Bishop, Tipton, Ind.
R. E. Smith, Amherst, Mass
R. P. Crawford, Pullman, Washington
Committee on Livestock Commerce — 1971

Dr. Glenn B. Rea, Chairman, Salem, Oregon

L. N. Butler, Jr., Phoenix, Ariz. C. T. Sanders, Kansas City, Mo.
J. H. Brashear, Oklahoma City, Okla. A. P. Schneider, Boise, Idaho
F. S. Lee, Brownlee, Nebraska R. Schnell, Dickenson, N.D.
F. W. Hansen, Springfield, Va. F. J. Schoenfeld, Salt Lake City, Utah
J. E. Hudelson, Topeka, Kansas Ingvard Svarre, Sidney, Montana
J. F. Andrews, Atlanta, Ga. F. W. Peterson, Omaha, Nebraska

Committee on Livestock Identification — 1971

Dr. S. H. Flora, Chairman, Brownsville, Texas

Leo G. Berg, Hyattsville, Md. Burton Eller, Denver, Colorado
Bernard Ebbing, Waterloo, Iowa Raymond Schnell, Dickenson, N.D.
Elmer Haus, Newport, Ky. Norman R. Swanson, Cheyenne, Wyoming
L. R. Barnes, Rushville, Ind. J. R. Bishop, Tipton, Ind.

Committee on Mastitis

Dr. J. S. McDonald, Chairman, Ames, Iowa

H. S. Byran, Urbana, Ill. E. J. Kersting, Storrs, Conn.
J. C. Davidson, Hyattsville, Md. R. J. Schroeder, South Gate, Calif.
R. J. Farnsworth, St. Paul, Minn. K. K. Peterson, Corvallis, Ore.
R. S. Guthrie, Ithaca, N.Y. Rufus Wiltbner, Washington, D.C.
R. I. Hostetler, Pullman, Washington

Committee on Meat and Poultry Hygiene — 1971

Dr. Charles H. Cole, Chairman, Lansing, Michigan

Dr. James K. Payne, Co-Chairman, Washington, D.C.

Export and Import:


Industry:

D. S. MacKenzie, Chicago, Illinois

Federal:

L. H. Burkert, St. Paul, Minn. O. L. Dodgen, Washington, D.C.
George Sperling, Arlington, Virginia W. H. Dubbert, Washington, D.C.
OFFICERS AND COMMITTEES

State-Federal Collaboration:

D. C. Breeden, Lincoln, Nebraska
C. H. Glotfelty, Madison, Wisconsin

State:

E. D. Baker, Madison, Wisconsin
L. R. Crowell, Albany, New York
M. L. Houston, Salem, Oregon

State:

Charles Cole, Lansing, Michigan
W. W. Rechner, Hyattsville, Md.
G. W. Lantis, Jr., Springfield, Ill.

Teaching Research and Public Health —
Universities and Training Instructions:

V. L. Dahl, Columbus, Ohio
D. C. Kelley, Manhattan, Kansas
J. A. Libby, St. Paul, Minn.

R. L. Parker, Atlanta, Ga.
M. A. Simmons, Denton, Texas
R. N. Terrel, Auburn, Alabama

Committee on Nationwide Eradication of Hog Cholera — 1971

Dr. D. L. Smith, Chairman, Indianapolis, Indiana
Dr. J. B. Taylor, Co-Chairman, Montgomery, Alabama

J. A. Baker, Ithaca, N.Y.
W. L. Bendix, Richmond, Va.
H. W. Dunne, University Park, Pa.
J. Marvin Garner, Des Moines, Iowa
R. E. Hall, Madison, Wis.
C. H. Mannasmith, Red Oak, Iowa
J. F. Andrews, Atlanta, Ga.
W. R. Prafka, Camden, Ind.
P. B. Doby, Springfield, Ill.
E. A. Butler, Des Moines, Iowa
J. E. Henderson, Austin, Tex.
M. J. Tillery, Hyattsville, Md.
L. N. Miller, Oklahoma City, Okla.
S. H. Flora, Brownsville, Texas

Committee on Nominations, Resolutions, and Internal Affairs — 1971

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

J. L. O’Harra, Reno, Nevada
J. F. Quinn, Lansing, Michigan
G. S. Kaley, Albany, New York

C. L. Campbell, Tallahassee, Fla.
J. W. Safford, Helena, Montana

Committee on Parasitic Diseases and Parasiticides — 1971

Dr. J. H. Brashear, Chairman, Oklahoma City, Oklahoma
Dr. W. C. Tobin, Co-Chairman, Denver, Colorado

D. W. Baker, Albuquerque, New Mexico
G. L. Crenshaw, Davis, California
J. L. Hourrigan, Hyattsville, Md.
J. L. Hopson, Albuquerque, New Mexico
H. F. Groves, Columbus, Ohio
H. B. McGrath, Kansas City, Mo.
A. C. Newman, Jr., Oplica, Ala.

R. L. Pyles, Albuquerque, New Mexico
H. R. Smith, Cincinnati, Ohio
I. H. Roberts, Albuquerque, N.M.
R. H. Singer, Bryan, Texas
D. E. Zinter, Beltsville, Md.
John Poole, Hyattsville, Md.
R. D. Radeleff, College Station, Tex.
## OFFICERS AND COMMITTEES

### Committee on Pharmaceuticals – 1971

Dr. S. F. Scheidy, Chairman, Philadelphia, Pennsylvania  
Dr. Hilmer L. Jones, Co-Chairman, Rahway, New Jersey

<table>
<thead>
<tr>
<th>Name</th>
<th>City</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. J. Anderson</td>
<td>Ft. Worth, Tex.</td>
</tr>
<tr>
<td>A. Freeman</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td>C. C. Beck</td>
<td>Ann Arbor, Mich.</td>
</tr>
<tr>
<td>H. L. Jones</td>
<td>Somerset, N.J.</td>
</tr>
<tr>
<td>Fred Kingma</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>G. T. Edds</td>
<td>Gainesville, Fla.</td>
</tr>
<tr>
<td>K. Mayer</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td>R. D. Radleff</td>
<td>College Station, Tex.</td>
</tr>
<tr>
<td>D. F. Green</td>
<td>Rahway, N. J.</td>
</tr>
<tr>
<td>James Hanson</td>
<td>St. Paul, Minn.</td>
</tr>
</tbody>
</table>

### Committee on Professional Education and Extension – 1971

Dr. R. C. Hammond, College Park, Maryland

<table>
<thead>
<tr>
<th>Name</th>
<th>City</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. C. Beck</td>
<td>Ann Arbor, Mich.</td>
</tr>
<tr>
<td>W. C. Burnet</td>
<td>Kansas City, Mo.</td>
</tr>
<tr>
<td>G. L. Crenshaw</td>
<td>Davis, Calif.</td>
</tr>
<tr>
<td>C. Dobbins</td>
<td>Athens, Ga.</td>
</tr>
<tr>
<td>N. B. Haynes</td>
<td>Ithaca, N.Y.</td>
</tr>
<tr>
<td>W. L. Henning</td>
<td>University Park, Pa.</td>
</tr>
<tr>
<td>R. I. Hostetler</td>
<td>Pullman, Wash.</td>
</tr>
<tr>
<td>Moses Simmons</td>
<td>Denton, Texas</td>
</tr>
<tr>
<td>T. P. Siburt</td>
<td>Blacksburg, Va.</td>
</tr>
<tr>
<td>K. Weinland</td>
<td>Lafayette, Ind.</td>
</tr>
</tbody>
</table>

### Committee on Public Health, Radiological Fallout and Toxicology – 1971

Dr. Richard L. Parker, Chairman, Atlanta, Georgia  
Dr. Robert H. Singer, Co-Chairman, College Station, Texas

<table>
<thead>
<tr>
<th>Name</th>
<th>City</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. Fagan</td>
<td>Westchester, Pa.</td>
</tr>
<tr>
<td>A. B. Park</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>I. M. Saturen</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>J. J. Steel</td>
<td>Atlanta, Ga.</td>
</tr>
<tr>
<td>E. E. Wedman</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>Ted Rea</td>
<td>San Salvador, S.A.</td>
</tr>
<tr>
<td>N. L. Meyer</td>
<td>Hyattsville, Md.</td>
</tr>
<tr>
<td>W. E. Jennings</td>
<td>Auburn, Ala.</td>
</tr>
<tr>
<td>Calvin Schwabe</td>
<td>David, Calif.</td>
</tr>
<tr>
<td>J. H. Stewart</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>E. C. Sharman</td>
<td>Hyattsville, Md.</td>
</tr>
</tbody>
</table>

### Committee on Public Relations and Local Arrangements – 1971

Dr. J. H. Brashear, Chairman, Oklahoma City, Oklahoma  
Norman Powers, Lake Luzerne, N.Y.

<table>
<thead>
<tr>
<th>Name</th>
<th>City</th>
</tr>
</thead>
<tbody>
<tr>
<td>W. L. Bendix</td>
<td>Richmond, Va.</td>
</tr>
<tr>
<td>Howard Obenchain</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>C. L. Campbell</td>
<td>Tallahassee, Florida</td>
</tr>
</tbody>
</table>

### Committee on Transmissible Diseases of Poultry – 1971

Dr. H. E. Goldstein, Chairman, Columbus, Ohio  
Dr. R. A. Bankowski, Co-Chairman, Davis, California

<table>
<thead>
<tr>
<th>Name</th>
<th>City</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raleigh Allen</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>R. G. Buzzell</td>
<td>Augusta, Maine</td>
</tr>
<tr>
<td>L. C. Grumbles</td>
<td>College Station, Tex.</td>
</tr>
<tr>
<td>J. E. Hanley</td>
<td>Dade City, Fla.</td>
</tr>
<tr>
<td>R. Hogue</td>
<td>Lafayette, Ind.</td>
</tr>
<tr>
<td>A. E. Janawicz</td>
<td>Monpelier, Vt.</td>
</tr>
<tr>
<td>T. L. Landers</td>
<td>Chamblee, Ga.</td>
</tr>
<tr>
<td>N. E. Nadler</td>
<td>Albany, N.Y.</td>
</tr>
<tr>
<td>B. S. Pomeroy</td>
<td>St. Paul, Minn.</td>
</tr>
<tr>
<td>W. Scholfield</td>
<td>St. Louis, Mo.</td>
</tr>
<tr>
<td>J. B. Thomas</td>
<td>Columbia, S.C.</td>
</tr>
<tr>
<td>J. B. Roberts</td>
<td>Little Rock, Arkansas</td>
</tr>
<tr>
<td>T. B. Ryan</td>
<td>Raleigh, N.C.</td>
</tr>
<tr>
<td>S. A. Moore</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>Claude P'ow</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>H. W. Towers</td>
<td>Dover, Delaware</td>
</tr>
</tbody>
</table>

### Committee on Rabies – 1971

Dr. R. K. Sikes, Chairman, Lawrenceville, Georgia  
Dr. E. M. Joneschild, Co-Chairman, Pierre, South Dakota

<table>
<thead>
<tr>
<th>Name</th>
<th>City</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. A. Carbrey</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>D. W. Dressen</td>
<td>Atlanta, Ga.</td>
</tr>
<tr>
<td>E. R. Quortrup</td>
<td>San Diego, Calif.</td>
</tr>
<tr>
<td>Marvin Goff</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>L. Brown</td>
<td>Lincoln, Neb.</td>
</tr>
<tr>
<td>James E. Christy</td>
<td>Charleston, W. Va.</td>
</tr>
<tr>
<td>P. B. Schnurrenberger</td>
<td>Springfield, Ill.</td>
</tr>
</tbody>
</table>
OFFICERS AND COMMITTEES

Committee on Salmonella — 1971

Dr. E. E. Erdman, Chairman, Madison, Wisconsin
W. E. Lyle, Co-Chairman, Madison, Wisc.

Dr. C. E. Boyd, Columbia, S.C.
Dr. W. C. Ferrall, Hartford, Conn.
H. G. Geyer, Fairfax, Virginia
J. R. Hay, Chicago, Ill.
John Walker, Hyattsville, Md.
Charles Hasserman, Des Plaines, Ill.
T. J. Grennan, East Greenwich, R.I.
J. G. Miller, Tifton, Ga.
William Dubbert, Washington, D.C.
N. W. Rodey, Mesa, Arizona
R. L. Parker, Atlanta, Ga.
G. B. Guest, Rockville, Md.
E. E. Grass, Sacramento, Calif.
J. S. Culbertson, Washington, D.C.
E. V. Jeszenka, Harrisburg, Pa.

Committee on Diseases of Sheep and Goats — 1971

Dr. J. L. Hourrigan, Chairman, Hyattsville, Md.
Dr. G. L. Crenshaw, Co-Chairman, Davis, Calif.

C. C. Beck, Ann Arbor, Mich.
W. W. Hawkins, Bozeman, Montana
J. E. Christy, Charleston, W. Va.
James Schoenfeld, Salt Lake City, Utah
A. L. Klingsporn, Bowie, Md.
J. H. Wommack, Citrus Heights, Calif.
L. R. Barnes, Pendleton, Ind.
W. W. Clark, Mission, Texas
H. E. Metcalf, Denver, Colorado
Donald Baker, Albuquerque, N. Mex.
W. A. Hickman, Pierre, S. D.
Blaine McGowan, Davis, Calif.
R. E. Simmons, Boise, Idaho
T. B. Snodgrass, Dallas, Tex.
Ward Van Horn, Buffalo, S.D.

Committee on State-Federal Relations — 1971

Dr. J. C. Shook, Chairman, Mechanicsburg, Pa.

W. L. Bendix, Richmond, Va.
J. R. Hay, Chicago, Ill.
T. A. Ladson, College Park, Md.
M. D. Mitchell, Pierre, S.D.
G. B. Rea, Salem, Oregon
W. C. Tobin, Denver, Colo.
H. W. Towers, Dover, Del.

Committee on Transmissible Diseases of Swine — 1971

Dr. D. P. Gustafson, Lafayette, Ind.
Dr. L. R. Barnes, Indianapolis, Ind.

R. A. Bankowski, Davis, Calif.
M. J. Barta, Chicago, Ill.
E. H. Bohl, Wooster, Ohio
E. A. Butler, Des Moines, Iowa
H. W. Dunne, University Park, Pa.
E. M. Dwyer, Boston, Mass.
James E. Fox, Ashland, Ohio
E. O. Haelterman, Lafayette, Ind.
Robert Hall, Madison, Wis.
J. B. Nance, Alamo, Tenn.
J. B. Torrey, Ames, Iowa
Miodrag Ristic, Urbana, Ill.
S. Nicholson, Baton Rouge, La.
L. F. Van Gorder, Hyattsville, Md.

D. A. Fuller, Des Moines, Iowa
Committee on Tuberculosis and Paratuberculosis — 1971

Dr. D. S. Ingraham, Chairman, Harrisburg, Pennsylvania
Dr. J. G. Flint, Co-Chairman, St. Paul, Minn.

V. H. Berry, Washington, D.C.
E. L. Brower, Trenton, N.J.
C. E. Boyd, Columbia, S.C.
E. Conn, Northern Ireland
O. J. Halverson, Salem, Oregon
A. F. Ranney, Hyattsville, Md.
A. B. Larsen, Ames, Iowa
R. M. Scott, Montpelier, Vt.

H. Lefkowitz, Buffalo, N.Y.
A. E. Lewis, Ottawa, Ont. Canada
A. R. McLaughlin, Madison, Wisc.
Rolland Paul, Des Moines, Iowa
A. P. Schneider, Boise, Idaho
P. L. Smith, Sacramento, Calif.
Charles Thoen, Rochester, Minn.
James B. Henderson, Austin, Texas

W. D. Yoder, Ames, Iowa
JOHN C. SHOOK
President-Elect

W. C. TOBIN
First Vice President

W. D. MITCHELL
President

OLIN H. TIMM
Second Vice President

W. L. BENDIX
Secretary-Treasurer
<table>
<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
</tr>
</thead>
<tbody>
<tr>
<td>25. Nov. 28-30, 1921</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. F. Crewe, Bismarck, N.D.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
</tr>
<tr>
<td>26. Dec. 6-8, 1922</td>
<td>Chicago, Ill.</td>
<td>*Dr. T. E. Munce, Harrisburg, Pa.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
</tr>
<tr>
<td>Date</td>
<td>Location</td>
<td>Speaker</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------</td>
<td>-------------------------------</td>
<td></td>
</tr>
<tr>
<td>Nov. 30-Dec. 1-2, 1927</td>
<td>Chicago, Ill.</td>
<td>*Dr. L. Van Es, Lincoln, Neb.</td>
<td></td>
</tr>
<tr>
<td>Dec. 4-6, 1929</td>
<td>Chicago, Ill.</td>
<td>*Dr. Chas. G. Lamb, Denver, Colo.</td>
<td></td>
</tr>
<tr>
<td>Dec. 3-5, 1930</td>
<td>Chicago, Ill.</td>
<td>*Dr. A. E. Wight, Wash., D.C.</td>
<td></td>
</tr>
<tr>
<td>Nov. 30-Dec. 1-2, 1932</td>
<td>Chicago, Ill.</td>
<td>*Dr. Peter Malcolm, Des Moines, Iowa</td>
<td></td>
</tr>
<tr>
<td>Dec. 6-8, 1933</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. T. Faulder, Albany, N.Y.</td>
<td></td>
</tr>
<tr>
<td>Dec. 5-7, 1934</td>
<td>Chicago, Ill.</td>
<td>*Dr. T. E. Robinson, Providence, R.I.</td>
<td></td>
</tr>
<tr>
<td>Dec. 24, 1936</td>
<td>Chicago, Ill.</td>
<td>*Dr. Walter Wisnicky, Madison, Wis.</td>
<td></td>
</tr>
<tr>
<td>Dec. 6-8, 1939</td>
<td>Chicago, Ill.</td>
<td>*Dr. J. L. Axby, Indianapolis, Ind.</td>
<td></td>
</tr>
<tr>
<td>Dec. 4-6, 1940</td>
<td>Chicago, Ill.</td>
<td>*Dr. H. D. Port, Cheyenne, Wyo.</td>
<td></td>
</tr>
<tr>
<td>Dec. 3-5, 1941</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. A. Crossman, Boston, Mass</td>
<td></td>
</tr>
<tr>
<td>Dec. 1-3, 1943</td>
<td>Chicago, Ill.</td>
<td>Dr. W. H. Hendricks, Salt Lake City, Utah</td>
<td></td>
</tr>
<tr>
<td>Dec. 6-8, 1944</td>
<td>Chicago, Ill.</td>
<td>Dr. J. M. Sutton, Atlanta, Ga.</td>
<td></td>
</tr>
<tr>
<td>Dec. 5-7, 1945</td>
<td>Chicago, Ill.</td>
<td>Dr. C. U. Duckworth, Sacramento, Calif.</td>
<td></td>
</tr>
<tr>
<td>Dec. 4-6, 1946</td>
<td>Chicago, Ill.</td>
<td>*Dr. William Moore, Raleigh, N.C.</td>
<td></td>
</tr>
<tr>
<td>Oct. 12-14, 1949</td>
<td>Columbus, Ohio</td>
<td>*Dr. T. O. Brandenburg, Bismarck, N.D.</td>
<td></td>
</tr>
<tr>
<td>Sept. 23-25, 1953</td>
<td>Atlantic City, N.J.</td>
<td>*Dr. T. Childs, Ottawa, Canada</td>
<td></td>
</tr>
<tr>
<td>Nov. 10-12, 1954</td>
<td>Omaha, Neb.</td>
<td>*Dr. T. C. Green, Charleston, W.Va.</td>
<td></td>
</tr>
<tr>
<td>Nov. 16-18, 1955</td>
<td>New Orleans, La.</td>
<td>Dr. H. F. Wilkins, Helena, Mont.</td>
<td></td>
</tr>
<tr>
<td>Nov. 28-30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. A. L. Brueckner, Baltimore, Md.</td>
<td></td>
</tr>
<tr>
<td>Nov. 13-15, 1957</td>
<td>St. Louis, Mo.</td>
<td>Dr. G. H. Good, Cheyenne, Wyo.</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Place of Meeting</td>
<td>President</td>
<td>Secretary</td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------------</td>
<td>------------------------------------------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>62. Nov. 4-6, 1958</td>
<td>Miami Beach, Fla</td>
<td>Dr. John G. Milligan, Montgomery, Ala.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>65. Oct. 3-Nov. 1-3, 1961</td>
<td>Minneapolis, Minn.</td>
<td>Dr. A. P. Schneider, Boise, Idaho</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>67. Oct. 15-18, 1963</td>
<td>Albuquerque, N.M.</td>
<td>Dr. T. J. Grennan, Jr., Providence, R.I.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>70. Oct. 10-14, 1966</td>
<td>Buffalo, N.Y.</td>
<td>Dr. C. L. Campbell, Tallahassee, Fla.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
</tbody>
</table>

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

HARRY E. GOLDSTEIN, D.V.M.

Almighty God, our Heavenly Father, we beseech Thee to be present at this Seventy-Fourth Annual Meeting of our Association, assembled in Thy name and presence.

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

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

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

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

President Wheeler, Distinguished Guests, Ladies and Gentlemen —

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

We, the living, sometimes fail to properly evaluate the works that come before us. In an era where man can visit the moon, and reach even further in the universe, we lose sight of what is near and dear to us — our fellowship.

There are no words, phrases, or paragraphs that can adequately convey properly the true sentiments of recognition for those who are no longer on our roster.

Their deeds, feats, and contributions speak for themselves.

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

LEON G. CLOUD — (Indiana Veterinary College 1915) — died October 24, 1969
HUGH HURST — (Colorado State University 1916) — died July 23, 1969
CLEMENS E. KORD — (Terre Haute Veterinary College 1912) — died August 30, 1970
A. K. KUTTLER — (San Francisco Veterinary College 1917) — died July 6, 1969
HARRY MCDANIEL — (Cincinnati Veterinary College 1913) — died March 10, 1970
ROBERT W. MENGES — (Ohio State University 1941) — died December 7, 1969
LOUIS H. MOE — (Ohio State University 1927) — died April 20, 1970
HERMAN C. RINHEART — (McKillip Veterinary College 1911) — died June 3, 1970
HAL J. ROLLINS — (Kansas City College 1916) — died August 30, 1970
FRANCISCO ROSENBUSCH — (Buenos Aires, Argentina 1934) — died February 1969
JEAN V. SMITH — (Cornell University 1929) — died April 9, 1970
HENDRICK VERSLUIJS — (Cornell University 1935) — Died June 8, 1970
ALPHA E. WARDLOW — (Ohio State University 1917) — died September 25, 1969
LEE A. WILCOX — (Indiana Veterinary College 1922) — died April 28, 1970
ASA WINTER — (Michigan State University 1921) — died July 1, 1969
FRANK E. ZIEGENBEIN — (Cincinnati Veterinary College 1920) — died May 1, 1969

May we bow our heads for a moment of silent prayer and tribute. Amen.
MESSAGE OF THE PRESIDENT-ELECT

by

M. D. Mitchell, D.V.M

United States Animal Health Association

Members of the United States Animal Health Association, Distinguished Guests, Ladies and Gentlemen.

It is indeed a great honor for me to address this select group. I feel that this is one of the most serious charges that I have ever been held responsible for and possibly at this time, I am more aware of my precarious position than anyone else in the room. I am grateful, however, that this is called the President Elect's Address, because, if my president elect's speech is not acceptable, I well never become President and as yet no one has told me just how they dispose of a poor president elect if he doesn't make the step up. I assume, by the time I leave this podium, I will find out just what does happen.

It's good to meet here in this historic city of "brotherly-love", however, I do hope there is some "sisterly-love" also, as would be a little ill at ease if only men would make a pass at me . . .

It may be of some interest to some of you here that there is a small tie between the State of Pennsylvania and my great state of South Dakota, which by the way is known as "The Land of Infinite Variety".

About ten years ago while on a field trip in the state (it was official business) I saw a large snapping turtle crawling across the highway. Being a hunter of all sorts — I stopped and picked up this huge twenty-five pound snapping turtle and took it home with me. I proudly showed it to my wife and suggested that we have it for dinner. She was pretty cute about this whole situation, especially when she stated that I would have to clean it before she could cook it. Inasmuch as I have never cleaned a turtle before, nor, as she later admitted, had she any idea on just how to cook it. By the time I got it ready she had ample time to go to the library and withdraw a book telling all about snapping turtles. This book was written by a man from this area and he certainly knew his snapping turtles. He not only gave all the various recipes for preparing turtles, but also stated, quote, "by far the best and meanest snappers come from South Dakota".

I would like to take a minute to thank, John Shook, his crew and Ella, our good-looking Secretary for the fine way they have arranged for all the facilities for this 74th Annual Meeting of this group and the 2nd Annual Meeting since the name was changed to the United States Animal Health Association. If their work is any indication of what is in store for the rest of the week, I know this is going to be a real successful meeting.

Rather than dwell on past accomplishments, I would prefer to let the Association stand on its own record. I would, however, like to express a few of my own ideas concerning the future accomplishments of this Association.

In the past, some Presidents of the Association, have made reference to our committees structure with regard to number, make-up and continuity. In some instances, I feel that we have overlooked some real talent which may be found in
our newer members. One reason could be that the newer member has not made known which area of regulatory veterinary medicine he is interested in - if any? and second, perhaps, due to an oversight he has just been passed over. I would like to see a policy established whereby a committee member would serve as Chairman of a committee for not more than three years in succession, with the exception, that there may be some extraordinary situation whereby it would be advantageous to keep a Chairman for a longer period of time. This decision could be left up to the President. I would also like to see each Chairman select, by one means or another, a Co-chairman, who could be called on to take over the Chairman’s duties, if for some reason it would become necessary. During my term I do plan to ask each Chairman to pick a Co-chairman from members of his committee. The T. B. Committee has done an outstanding job; however, I do feel that there is one stumbling block which has been slowing down their efforts and I refer to the depopulating of cattle herds which are known to be infected with Bovine Tuberculosis. I feel that we should strive to change the thinking of the general public so that they will think of Bovine Tuberculosis in the same light as F and M, Rinderpest or any other exotic disease. At the present time, even Hog Cholera is viewed more as an exotic disease and treated as such, than is Bovine Tuberculosis. If F and M was diagnosed in one of our herds at this time, I’m sure that there would not be a moments hesitation to depopulate the herd, nor, would we try to think of some excuse as to why we should delay disposing of the infected herd. Still, as far as Bovine Tuberculosis is concerned even some of us regulatory people are slow in attempting to depopulate these herds. The incidence of this disease is now at a low point and the cost in dollars would be considerably less if we slaughtered all the animals and paid the owners full appraisal value, than, it would be to test the herd until we feel it is clean. I would hope that the T. B. Committee be the prime mover to start a campaign of some sort to change the thinking of not only the cattle owner, but, also the State and Federal Regulatory personnel as well. Maybe they need the services of a psychiatrist?

The committee on Anaplasmosis has a real serious charge, due to the fact, that there is a sharp division in the thinking on the part of the industry regarding this disease and what should be done if anything? however, I believe the make-up of this committee is well enough balanced so one faction cannot run away with the ball.

The committee on Livestock Commerce has added responsibilities, due to the changes in the marketing procedures of livestock. I feel that the committee should look at the whole picture of livestock marketing as well as auction markets and stockyards. I predict that in the near future we will see livestock bought and/or sold much in the same way stock and bonds are now handled (through a brokerage exchange). With all the advances made in computers and data processing I think it very possible and probable that in the near future one living in Iowa could call a broker and tell him that he wants to buy a number of cattle; state the weight, breed and so forth; receive a price quote; purchase the animals; have them delivered and never leave his home in Iowa. The same thing only in reverse could happen if a
rancher in Montana wanted to sell his cattle, he would simply list them with a brokerage firm and sell them just as you would sell one hundred shares of American T & T. Therefore, it behooves us to get our laws and regulations geared so this type of movement can take place and for once maybe we can be ready in advance and not way behind the progress of the marketing procedure.

To the committee on Evaluation and Development of State-Federal Programs, I would say that they have done a yeomans service in at least settling down some of the bickering which crops up between State and Federal people. I think the greatest single achievement they can accomplish is to encourage each state to take steps to be more responsible for projects within their own borders and do less yelling and asking for the Federal Government to do what rightfully is the States responsibility. This should include money and legislative authority. When each state accepts its full obligation there will be no need to fear the usurping of states rights by the Federal Government as now exists in some areas. I believe I detect a philosophy and some action within the ANH Division which has been alluded to by past Presidents of this Association. There seems to be more emphasis on training specialists who can be moved about from one state to another when needed and less effort put forth on building a large force of people in each state. I have felt for a long time that this is the proper approach. Certainly, it would seem more feasible for the Federal Government to have a special team equipped and ready to move any place in these United States, than for each state to try to set up the same team, thus, winding up with maybe fifty such groups when five or six groups would be sufficient.

To the U.S.D.A. I would like to express my thanks and appreciation for the services they have rendered both within the State of South Dakota and the several other states in these United States. I would suggest, however, that you be a little more aggressive in using your authority regarding Federal Quarantine. I believe you are rendering a dis-service to some states by being slow to use this authority. For example, in some instances, a state for one reason or another fails to control a disease within its borders and/or allows this disease to be exported to another state. It is my belief that the entire state, in this case, should be placed under Federal Quarantine until such time as it is demonstrated that the state involved can or will take care of its own problem as it should have in the first place. I also believe that this would be an incentive for such a state to speed up its activities to get the disease under control. This could, however, cause a need for additional money from the states General Fund or require additional legislation; however, in the meantime, the neighboring states would not continually be exposed to the threat of importing a disease. This procedure should pertain to any disease from Brucellosis to Hog Cholera and/or any of the others in between which we are now spending public money and providing man power for the purpose of eradication.

The U.S.D.A. has been engaged with several states in a Screwworm Eradication Program. Those states in the south and southwest are probably more aware of the needs for this program than those of us in the north and eastern part of the United States; however, I know from personal experience that this parasite can be transported to the northern states during the summer months. I do hope that all
states will support the ANH in order that they can succeed with this program. In addition, I am convinced that before this program is completed there will have to be a shift in the area or zone of activity. I have felt for sometime that this zone should be moved to the south, in fact, quite aways south. I understand that already there has been considerable investigation concerning the feasibility of setting up a barrier across the Isthmus of Tehuantepec. This information points up the fact that it would not only be to our advantage to move this barrier further south of the United States border, but, would also be less costly in dollars and cents.

I would be remiss if I failed to mention the C&MS with respect to the Wholesome Meat Act. I hope that you people will always be aware of the problems we are faced with in our attempt to implement the State Meat Inspection Program. Up to this point, I do believe you have been very patient and at least in our part of the country, have had some real good men who are really doing a good job of advising and assisting us State people. I would admonish you to take seriously, your membership in this Association, and I believe you will thus be in a good position to profit from the advice of others and/or even gain support for somethings which otherwise you may have resistance. You've all probably heard the expression, "EAT, DRINK and be MERRY", well I'd like to make the following suggestion: Inasmuch as God created the world and all that is in it, he also created the day. This day can be divided into three equal parts. If during the next few days each of us here spends eight hours at labor and eight hours for rest we will all have eight hours left for entertainment and from what I've heard about Philadelphia — eight hours entertainment a day will be about all I can take. This schedule if followed should insure a very fruitful and worthwhile meeting . . .

One custom of this Association is to present the outgoing President with a momento to remind him of our appreciation for his services. This, I feel is one of the more pleasant tasks which I have assumed this year; therefore, I would like to present you, Dr. Wheeler, with this certificate for you to hang on the wall to prove to the public that you did serve as President of the United States Animal Health Association and in addition, this key which I hope you will wear with equal pleasure to yourself and also as an honor to the Association. I would, however, admonish you that it will be a breech of etiquette to leave this key in a hotel room or exchange it for a pawn ticket.

On behalf of the Association and myself, I would like to say thank you for the dedicated service you have rendered to this organization and we all hope and expect you will continue to contribute your services.
UNITED STATES ANIMAL HEALTH ASSOCIATION

1970 REPORT OF THE SECRETARY AND TREASURER

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

The Secretary is happy to greet all the members and guests at this, the seventy-fourth annual meeting of the United States Animal Health Association, in the City of Brotherly Love, Philadelphia. We hope that you have all found accommodations that are satisfactory and pleasant. We had planned a trip through some of historic restored Philadelphia and culminating in an outdoor-indoor refreshment session, covering both the liquid and solid type. We regret that this fell through due to problems with the contractor. On the other hand, we have an extremely full meeting, and this will permit those in attendance a little more time to explore this fascinating historic city.

Mention was made last year of the number of our individual memberships. As nearly as the Secretary's office can figure, we have some 1,146 names that are, or at one time were, members. For the previous year, we had 863 paid members and approximately 283 who were sent dues notices but who made no response. During this past fiscal year, we received dues from 913 individual members, and it appears that approximately 80 for which we have no record of dues payment for the last three years. I would recommend that some method be devised for dropping members who do not keep up their dues, to allow us to keep an absolutely accurate list. There are always some additions and some of whom we lose track, but for an organization of this size, it seems to the Secretary that this is an excessive number of non-active or non-dues-paying members. The Secretary will, however, present to the Executive Committee for consideration applications that he has received from a number of individuals seeking membership and from one organization applying for allied membership. The Secretary is, of course, extremely pleased that the Consumer and Marketing Service of the United States Department of Agriculture now has applied for and been granted official membership and a seat on our Executive Committee. The Secretary has been informed that the Bureau of Veterinary Medicine of the Food and Drug Administration is bringing its regional supervisors to Philadelphia to meet with us, and it is hoped that they will consider becoming an official member of this Association. We think that our bases of interest and activity are similar enough to justify a much closer relationship that we have experienced in the past, and we hope that they share these sentiments.

As you know, the next meeting of our Association is at the Hotel Skirvin in Oklahoma City, October 24-29, 1971. The following year the Association has voted to go to Florida; and at the invitation of Dr. C. L. Campbell, the State Veterinarian of Florida, who will be our host, the Secretary this spring visited Florida and looked at several hotels in the Miami Beach area that had made a bid for this meeting. After careful consideration, the Secretary, with the agreement of Doctor Campbell, selected The Americana at Bal Harbour, Miami Beach, and the meeting is set up for November 5-10, 1972. This is a beautiful hotel with superb
accomodations, and in early November the weather should be just about right. I am sure the Association will enjoy a most profitable and pleasant meeting in Miami Beach.

You will note that our registration fees have been increased for this year. This was done by action of the Board of Directors at its meeting in connection with the annual convention of the American Veterinary Medical Association in Las Vegas this past summer. Our individual dues remain at $10; but as expenses are going up and as our commitments increase, additional revenue is becoming necessary. One of the areas that need revenue is our relationship with the American Association of Veterinary Laboratory Diagnosticians. This organization, as you know, is a direct affiliate of the United States Animal Health Association. They hold their meeting immediately prior to ours. Most of them are associated with us in our respective states and positions. They come to our meetings and pay our registration fee, for which we supply them with meeting facilities and publish their Proceedings. In addition, we provide them with $200 a year for office expenses. This organization is growing, both in size and importance, and they find this budget is entirely inadequate. Let us hope that some means of relieving this problem will be found at this meeting.

Attached to this report is a copy of the Report of the Treasurer for the fiscal year ending September 30, 1970. I attach also a statement in the form of a letter from our accountant. In the previous fiscal year, the accountant and Treasurer decided to close the books on September 25, which left some accounts payable unpaid that were picked up after the beginning of this fiscal year, beginning October 1, 1969. This year we have closed the books and our accounts as of the close of business in fact September 30, and we have taken care of this year's accounts right up through all the bills that have come in. The actual cash statement shows that our expenditures for this past fiscal year exceeded our revenue by $4,913.32. This, however, is a technicality, because we paid some of the previous fiscal year's bills after the close of that year, which circumstance reflects itself in our current statement as an expenditure out of the 1970 fiscal year. This will be adjusted in the next statement, and it will show actual cash receipts and expenditures for the current year. Among the highlights of our financial statement, you will note that our individual dues went up slightly. Our official dues went up in the sum of $200. The sale of Proceedings fell off rather sharply. This is due primarily to the trouble we are having delivering Proceedings out of the country. The previous Secretary-Treasurer warned us that he had had trouble continually in this regard, and the present incumbent is finding this all too true. We have not yet devised a method to guarantee delivery of these Proceedings and receipt of the sale price. We are going to keep trying, however. Receipts from the sale of reprints fell off sharply too, as reflected by this statement; but we have nearly $4,000 in reprint orders that have not been paid for as yet, because we were very late in getting a great many of our reprints from the printer. Printing costs, as you will note, have increased almost $1,000, with virtually no increase in the amount of printing. Office supplies are up almost 50%. Communications have increased and are up more
than 50%. I have brought approximately 100 sets of our financial statement to the meeting, and for as long as they last, they will be available to any member who wants one. Any member desiring an individual copy may let me know, and our office will be glad to supply him with one. We are solvent; we are in a position to pay our bills; and we have a respectable cash balance.

UNITED STATES ANIMAL HEALTH ASSOCIATION
1444 East Main Street
Richmond, Virginia 23219

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
FOR PERIOD OCTOBER 1, 1969 THROUGH SEPTEMBER 30, 1970

CASH BALANCE – OCTOBER 1, 1969:

<table>
<thead>
<tr>
<th>Account Description</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern Bank and Trust Company</td>
<td>$8,397.03</td>
</tr>
<tr>
<td>Richmond, Virginia (Savings)</td>
<td></td>
</tr>
<tr>
<td>Southern Bank and Trust Company</td>
<td>198.90</td>
</tr>
<tr>
<td>Richmond, Virginia (Checking)</td>
<td></td>
</tr>
<tr>
<td>Southern Bank and Trust Company</td>
<td>222.66</td>
</tr>
<tr>
<td>Richmond, Virginia (Local Arrangements Acct. – Savings)</td>
<td></td>
</tr>
<tr>
<td>Trevose Savings and Loan Association</td>
<td>1.00</td>
</tr>
<tr>
<td>Morrisville, Pennsylvania</td>
<td></td>
</tr>
<tr>
<td>Sandia Savings and Loan Association</td>
<td>1.00</td>
</tr>
<tr>
<td>Albuquerque, New Mexico</td>
<td></td>
</tr>
</tbody>
</table>

Total Cash Balance = $8,820.59

INCREASED BY CASH RECEIPTS:

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual Dues</td>
<td>$9,130.00</td>
</tr>
<tr>
<td>Official Dues</td>
<td>5,200.00</td>
</tr>
<tr>
<td>Proceedings</td>
<td>2,489.20</td>
</tr>
<tr>
<td>Reprints</td>
<td>2,734.54</td>
</tr>
<tr>
<td>Registration Fees</td>
<td>7,125.00</td>
</tr>
<tr>
<td>Foreign Animal Books</td>
<td>30.00</td>
</tr>
<tr>
<td>Interest Income</td>
<td>1,330.22</td>
</tr>
</tbody>
</table>

Total Increase = $28,038.96

TOTAL BEGINNING BALANCE AND RECEIPTS = $36,859.55
REPORT OF SECRETARY-TREASURER

UNITED STATES ANIMAL HEALTH ASSOCIATION
1444 East Main Street
Richmond, Virginia 23219

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR PERIOD
OCTOBER 1, 1969 THROUGH SEPTEMBER 30, 1970

DECREASED BY EXPENDITURES:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual Meeting</td>
<td>$ 2,773.89</td>
</tr>
<tr>
<td>Printing</td>
<td>12,272.59</td>
</tr>
<tr>
<td>Office Supplies</td>
<td>666.64</td>
</tr>
<tr>
<td>Salary</td>
<td>11,237.50</td>
</tr>
<tr>
<td>Social Security Tax</td>
<td>655.55</td>
</tr>
<tr>
<td>Communication</td>
<td>2,127.02</td>
</tr>
<tr>
<td>Travel:</td>
<td></td>
</tr>
<tr>
<td>Dr. John L. O'Harra</td>
<td>435.78</td>
</tr>
<tr>
<td>Dr. Frank B. Wheeler</td>
<td>820.77</td>
</tr>
<tr>
<td>Dr. W. C. Tobin</td>
<td>284.16</td>
</tr>
<tr>
<td>Mr. Ralph Bishop</td>
<td>128.78</td>
</tr>
<tr>
<td>Dr. M. D. Mitchell</td>
<td>312.00</td>
</tr>
<tr>
<td>Dr. W. L. Bendix, Secretary-Treasurer</td>
<td>300.50</td>
</tr>
<tr>
<td>Ella R. Blanton, Office Secretary</td>
<td>118.65</td>
</tr>
<tr>
<td>Miscellaneous Expense</td>
<td>485.23</td>
</tr>
<tr>
<td>Office Equipment</td>
<td>139.88</td>
</tr>
<tr>
<td>Bank Service Charge</td>
<td>33.98</td>
</tr>
<tr>
<td>Federal and F.I.C.A. Tax withheld</td>
<td></td>
</tr>
<tr>
<td>in period 10/1/69 and paid</td>
<td></td>
</tr>
<tr>
<td>during period 10/1/69 - 9/30/70</td>
<td>159.36</td>
</tr>
</tbody>
</table>

$32,952.28

CASH BALANCE – SEPTEMBER 30, 1970:

<table>
<thead>
<tr>
<th>Account</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern Bank and Trust Company</td>
<td></td>
</tr>
<tr>
<td>Checking Account</td>
<td>381.59</td>
</tr>
<tr>
<td>Savings Account</td>
<td>3,287.96</td>
</tr>
<tr>
<td>Local Arrangements Account</td>
<td>235.72</td>
</tr>
<tr>
<td>Trevose Savings and Loan Association</td>
<td></td>
</tr>
<tr>
<td>Morrisville, Pennsylvania</td>
<td>1.00</td>
</tr>
<tr>
<td>Sandia Savings and Loan Association</td>
<td></td>
</tr>
<tr>
<td>Albuquerque, New Mexico</td>
<td>1.00</td>
</tr>
</tbody>
</table>

$ 3,907.27
UNITED STATES ANIMAL HEALTH ASSOCIATION
1444 East Main Street
Richmond, Virginia 23219

SUMMARY OF OPERATIONS
FOR PERIOD OCTOBER 1, 1969 THROUGH SEPTEMBER 30, 1970

REVENUE:
Total Cash Receipts $28,038.96
Less — Expenditures 32,952.28
Excess of Expenditures over Revenues $ 4,913.32

NET WORTH — SEPTEMBER 30, 1970

<table>
<thead>
<tr>
<th>Account</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accounts Receivable</td>
<td>$5,894.08</td>
</tr>
<tr>
<td>Balance</td>
<td></td>
</tr>
<tr>
<td>Southern Bank and Trust Company, Richmond, Virginia</td>
<td></td>
</tr>
<tr>
<td>Checking Account</td>
<td>381.59</td>
</tr>
<tr>
<td>Savings Account</td>
<td>3,287.96</td>
</tr>
<tr>
<td>Local Arrangements Account</td>
<td>235.72</td>
</tr>
<tr>
<td>Balance</td>
<td></td>
</tr>
<tr>
<td>Trevose Savings and Loan Association</td>
<td>1.00</td>
</tr>
<tr>
<td>Morrisville, Pennsylvania</td>
<td></td>
</tr>
<tr>
<td>Balance</td>
<td></td>
</tr>
<tr>
<td>Sandia Savings and Loan Association</td>
<td>1.00</td>
</tr>
<tr>
<td>Albuquerque, New Mexico</td>
<td></td>
</tr>
<tr>
<td>Petty Cash Fund</td>
<td>25.00</td>
</tr>
<tr>
<td>Deposit — C&amp;P Telephone Company, Richmond, Virginia</td>
<td></td>
</tr>
<tr>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Inventory — Supplies and Proceedings</td>
<td>2,138.61</td>
</tr>
<tr>
<td>U.S. Treasury Bonds</td>
<td>20,000.00</td>
</tr>
<tr>
<td>Furniture and Fixtures</td>
<td>932.76</td>
</tr>
<tr>
<td>Net Worth — September 30, 1970</td>
<td>$32,997.72</td>
</tr>
</tbody>
</table>

ANALYSIS OF CHANGE IN NET WORTH

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net Worth, September 30, 1969</td>
<td>$37,911.04</td>
</tr>
<tr>
<td>Decreased by:</td>
<td></td>
</tr>
<tr>
<td>Excess of Expenditures over Revenues for fiscal year ended September 30, 1970</td>
<td>4,913.32</td>
</tr>
<tr>
<td>Net Worth, September 30, 1970</td>
<td>$32,997.72</td>
</tr>
</tbody>
</table>

Henry H. Budd
Accountant
Dr. W. L. Bendix  
Secretary-Treasurer  
United States Animal Health Association  
1444 East Main Street  
Richmond, Virginia 23219

Dear Dr. Bendix:

You will recall that we closed the books for the United States Animal Health Association for the fiscal year ending September 30, 1969 on September 25, 1969, in order that we might have the statement prepared for you to take to Milwaukee for the Annual Meeting.

There were a number of invoices which were received between September 25 and September 30 covering expenses which should have been paid for the fiscal year ending September 30, 1969. These had to be carried over and paid in the fiscal year ending September 30, 1970.

Due to the closing of the books on September 25, 1969, and carrying over of certain invoices caused an excess of expenses over revenues for the fiscal year ending September 30, 1970. Also, we were late in receiving reprints from the printer during the fiscal year ending September 30, 1970 which caused a decrease in revenue for this fiscal year.

We trust that this information will satisfactorily explain, “excess of expenses over revenues” in the amount of $4,913.32.

Very truly yours,

Henry H. Budd  
Accountant
President Wheeler, convention delegates and guests, ladies and gentlemen. It is a pleasure to welcome you to this, the 74th annual meeting of the United States Animal Health Association, and to our wonderful state of Pennsylvania.

It is especially gratifying for me, as a representative of a great agricultural state, to greet your organization that is serving agriculture so effectively. Governor Raymond P. Shafer, and all Pennsylvanians, join me in a sincere and warm welcome on this occasion.

We are proud of Pennsylvania that is so rich in history and tradition. Here in Philadelphia — a city that was founded by William Penn in 1682 — the First Continental Congress met in 1774.

Here the Declaration of Independence was adopted in 1776, and here the Constitution was drafted in 1787.

Only a few miles away is Valley Forge, a monument to the courage and devotion of the men who fought for our independence.

And only a hundred miles to the west is Gettysburg where the fate of this nation was decided in three terrible days of fighting that marked the turning point of the Civil War.

It is any wonder that — in 1976 — the 200th anniversary of this nation will be celebrated here in Philadelphia, the cradle of American independence.

The products of Pennsylvania mines, factories and farms were important to the success of our struggle for independence, and agriculture was a key factor in developing the economy of this young nation.

We are proud of Pennsylvania’s agricultural endeavors and accomplishments.

Today, we rank first in the nation in production of mushrooms and cigar leaf tobacco; third in production of maple syrup; third in the value of chickens and fourth in value of milk cows and heifers; and fifth in production of milk and eggs.

In food processing, Pennsylvania ranks first in the manufacture of ice cream, pretzels, potato chips, and sausage and scrapple.

We also have another important first of particular interest to you group — the first meat inspection law in the nation.

It was enacted by the Provincial Assembly of Pennsylvania in 1727, forty-nine years before the Declaration of Independence was signed. Its purpose was to prevent unfair practices in packing beef and pork for export.

The law provided stiff penalties for violations and required the inspector to place his brand mark on barrels of approved meat, much the same as inspectors today stamp meat that has passed inspection.

In mentioning meat inspection and meat hygiene, it is only natural to think of the years of work and the widespread efforts aimed at the eradication, control and
prevention of diseases of livestock and poultry.

Your organization has had a leading role in this work, and your activities have contributed a great deal to the progress achieved in this field. More important, you are to be commended for exerting these efforts so effectively without impeding normal traffic through channels of trade.

The dedicated professional work of this organization has been a key factor in establishing and maintaining the high level of production from healthy livestock that is enjoyed in this country today.

Now, more than ever, we must recognize the tremendous pressures that are being exerted on the nation's meat and poultry industries because of the intensified interest in consumer protection. The demand for clean, wholesome meat and meat products must be met at once if we are to provide adequate, effective protection for both producer and consumer.

Your organization, the United States Animal Health Association, has demonstrated over the years how industry and the veterinary profession can work hand-in-hand to accomplish a common goal for the benefit of everyone.

As an example of the results to be achieved through cooperation, we need only look at the long fight to stamp out brucellosis.

Today, the entire Northeastern United States is virtually brucellosis free. Only one county stands in the way of achieving this goal. It is Washington County in the western part of Pennsylvania where a single herd has been a problem for several years. Our normal efforts have been unavailing and we are now initiating legal action to resolve this problem as quickly as possible. When it is cleared up, not only Pennsylvania but the entire Northeast will be free of this costly livestock disease.

Again, I am happy to welcome you here for the first annual meeting of your association in Pennsylvania. I hope your meeting will be most successful, and your visit to our state so enjoyable that you will not wait another 74 years before returning.

I would like to suggest that you plan to visit many of our interesting and historic spots in this area and, perhaps, tour the unique town of Hershey and the Pennsylvania Dutch country nearby. I am sure you will find the trip interesting and worthwhile.
RESPONSE TO ADDRESS OF WELCOME

J. H. Brashear, Oklahoma State Veterinarian

Thank you, Mr. Bull, thank you for making us feel welcome to Pennsylvania and the city of Philadelphia. Also, we thank Governor Shafer and other Pennsylvanians who have greeted us (through you) so sincerely.

It is my privilege to respond in behalf of the United States Animal Health Association and I assure you that the pleasure of being here is all ours. Those of us who have not visited Pennsylvania before have been looking forward to this occasion and others who have been here are happy to return. We know that you sincerely want us to feel welcome here and that all you tell us is true and not just some more Bull.

I will especially enjoy my visit because I have always wanted to visit the birthplace of some of my ancestors. My grandmother was a “hard-headed” Pennsylvania Dutchman and I have heard many things concerning this state and the people in it.

You mentioned that Philadelphia was founded by William Penn in 1682; I would like to elaborate on that. Being a native of Oklahoma, I naturally compare places that I visit to Oklahoma, and I find that, historically, Pennsylvania and Oklahoma have one thing in common, and that is in the manner in which they came into being.

Oklahoma was settled and made civilized by Indians and Outlaws that were unwanted in other parts of the United States, and Pennsylvania was founded as a colony by William Penn and his followers who were unwanted in England.

It seems as though Mr. Penn was a great aggravation to the King and other dignitaries of England because of his religious beliefs and spent a lot of time in jail because of this. King Charles II owed old Admiral Penn a debt in the neighborhood of about $80,000 and agreed to pay this debt to the Admiral’s son William by giving him a strip of wilderness land in America west of the Delaware River between New York and Maryland. William Penn accepted this territory and named it Sylvania meaning “woodland”.

The old King still wanted to push his weight around and thought it should be called Penn in honor of the Admiral, so William compromised and named it Pennsylvania meaning “Penn’s Woods”. His wisdom and caution in matters of planning provided the harmony and good government which made Philadelphia “the city of Brotherly Love”.

From this beginning Pennsylvania took its place in the United States and has continued to contribute more than its fair share to this Nation. Your statements concerning this State are very conservative. So that you will not be accused of being boastful, I will mention some more accomplishments of Pennsylvania.

It was once the Keystone of the original thirteen states and has now become the Keystone of the industrial life of the United States. Such industries as iron and steel, coal, shipbuilding, and textiles are of great importance.

It leads the states in the value of coal, cement, and slate. It is first in iron and steel products, which center in Pittsburgh, the steel capitol of the world. Steel from
the glowing crucibles of Pittsburgh’s blast furnaces goes to almost every country to build bridges, railroads, and countless tools and machines. Pennsylvania makes sixty percent of the nation’s tin plate. Coal and iron are only two of the minerals that make up the enormous wealth of its mines. The state produces practically the nation’s entire supply of anthracite (hard) coal. It is second in the total value of manufactured products. It also produces one-fourth of the nation’s textiles. Ships from Chester and Philadelphia sail the seven seas.

Pennsylvania is the only state with commercial water outlets on the Great Lakes, to the Gulf of Mexico and through the Atlantic Ocean. You mentioned that Pennsylvania was very important years later in serving this country in times of conflict through uses of its industries and resources.

Men of Pennsylvania such as Daniel Boone, Benjamin Franklin, and many others have helped make this nation what it is today.

Your statement that Pennsylvania is a leader in the livestock industry and is doing its share in producing good, healthy animals, is very evident. The United States Animal Health Association is proud of progress made in Pennsylvania in disease eradication and in the control of the spread of diseases. Other states have no fear of disease when they accept livestock from this state. We of the United States Animal Health Association are honored to be welcome to the State of Pennsylvania and are looking forward to visiting all the places you mentioned where important events happened that played such a tremendous role in the development of this great nation. And again, Mr. Bull, I assure you it is our pleasure to visit a place of such great distinction.
REPORT OF THE
UNITED STATES ANIMAL HEALTH ASSOCIATION
COMMITTEE ON NOMINATIONS, RESOLUTIONS,
AND INTERNAL AFFAIRS

The Committee on Nominations, Resolutions, and Internal Affairs met in the Secretary’s Suite on Thursday afternoon, October 22nd, and Friday morning, October 23rd. The following members were present: John L. O’Harra, Chairman, C. L. Campbell, T. J. Grennan, Grant S. Kaley, J. W. Safford, and John F. Quinn. Mr. Wilson Powell rendered able assistance as recording secretary.

INTERNAL AFFAIRS

This Committee commends the Secretary’s Office on the arrangements for this the 74th Annual Meeting of the United States Animal Health Association. However, it recommends that the Registration Desk be staffed throughout the meeting to facilitate registration of late comers and to serve as a central clearing house for information, messages, and other services for those in attendance at the meeting. This Committee also recommends that the election and installation of officers of the Association be held earlier in the meeting when the attendance is at its peak.

Your Committee requests the Secretary to express thanks to all of those involved in the arrangements and success of the meeting.

RESOLUTIONS (16 this year)

Resolution

WHEREAS, Venezuelan Equine Encephalomyelitis (VEE), an arthropod-borne zoonosis, constitutes a major economic and public health threat wherever epizootics occur; and
WHEREAS, a serious epizootic of VEE has occurred in Central America and Mexico in 1969-70; and
WHEREAS, VEE poses an imminent threat to the equine industry of the United States; and
WHEREAS, quarantine and slaughter methods used in control and eradication of animal diseases are not generally applicable to arboviral zoonotic diseases; and
WHEREAS, an experimental attenuated live vaccine, made available by the U.S. Department of Defense and used in Central America and Mexico, appears to have been safe and effective in preventing the spread of the 1969-70 VEE epizootics; and
WHEREAS, this vaccine is not currently commercially available in the world; and
WHEREAS, no stockpile of this vaccine currently exists; and
WHEREAS, inactivated vaccines prepared from virulent VEE viruses have been shown to cause disease and have provided inadequate protection; and
WHEREAS, vaccination of equidae is essential to prevent the spread of VEE epizootics, it is also recognized that other methods such as vector control, restriction of the movements of hosts such as equidae, man, dogs, and other mammals may play a part in preventing the spread of VEE; NOW THEREFORE BE IT

RESOLVED, That the U.S. Department of Agriculture begin immediate negotiations to prepare through cooperation with the Department of Defense ten million doses of VEE vaccine to provide a stockpile for use in the United States, when the disease erupts in the United States.

RESOLVED FURTHER, That the U.S. Department of Agriculture pursue under a highest priority, a research project to obtain data required for the commercial production and use of VEE vaccine in the United States.

Resolution

WHEREAS, The complement fixation test has been shown to be a practical aid to the diagnosis of Equine Piroplasmosis; and
WHEREAS, the American Association of Laboratory Diagnosticians has recommended that the C.F. test be recognized as the official diagnostic test for equine piroplasmosis whether caused by Babesia caballi or Babesia equi until such time as a more accurate test is developed; NOW, THEREFORE, BE IT
RESOLVED, That the U.S. Animal Health Association recommend that the complement fixation test be adopted as the official test for the diagnosis of equine piroplasmosis by all animal health regulatory agencies in the United States.

Resolution

WHEREAS, it has been demonstrated that horses recently imported into the United States have been found affected with EP caused by Babesia caballi and/or Babesia equi; and
WHEREAS, horses offered for importation have often been found infested with tropical horse ticks (Dermacentor nitens); and
WHEREAS, during the last sixty days since the initiation of test procedures more than forty percent of horses offered for importation from Central and South American and Caribbean countries have been found affected with EP, and in some cases infested with vectors of this disease, namely Dermacentor nitens; NOW, THEREFORE, BE IT

RESOLVED, That the U.S. Animal Health Association take cognizance of and endorse action recently initiated by the U.S. Department of Agriculture in amending Title 9 of the Code of Federal Regulations on importation requirements, as follows:
1. All equidae, except those born in Canada, including zebras, be freed of ticks in the country of origin, and upon arrival in the United States be inspected, found free of ticks and receive a precautionary treatment with a permitted tickicide.

2. Prior to being released for entry, such equidae be subjected to the CF test for EP and handled in the following manner:
   a. Animals not classed as reactors to CF test be allowed to enter if otherwise eligible.
   b. Animals classed as reactors to the CF test be refused entry.

RESOLVED, FURTHER, That the procedure adopted by the U.S. Department of Agriculture in that interim before the foregoing amendments to the federal regulations become effective, which provides that equidae now being imported into the United States and destined for Florida meet prescribed negative EP and vector requirements, be immediately extended by the Director of the Animal Health Division to encompass all states.

Resolution

WHEREAS, it has been shown that horses recently moved interstate from equine piroplasmosis endemic areas into 12 states where E.P. has not been considered to be endemic have been found to be affected with E.P.; and

WHEREAS, it has been shown that horses recently moved interstate have been found to be infested with tropical horse ticks upon arrival in four states not considered to be tick infested or endemic for E.P.; NOW, THEREFORE, BE IT

RESOLVED, That the United States Animal Health Association support a concerted effort to amend the code of federal regulations to measureably reduce the interstate spread of equine piroplasmosis, such amendments in the regulations to be made within one year.

Resolution

WHEREAS, the U.S.A.H.A. has received information of a proposed reorganization within the U.S. Department of Agriculture which would affect the type of personnel that would be enforcing regulations pertaining to animals and animal products brought into the United States from foreign countries or exported from the United States and

WHEREAS the reorganization may result in non-veterinary personnel being placed in line functions of USDA where judgments are made concerning or pertaining to the health status of animals offered for import into or export from the United States and

WHEREAS such non-veterinary personnel may not have the expertise to make decisions that will assure that animals or animal products imported into or exported from the United States will not threaten the livestock population of the United States and countries receiving exports from the United States.
THERE BE IT RESOLVED by the USAHA here assembled that, in the interest of protecting the United States livestock industry from foreign animal disease and in the interest of protecting the livestock industries of countries receiving exports from the United States from U.S. livestock diseases, the responsibility for determining the health status of animals and the disposition of animal products entering or leaving the United States and policies affecting the international movement of such products through the United States be reserved to members of the veterinary profession who are trained and experienced in such matters and

BE IT FURTHER RESOLVED that the exercise of this responsibility and all decisions pertaining thereto should not be delegated to other regulatory officials and

BE IT FURTHER RESOLVED that a copy of this resolution be sent to the U.S. Secretary of Agriculture and the major livestock organizations in this country.

From the Committee on Foreign Diseases.

Resolution

The committee on Transmissable Diseases of Poultry submit the following resolution for your consideration.

WHEREAS, Duck Virus Enteritis (Duck Dutch Plague) occurs in many foreign countries; and

WHEREAS, waterfowl infected with this disease shed the virus for at least 45 days and infection can spread by direct or indirect contact; and

WHEREAS, this disease was eradicated from commercial waterfowl in the United States only following great costs to the industry and public funds; now, therefore, be it

RESOLVED that the Code of Federal Regulations, Title 9, be amended to require all imported waterfowl, prior to release for use or from quarantine, be tested and found negative for Duck Virus Enteritis.

Resolution

A genus specific fluorescent antibody conjugate and counterstain have been developed by Diagnostic Bacteriology, Diagnostic Services, Animal Health Division, for the detection of salmonella in animal feeds and by-products. Recent data indicate that the technique has considerable promise as a definitive procedure. Until the evaluation of the technique is completed, the Committee recommends that it be officially approved as a salmonella screening procedure.

Resolution

WHEREAS, We believe that diagnostic reagents are an integral part of every laboratory; and

WHEREAS, We believe that many diagnostic reagents now in use by veterinary
diagnostic laboratories do not meet minimum quality standards; and

WHEREAS, Diagnostic reagents are not tested and licensed; therefore,

RESOLVED, That all diagnostic reagents sold or produced for interstate
shipment in the United States and for use in veterinary diagnostic laboratories, be
tested to insure minimum standards of accuracy and dependability by the
Veterinary Biologics Division with the support of the Animal Health Division. —
Salmonella Committee

Resolution

The following resolution was adopted by the Anaplasmosis Committee on
October 19, 1970:

WHEREAS, an increasing number of foreign countries are placing restrictions
on the importation of American cattle because of Anaplasmosis, and

WHEREAS, interstate restrictions on the movement of cattle infected with, or
exposed to, Anaplasmosis are becoming more common, and

WHEREAS, there is an increasing concern about Anaplasmosis among
cattlemen of the Northwest region of the United States where the tick is considered
to be an important vector and wildlife may act as reservoirs of infection.

THEREFORE, BE IT RESOLVED that the U.S.A.H.A. request the U.S.
Department of Agriculture to promptly inaugurate pilot projects in several herds in
representative states of the Northwestern region to determine the feasibility of
establishing and maintaining Anaplasmosis-free herds for periods of five or more
years by the use of recommended procedures.

Resolution

Resolution on *Streptococcus agalactiae* infected cows from the U.S.A.H.A.
Mastitis Committee:

RESOLVED, That the U.S.A.H.A. strongly recommends that the USDA
appoint a committee to devise; with the aid of consultants from the U.S.A.H.A.,
N.M.C., and A.V.M.A.; a protocol for controlling the interstate movement of dairy
cattle over 18 months of age infected with *Streptococcus agalactiae*.

Resolution

WHEREAS, trichinosis has been a public health hazard for at least a century; and

WHEREAS, most European nations perform a routine trichina examination at
slaughter as part of their meat inspection programs; and

WHEREAS, the United States, with one of the highest known trichinosis-
infection rates in the world, has no program for trichina inspection or eradication; and

WHEREAS, the pooled-sample, artifical digestion technique for trichinosis
detection has been recently developed; and,

WHEREAS, the pooled-sample method has been tested in an extensive field trial; and,

WHEREAS, the field trial results show that the method meets or exceeds the accepted standards of accuracy for detecting infection capable of causing illness in humans; and

WHEREAS, the pooled-sample method can be conducted without interrupting normal slaughtering procedures; and

WHEREAS, this method is readily adaptable to high-speed slaughter procedures; and,

WHEREAS, this method will permit the traceback of trichina-infected animals to point of origin is essential to trichinosis eradication:

THEREFORE, BE IT RESOLVED, that the United States Animal Health Association in convention assembled at Philadelphia, Pennsylvania, October 19-23, 1970, urges the United States Department of Agriculture to work with the States in developing and implementing without further delay a program for the control and eradication of trichinosis.

Resolution

WHEREAS the complex syndrome of swine dysentery is causing great economic loss to this nation's swine industry, and

WHEREAS the causative factor of swine dysentery is unknown, and

WHEREAS preventative, therapeutic and regulatory measures have proved ineffective,

THEREFORE be it resolved that the United States Animal Health Association request the Secretary of Agriculture to give some serious consideration to the financial needs for research on swine dysentery.

Resolution

WHEREAS, consumption of meat from animals and poultry, containing biological residues (as defined in the Federal Meat Inspection Regulations), may be injurious to human health, and

WHEREAS, consumption of meat from animals and birds, infected with zoonotic diseases, may be inimical to human health, and

WHEREAS, legislative authority is not uniformly provided for the quarantine and disposition of animals and birds containing biological residues or infected with such zoonotic diseases, which are intended for slaughter.

THEREFORE BE IT RESOLVED that the United States Animal Health Association urge the Congress of the United States and those State legislatures, which have not already done so, to enact legislation to provide the mandatory quarantine and disposal of animals and poultry so affected.
Resolution

WHEREAS, International trade in meat and poultry products is essential to the National welfare, and
WHEREAS, consumers have a right to expect that all imported meat and poultry products sold are wholesome and prepared under standards equal to the U.S. Standards, and
WHEREAS, there is no substitute for adequate veterinary ante-mortem and post-mortem inspection at the plant of origin by veterinarians and inspectors in that country,
THEREFORE BE IT RESOLVED that the Department of Agriculture be commended for its plan to improve its foreign review program by assigning highly qualified veterinarians to foreign posts and,
BE IT FURTHER RESOLVED that the veterinarians so assigned should be of a rank commensurate with their responsibility for being the primary representative of the United States in the area of meat and poultry inspection in the foreign country.

Resolution

WHEREAS there have been efforts on the part of a limited number of Congressmen to amend and strike “scrapie and” from the basic act of May 24, 1884, and
WHEREAS such an amendment would greatly restrict the action of animal disease regulatory agencies and defeat the purpose of the eradication program, and
WHEREAS research has disclosed that there are certain similarities of scrapie of sheep and goats, transmissible mink encephalopathy of mink, and kuru or Creutzfeldt-Jakob disease of man.
THEREFORE BE IT RESOLVED that research be continued to develop more information on these diseases including the possible human health aspects of scrapie and transmissible mink encephalopathy, and that the USAHA opposes bills in Congress which will make control of scrapie more difficult.

Resolution

WHEREAS, certain ectoparasites are being eradicated by use of Toxaphene; and
WHEREAS, Toxaphene does not create the persistent residue problems as chlorinated hydrocarbons in general; and
WHEREAS, Toxaphene is and has been the standard insecticide for the control of several ectoparasites in livestock, resulting in considerable economic benefits to the livestock industry, as well as contributing to the economic advantages to the meat consumers; and
WHEREAS, it has been proposed in the Federal Register that Toxaphene be included in a list of persistent chlorinated hydrocarbons listed; NOW, THERE-
FORE,

BE IT RESOLVED that the United States Animal Health Association advise the Food and Drug Administration that we oppose such action pertaining to Toxaphene.

BE IT FURTHER RESOLVED that this Association offer to consult with FDA in developing sound, standard recommendations for the use of Toxaphene prior to such action being taken.

The Association secretary is requested to prepare Resolutions of appreciation to the many who have contributed to the high level of this meeting and to distribute same.

This Committee was faced with filling the position of President-Elect other than by orderly accession this year due to the untimely death of Dr. Jean Smith the incumbent in this office.

NOMINATIONS

Your Committee submits the following nominees for officers of the United States Animal Health Association: To be installed as President — Dr. M. D. Mitchell; For President-Elect — Dr. John C. Shook; For First Vice President — Dr. W. C. Tobin; For Second Vice President — Mr. Olin Timm; For Treasurer — Dr. W. L. Bendix.

Your Committee submits the following nominees for regional industry representative: Northeast: Dr. E. S. Braynt of Maine, Dr. William Henning of Pennsylvania; West: Mr. Bob Larrimore of Wyoming, Mr. Archie Wilson of Montana; Central: J. W. Bishop of Indiana, Ward Van Horn of South Dakota; South: Joe Finley of Texas, Jim Nance of Tennessee.

I move the Committee report be accepted and the named Individuals be placed in Nomination.
REPORT OF THE COMMITTEE ON
ANIMAL VIRUS CHARACTERIZATION

S. McConnel, College Station, Texas, Chairman; C. J. York, San Diego, California, Executive Secretary


The Animal Virus Characterization Committee has had several meetings during the past year, with at least part of the membership attending and the remainder participating by exchange of information. The activities of the Committee have centered around four main features: (1) the completion of an animal reference virus catalogue; (2) the assembling of specific characterization data on a virus representative for each type of virus; (3) establishing and participating in internal working teams covering specific virus groups; and (4) maintaining a center for virus data collection.

The animal reference virus catalogue is an effort to place all the known, well understood animal viruses in their proper classification group and to select for each virus a specific strain to represent that virus. These strains were selected as the ones that have all the major characteristics for that particular virus, possess satisfactory antigenicity so that they can serve as the serotype, and have all the necessary features of workability. For example, if a number of strains exist for a given virus and one of these can be propagated better in tissue culture and makes a good antigen, then this strain would be chosen as the reference. The strain selected may be the first reported isolate of the particular virus or it may be a recent isolate having more useful properties than those studied earlier. For these reasons, the selected reference virus should not be termed prototype although they may be one and the same in some instances.

In drafting the animal reference virus recommendations, data on several hundred viruses were examined, and over 200 viruses were selected and placed in fourteen virus groups following as near as possible the classification scheme of the International Committee on Nomenclature of Viruses. To allow for rapid publication and widespread distribution, the complete catalogue of reference virus selections will be published through the courtesy of the American Journal of Veterinary Research. A summary of the virus recommendations is presented here:

Adenovirus: 10 representative viruses.
Arenovirus: (new group): only one animal virus at this time.
Coronavirus (new): 2 viruses. Infectious Bronchitis is the best example.
Herpesvirus: 19 viruses. This is an imcomplete group, with new isolates being
discovered frequently.

Iridovirus (new): 3 viruses, primarily amphibian, fish, and some insects. African Swine Fever is the only mammalian representative.

Leukovirus: 14 viruses at the moment, including avian leukosis.

Myxovirus: 11 viruses, primarily Influenzas.

Papovavirus: 7 viruses. These are tumor-producing agents, including warts.

Paramyxovirus: 10 viruses. They include many that were in the Myxovirus group at one time, i.e., Newcastle, Rinderpest and Canine Distemper.

Paravovirus: 3 viruses. This is a new group consisting of some of the smallest known viruses. Infectious Panleukipenia of cats is the most important.

Picornavirus: a very large group with 61 viruses included, with many more to be added as research continues. It consists of such important ones as Foot-and-Mouth disease.

Poxvirus: 19 viruses.

Reovirus: 29 viruses including Bluetongue and African Horsesickness.

Rhabdovirus: 6 viruses including Rabies and Vesicular Stomatitis.

Unclassified: 8 viruses such as Hog Cholera and Bovine Virus Diarrhea. These will be placed in proper classification groups as their characteristics are better understood.

This list does not cover all viruses. There are many more that are not understood enough to designate reference strains at this time.

In making these recommendations, the Committee believes that widespread use of virus reference strains would provide a basis for specific reagent preparation, facilitate the conduct of viral diagnostic efforts and research programs, be a basis for comparison of results between laboratories, a means of standardization for vaccine development and control, and an aid in regulatory programs.

In spite of the work that has been carried out in many laboratories, specific information is often lacking for many virus groups, and international accord is often missing. Therefore, in cooperation with the Veterinary Public Health Division of the World Health Organization, a series of small working teams have been established consisting of men actively engaged in work with a particular virus group. By exchanging antisera, antigens and data, these men will be in a position to clarify areas where confusion still exists and make new recommendations. Eleven such teams are currently active, covering most of the virus groups just mentioned.

Making specific reference virus recommendations is only the first step in understanding virus characterization. For each virus there are at least 50 or more points that need to be considered, ranging from particle size or nucleic acid composition to disease potential and geographic distribution. Accordingly, the Committee is continuing to compile data on each of the major viruses in the catalogue. We anticipate this data to be our next publication in order to allow for its wide usage and application to virus problems.

To assure that this data has continuing value and is continually updated, a center is being maintained at the laboratory of one of the Committee members.
How to retrieve this information and make it available on a more or less weekly basis to all who need it is one problem not yet resolved, but the Committee feels the subject is important enough to demand a continuing effort.

In view of the constant discovery of new viruses, improvements in research methods leading to new characterization data, the need for application of this information to diagnostic methods, and standardization of both reference reagents and vaccine development, it is recommended that an Animal Virus Characterization Committee be maintained to keep the Association abreast of developments in this rapidly changing field. Furthermore, in view of the continual and accelerated movement of animals, material and people on an international basis, presenting a constant threat of disease spread both internally and internationally, it is felt that continual exchange of virus characterization data is one important way to provide surveillance on known zoonoses and detect emerging diseases.
REPORT OF THE COMMITTEE ON LIVESTOCK COMMERCE

G. B. Rea, Salem, Oregon, Chairman

Mr. Chairman:
This is the first year of activity for the Committee on Livestock Commerce. Its purpose is to carry on the functions formerly satisfied by the Committee on Laws and Regulations and the Committee on Livestock Markets and Transportation. It was also to broaden its scope to include all facets of that part of the livestock industry which pertains to the exchange of ownership of animals and their movement from one area or state to another.

The first charge given us by President Wheeler was to consider a new and more functional system of certifying to the health of animals in interstate trade than that which is presently used i.e. interstate health certificates and permits. Our group discussed this subject at great length. We all realize that it deserves our most careful study and consideration.

As a result of our perusals and in view of our present lack of a positive means of identification, we recommend that this subject continue to be studied by the A.N.H., the U.S.A.H.A. and the Livestock Industry, to the end that the original request made of this committee may eventually be satisfied.

In line with the afore consideration it was pointed out that several states have relaxed their importation regulations with regard to feeder cattle. In one or more states no H.C.'s or permits are required on feeder steers and only minor requirements; such as prior permits, are placed and certain ages of feeder females. We acknowledge the practicality of this action and suggest that the several states review and apply such relaxations as may be applicable to their local situation.

The problem of animal identification is a hold over from both of parent committees. It was again discussed at length with consideration being given to a complete new system of brands which could be extended to international proportions. It was the general agreement that this was a matter of grave concern to the entire livestock industry as it pertains to disease control, theft, taxation, and general record keeping. The Committee does not, at this time, have a specific recommendation. It does recognize that to eventually satisfy the aforementioned requirements for animal identification a total combined effort on the part of the producer, the marketing industry, and the regulatory official is essential.

Your committee will continue to study the ramifications and probe the various
possible solutions to both the health certification and livestock identification problems as they pertain to interstate trade to the end that a safe and practical positive recommendation may eventually be made.

The general standards, for approval of Livestock Markets, as recommended by the former committee in 1968 were discussed. These standards related to facilities utilized in the business of livestock markets and livestock dealers. Your committee commends those states which have adopted these recommended general standards. Further, it submits that these minimum standards are as valid and appropriate today as they were when first adopted. Therefore, it urges that these same standards be adopted by all states; and that the Animal Health Division apply them in approving facilities used in interstate movement of all classes of livestock in lieu of the present various specific approvals.

In 1969, the former committee on Laws and Regulations made recommendations regarding the indemnification of animals slaughtered due to the outbreaks of foreign diseases when in interstate channels of trade. This committee was advised that Federal regulations have been amended to accomplish this recommendation. The committee commends the Animal Health Division, USDA, for this action.

It has been reported to this committee that zoo veterinarians are in the process of adopting a standard health certificate for use in the movement of zoo animals between approved zoos. It was brought out in the discussion that such movement of zoo animals is under the jurisdiction of various departments of state governments. Therefore, the committee recommends that the zoo veterinarians review this matter with the appropriate local officials for the purpose of conforming with the existing laws of the state of destination.

Your committee again recommends the adoption by all states of the uniform health certificate proposed by the U.S.L.S.A. in 1964, with the following change in the veterinarian’s certification as recently proposes by the A.V.M.A. The recommended veterinarian’s certification reads as follows:

“I have inspected the animals described hereon and find them to be free from visible signs of infectious, contagious, or communicable disease. The vaccinations and results of tests are as indicated above.

__________________________
Veterinarian

__________________________
Date

We urge the responsible livestock regulatory officials of the several states to adopt the aforementioned uniform health certificate, as amended, when their existing supplies are depleted.

Mr. Chairman, this constitutes the first report of the Committee on Livestock Commerce. I respectfully submit it for approval by the Executive Committee.
REPORT OF THE COMMITTEE

Glenn B. Rea, Chairman
L. N. Butler
F. S. Lee
W. F. Peterson
J. F. Hudelson
J. B. Roberts
Dean E. Flagg
C. T. Sanders
R. Schnell

Ingvard Svarre
J. H. Brashear
E. E. Montgomery
F. W. Hansen
W. W. Bird
J. F. Andrews
D. L. Smith
A. P. Schneider
F. J. Schoenfeld
G. G. Bierman
REPORT OF THE
ANIMAL WELFARE COMMITTEE

Grant S. Kaley, Albany, New York, Chairman

Model State Law

With reference to the Model State Law which appeared as a part of the last previous committee report, we are pleased to announce that the proposal was approved by the Council of State Governments for distribution to legislative service agencies. The Committee urges the membership of the U.S. Animal Health Association to take an active interest in encouraging the introduction and adoption of this law by the several state legislatures.

Wild and Exotic Pets

It has been called to the attention of your committee that the capture, importation and sale to individuals as pets of wild species, both exotic and native is increasing. It is the sense of this committee that legislative bodies should act to adopt statutes or strengthen existing statutes with a view to discouraging the capture, interstate movement and sale of such animals to individuals not capable of providing them with adequate housing, care and food. The committee would point out that the sale of such animals to individuals can constitute a hazard to the health and safety of the public as well as to the welfare, care and preservation of such species.

U. S. Public Law 89-544

The United States Department of Agriculture is to be commended for its conscientious effort to attain effective enforcement of the provisions of P.L. 89-544. The Department has been handicapped by problems related to authority. The committee supports the objectives of proposed federal legislation which would broaden the coverage provided by P.L. 89-544 and strengthen the hand of the department in dealing with violations.

Soring of Horses

In a previous report the committee expressed support of pending federal legislation designed to make illegal soring of horses. Inasmuch as this legislation is again before the Congress for consideration it seems appropriate to reaffirm its support of this proposal.
Zoos and Menageries
Your committee has been informed of numerous complaints concerning inhumane and unsanitary conditions at zoos and menageries where wild animals are exhibited outside their natural habitat. It is recommended that model legislation be prepared for the purpose of regulating and controlling such establishments with the objective of assuring that the animals, birds and reptiles confined in such places receive shelter, care and food commensurate with the species involved.

Air Transportation
It has been brought to our attention that many animals are transported by air under conditions which are not only inhumane but which actually endanger the life of the animals. Further consideration to the handling of living animals by common carriers and recommendation in this area will be forthcoming in a future committee report.
DISTRIBUTION OF VIRUS IN BLOOD COMPONENTS DURING VIREMIA OF BLUETONGUE

A. J. Luedke

In the pathogenesis of viral infections in host animals the researcher is concerned with 4 phases of the virus: 1) entry into the host, 2) spread within the host, 3) localization in target organs, and 4) release from the host. Blood of bluetongue (BT)-infected animals is an important factor relative to phase 2 and, because of the transmission of the virus by hematophagous insects it is also an important factor in phase 4. Although a viremia was originally known to exist in bluetongue-infected sheep and cattle in South Africa it was only recently adequately established and characterized for sheep, cattle, goats, white-tailed deer, and elk. As early as 1905, Spreull found that serum of infected sheep was as infective as whole blood. This fact persisted throughout the BT literature for the past 60 years and apparently was never questioned because of the absence of a sensitive and quantitative direct viral assay procedure for blood from infected animals. Pini et al. in 1966 reported that the unmodified virus was closely associated with the buffy coat of infected sheep from postinfection (PI) days 3 to 13 as assayed in baby hamster kidney cell cultures. They further found that the number of viral isolations decreased rapidly after the day of peak temperature in the animal. Virus was also detected in low concentration in the plasma, but it was pointed out that the use of the buffy coat suspension would increase the probability of isolation of BT virus from field samples.

According to Peebles, there are many reports of viremia in various diseases of man and animals but most do not establish the locations of the virus in blood components. During the short viremic period in infected humans, he recovered measles virus predominantly in a washed leukocyte fraction and occasionally from erythrocytes and plasma but at a much lower viral concentration. His hypothesis was that measles virus might be predominantly located in an intracellular position or at least firmly adherent to cell membranes in such a fashion as to be protected from neutralization by rising serum antibody. Poliomyletis, vaccinia in rabbits, and fowl plague virus were reported to act in a manner similar to measles virus.

Virus-leukocyte interactions have been well documented with respect to adsorption of virus to the leukocytes or phagocytosis and replication of virus in leukocytes. However, there have been few determinations of the amount of infectious virus in erythrocytes, buffy coat, and plasma during various stages of viremia. In one study the buffy coat contained most of the virus, for a brief period during reovirus viremia in mice. Heuschele found African swine fever virus in all fractions of blood on PI day 3. His study was restricted to the first 4 PI days. Howe
and Morgan\textsuperscript{5} found that Sendai virus adsorbed to the erythrocytes and caused invaginations in the plasma membrane and subsequently apparently sealed itself into a segment of the membrane. They postulated that under these conditions the virus would be intracellular and escape the neutralizing effect of serum antibodies.

A coexistence of BT virus and its serum neutralizing antibody has been reported in infected sheep and cattle\textsuperscript{9, 13} Preliminary studies further indicated that BT viremia was associated with cells rather than plasma, but all initial attempts to elute virus from the blood cells were unsuccessful. It was necessary therefore, to first quantitate the virus in blood components during the early, acute, and convalescent stages of BT infection. This study would not only increase our understanding of the distribution of the virus in the blood but it would also improve our BT viral isolation techniques, especially during the latent period of infection.

**MATERIALS AND METHODS**

*Animals.* -- Ten yearling Warhill wethers were used in the first experiment and 3 yearling Warhill wethers, 3 two-year-old Saanen goats (2 castrated males and 1 female), three 8-month-old Holstein cattle (2 females and 1 steer), and 1 one-month-old Holstein bull calf were used in the second experiment.

*Experimental Procedure.* -- The animals were exposed to pathogenic BT virus by intradermal and subcutaneous inoculation of 4 ml. of BT virus-infected blood. In the first experiment, 10 of the sheep were all inoculated at the same time while in the second experiment the cattle, sheep, and goats were inoculated 2 at a time. The animals were observed for at least 3 days prior to inoculation in order to determine the reference body temperature, leukocyte count, and packed cell volumes. Samples of heparinized blood (50 ml.) for fractionation were obtained on postinfection (PI) days 3, 7, and 14 for the first experiment and on PI days 1, 3, 5, 7, 9, 11, 14, 21, and 28 for the second experiment. Samples of blood preserved in equal volumes of a preservative solution OPG\textsuperscript{*} were obtained on PI day 28. Pre- as well as postinoculation serum samples were obtained.

*Blood fractionation procedure.* -- Venous blood was drawn under sterile conditions into a 50 ml. glass syringe and then the needle was removed and the blood gently expelled into a sterile 50 ml. tube containing 0.5 ml. (500 U.S.P. units) heparin solution. The tube was inverted several times. In the laboratory the whole blood was centrifuged at 2800 r.p.m. for 30 minutes at 4\textdegree{} C. The plasma was aspirated with cannula and syringe and 10 ml. saved. The buffy coat was carefully removed with cannula and syringe and put into Wintrobe tubes. The Wintrobe tubes were centrifuged at 1500 r.p.m. for 5 minutes, plasma removed, and the buffy coat aspirated with cannula and syringe and put into 9 ml. of PBS making approximately a 10\textsuperscript{-1} dilution. The 50 ml. tube containing the packed erythrocytes was filled with

\textsuperscript{*}Anticoagulant preservative solution consisting of potassium oxalate, 5 Gm.; phenol, 5 Gm.; glycerin, 500 ml.; and distilled water, 500 ml.
phosphate buffered saline (PBS) and mixed thoroughly. The red cell suspension was centrifuged at 2800 r.p.m. for 20 minutes and the PBS removed with cannula and syringe and replaced with fresh PBS. This procedure was repeated two more times and the 10 ml. of the packed cells saved. All dilutions were made with PBS and the lowest dilution inoculated for each fraction was $10^0$ plasma, $10^{-1}$ buffy coat, and $10^{-1}$ erythrocytes; 0.1 ml. of each dilution was inoculated intravascularly into a set of 6 embryonating chicken eggs (ECE). Prior to viral assay all of the samples were treated by sonification with a 125 watt, 20-kc sonifier* for 30 seconds with an attached 1/8 micro-tip horn at an instrument setting of 4.

_Virus._ — A Wyoming BT viral strain designated 62-45S (previously BT 262) obtained from sheep in an epizootic of BT was used in the first experiment. Inocula consisted of infected ovine blood (10th serial passage) preserved in an equal volume of OPG. An Idaho BT viral strain designated 63-66B (previously BT ox 183) was used in the second experiment. Inocula consisted of infected ovine blood (5th serial passage), infected cattle blood (3rd serial passage), and infected goat blood (4th serial passage) preserved in equal volumes of OPG solution. Each inoculum had a viral titer of 4.5 to 5.5 ($\log_{10}$ ELD$_{50}$/1 ml.) as assayed by the intravascular inoculation technique as described.

_Clinical observation._ — Body temperatures were recorded twice daily. Daily observations for clinical signs of disease were continued for 28 days in all of the animals.

_Hematologic observations._ — Blood samples from all animals were collected on PI days 0 to 11, 14, 21, and 28. Leukocyte counts were made with an electronic cell counter, and packed cell volume was determined by the standard microhematocrit method.

_Viral assay._ — Infectivity of BT virus from the blood fraction samples was determined in 10 to 11-day-old embryonating chicken eggs by the intravascular inoculation technique as previously described. Fifty per cent end points were calculated by the method of Reed and Muench.

_Immunodiffusion tests._ — The presence or absence of precipitin antibodies in the serum was determined before exposure and on the 28th postexposure day. The test was conducted by the micro agar gel precipitin test, using a soluble BT viral antigen.

*Model S-125 Branson Instruments, Inc., Danbury, Conn.
In experiment I in sheep, during the acute stage of the disease, the washed erythrocytes yielded up to 10 to 13 times the virus concentration of the buffy coat and up to 10,000 times the viral concentration of the plasma fraction (Table 1). In early and convalescent stages of the disease the virus was consistently obtained only from the washed erythrocyte fraction. Serum BT virus neutralizing and precipitin antibody were present at the convalescent stage of the infection.

In experiment II the washed erythrocytes yielded 10 to 100 times the viral concentration of the buffy coat and 1,000 to 1,000,000 times the virus concentration of the plasma during the acute stages of the disease in sheep, cattle, or goats (Tables 2, 3, 4). Plasma of sheep contained minimal amounts of BTV only during the early and acute stages of the disease. In cattle and goats the plasma contained less virus than in sheep at any one time but it can be detected more frequently during the course of the disease. For example, the virus was detected in 9 of the 27 plasma samples from sheep (Table 2) and always in low titer. With cattle (Table 3), the virus was detected in 17 of the 27 plasma samples but it usually could not be titered. Goat plasma samples gave results similar to those obtained from cattle (Table 4).

Buffy coat fractions from sheep, cattle, and goats contained titratable concentrations of virus during the early, acute, and early convalescent stages of the disease (Tables 2, 3, 4). However, the washed erythrocyte fractions usually contained from 1 to 3 log\(_{10}\) higher concentration of virus than the buffy coat during the same stage of the disease. Furthermore, the washed erythrocyte samples on PI day 21 and 28 were the only fractions that consistently contained a titratable quantity of virus. The 3 cattle washed erythrocyte fractions had log\(_{10}\) viral titers of 2.3, 3.6, and 4.0 on PI day 28 while 2 of the sheep and only 1 of the goats had erythrocyte fraction viral titers on PI day 28.

The month-old calf infected with strain 63-66B BT virus had a viremia with viral isolations very similar to those recorded for B47 (Table 3); the washed erythrocytes viral titer on PI day 28 was 4.5.

Graphic representation and summarization of the BT virus distribution in the 3 blood fractions of the blood of B47, is presented in figure 1. It demonstrates the minimal concentrations of BT virus in the plasma, a much greater concentration in the buffy coat fractions, and the greatest concentration in the washed erythrocyte fractions, especially at PI day 21 and 28. This typifies the viral distribution in all the animals of each species tested.

Viral assays of the non-fractionated blood in OPG samples collected on PI day 28 from 10 animals in experiment II were negative for isolation of the virus. This was verified by repeating the test with several of the samples and inoculating up to 24 embryonating chicken eggs per sample. Reassay was also conducted on several of the blood samples collected from animals on the same day that the washed erythrocyte fraction had high viral concentration but again no virus was isolated from whole blood in OPG.

All of the serum samples collected on PI day 28 from the 10 animals in experiment II contained BT precipitin antibodies as shown by positive AGP tests.
DISCUSSION

The results of BT viral assay of the plasma,uffy coat, and washed erythrocyte blood fractions obtained from sheep, cattle and goats indicates that BT viremia is predominantly associated with cells rather than plasma. Spruell’s finding in 1905\textsuperscript{19} that sheep serum was just as infective as whole blood and Pini et al.'s\textsuperscript{5} finding that unmodified BT virus was closely associated with the buffy coat were not erroneous since the present study determined that the virus could be found in all 3 blood fractions. The fractionation procedure used did not exclude erythrocytes at all times from the buffy coat fraction. For example, viruses can be adsorbed to leukocytes, phagocytized by and replicated within leukocytes.\textsuperscript{3} Also, it has been documented that the buffy coat fraction contains cellular particles other than leukocytes or platelets that are capable of carrying virions.\textsuperscript{7} In order to develop a procedure for blood fractionation it was not considered essential to eliminate trapped erythrocytes in the buffy coat fraction by excessive manipulation. Furthermore, in preliminary studies, as many as 3 washings of the buffy coat gave essentially the same results as obtained by the procedure described. In many instances the cohesive and sticky buffy coat was white in the early and convalescent stages of the disease whereas during the peak viremia stages of the disease it had a reddish tinge. Since a hemolytic anemia occurred in the BT-infected sheep\textsuperscript{12} it seemed logical to conclude that the discoloration was due to adsorption of pigments from the hemolysis of erythrocytes rather than adsorbed or phagocytized BT-infected erythrocytes. In any event, virus isolation from the buffy coat was negative at times when high concentrations of virus were still detectable in the erythrocyte fraction. Thus, the procedure was accurate in a practical sense and is a technique which should prove useful and could be applied at most diagnostic laboratories.

The most significant aspects of the findings reported are concerned with the diagnostic applications and potential reservoir sites of the virus. Since BT disease, at certain stages, resembles vesicular diseases such as foot-and-mouth disease, differential diagnosis is essential especially in sheep and cattle and failure to isolate the virus may lead to an inaccurate diagnosis. Standard methods of unmodified BT viral assay in embryonating chicken eggs as previously described\textsuperscript{2, 9, 13} and as used on the blood samples collected in OPG on PI day 28 in this study gave negative results. In reality, the whole blood samples were not BT virus-free as infectious virus was demonstrated to be present in the red blood cells. It is not known whether this virus could initiate an infection cycle by transmission to insect vectors but apparently an ideal epidemiological “iceberg phenomenon” exists in regard to the inapparent clinical response of cattle and goats to the virus. Another very important latent feature of BT disease appears to be unfolding in the state of Minnesota. The Denver laboratory has made only 1 BT viral isolation from Minnesota which was from cattle, but recent serologic evidence suggests that the virus is prevalent throughout the state.\textsuperscript{1} Thus, BT virus probably should be considered ubiquitous, not only because it has been isolated from 4 continents but
because it seems to be widely distributed in cattle in Minnesota as an apparent masked disease entity.

Coexistence of BT virus and specific serum neutralizing antibody has already been documented for sheep\(^9\) and cattle\(^{13}\) on PI day 21. This coexistence was demonstrated utilizing whole blood in OPG from which BT virus was isolated as late as PI day 31 and 26 from BT-infected sheep and cattle, respectively. The virus was previously isolated as late as PI day 21 from goats.\(^{11}\) Also, the virus was isolated from BT-infected cattle on PI day 50 via sheep inoculation but not by intravascular inoculation of embryonating chicken eggs.\(^{13}\) In the present study it was not deemed necessary to demonstrate a rising serum neutralizing antibody titer which regularly occurs with BT virus infection in sheep, cattle, and goats by PI day 21. For purposes of this study the positive AGP test on PI day 28 was considered ample evidence of the test animals immunologic competence. Finding of high concentrations of BT virus in blood of infected animals when specific antibodies are present may indicate a firm adsorption of the virus to the erythrocyte membrane or localization within or outside of the membrane so that the virus is protected and precludes its neutralization. The firmness of the virus-erythrocyte relationship was further demonstrated when numerous preliminary attempts were made to elute the virus from the erythrocyte. Elution was attempted by washing BT-infected erythrocytes with various buffers at varying pH and temperature conditions, by addition of lipase, and by aging, but without success. Attempts were not made to exhaust all potential procedures but preliminary evidence indicates that elution, if possible, is not a simple process and BT virus may be sealed in the erythrocyte plasma membrane as previously demonstrated for Sendai virus.

It is apparent from the concentrations obtained in the erythrocyte fraction during the early, acute, and convalescent stages of the disease that the erythrocyte is the primary cell involved in the pathogenesis and transport of the virus in the blood. Isolation of minimal concentration of infective virus from the plasma is believed due to destruction of erythrocytes by the infectious process as hemolytic anemia routinely occurs in infected sheep. It would appear that the erythrocyte destruction was more severe in goats and cattle as evidenced by more isolations of virus from the plasma samples. However, cattle and goats do not routinely develop as severe a reduction in the packed cell volumes of blood during the course of the infection as recorded for sheep. Fragility of the cellular elements in the blood of cattle and goats versus sheep may also have caused this obvious difference. In sheep the buffy coat fraction becomes negative for infective virus as early as PI day 14 (Experiment I) and this might indicate that leukocytes are phagocytizing BT virus-infected erythrocytes until such time as the leukocytes cannot recognize an infected erythrocyte. If replication or adsorption of virus was occurring within the buffy coat fraction, different results should have been observed.

Two strains of BT virus were used in the 2 experiments with the same virus distribution occurring in the blood of the infected sheep for each strain. Although the other 4 BT viral strains\(^{14}\) have not been tested in animals, there is no reason to believe they would produce a major difference in virus distribution in blood
components. BT virus strain 63-66B (formerly BT ox 183) was used in the second experiment because it is the only strain of BT virus tested at the Denver Laboratory that has consistently produced a high viremia in sheep, cattle, and goats.

The use of washed erythrocytes as inocula in etiologic investigations now appears not only to be appropriate, but mandatory if one wants to achieve the greatest efficiency in diagnosis of bluetongue in any suspect domestic ruminant. The procedure would seemingly be of great benefit in the state of Minnesota to extend the etiologic boundary¹ as well as in many other states where only a few nidus of infections appear to exist. Although no conclusive statement can be made here concerning known susceptible wild ruminants, one can readily speculate that the same virus-erythrocyte relationship exists as occurs in the 3 domestic ruminants reported.

SUMMARY

Bluetongue viremia, in sheep, cattle, and goats as determined over 28 postinfection days, is associated primarily with cells rather than plasma. Quantitative assay of virus in the plasma, buffy coat, and washed erythrocyte fractions showed that there was 10 to 100 times the concentration of virus in the washed erythrocytes than the buffy coat fractions and 1000 to 1,000,000 times the concentration as compared to the plasma fractions during the acute stages of the disease. On postinfection day 28, when blood contained specific BT antibodies, the whole blood samples in OPG were negative for isolation of BT virus, whereas washed erythrocyte fractions yielded log₁₀ viral titers as high as 4.5. Thus, bluetongue virus appears to be very closely associated with the erythrocytes during the early, acute, and convalescent stages of BT infection in sheep, cattle, and goats.
TABLE 1

Bluetongue In Sheep:
The Mean and Range of Viral Titers on Postinfection Days 3, 7, and 14
For 10 Sheep Infected with Bluetongue Viral Strain 62-45S

<table>
<thead>
<tr>
<th>BLOOD FRACTION</th>
<th>POSTINFECTION DAY</th>
<th>3 (Early)</th>
<th>7 (Acute)</th>
<th>14 (Convalescent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>Mean</td>
<td>5.45* (284,325)</td>
<td>5.70 (503,613)</td>
<td>3.63 (4,272)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2.0 - 6.3</td>
<td>3.4 - 6.4</td>
<td>1.8 - 4.4</td>
</tr>
<tr>
<td>Buffy Coat</td>
<td>Mean</td>
<td>3.38 (2,443)</td>
<td>4.63 (42,851)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.0 - 4.3</td>
<td>2.5 - 5.3</td>
<td>0</td>
</tr>
<tr>
<td>Plasma</td>
<td>Mean</td>
<td>2.62 (425)</td>
<td>2.0 (105)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.0 - 3.5</td>
<td>0.0 - 2.4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Titers Are Shown As Median Embry Lethal Dose Per Milliliter.
TABLE 2

Bluetongue in Sheep:
Viral Titers* in 3 Infected Yearling Sheep For a Number of
Selected Days After Inoculation from 3 Blood Fractions
As Assayed by Intravascular Inoculation of
Embryonating Chicken Eggs

<table>
<thead>
<tr>
<th>DAY AFTER INOCULATION</th>
<th>SHEEP NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHEEP 2562</td>
</tr>
<tr>
<td></td>
<td>Buffy Plasma Coat</td>
</tr>
<tr>
<td></td>
<td>RBC's</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>1.5</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>-</td>
</tr>
</tbody>
</table>

* Titers Are Shown As Median Embryo Lethal Dose Per Milliliter.
X Means Bluetongue Virus was isolated.
— Means NO Bluetongue Virus was Isolated.
** Blood in OPG Samples Obtained on Day After Inoculation 28 For All 3 Sheep Were Negative For Isolation of the Virus.
### TABLE 3

Bluetongue in Cattle: Viral Titers* in 3 Infected 8-Month-Old Calves For a Number of Selected Days After Inoculation From 3 Blood Fractions As Assayed by Intravascular Inoculation of Embryonating Chicken Eggs

<table>
<thead>
<tr>
<th>DAY AFTER INOCULATION</th>
<th>B 46 Buffy Coat</th>
<th>B 46 RBC's</th>
<th>B 47 Buffy Coat</th>
<th>B 47 RBC's</th>
<th>B 48 Buffy Coat</th>
<th>B 48 RBC's</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>X</td>
<td>3.6</td>
<td>X</td>
<td>2.0</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>1.4</td>
<td>4.0</td>
<td>5.1</td>
<td>3.6</td>
<td>6.0</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>X</td>
<td>3.8</td>
<td>5.3</td>
<td>4.6</td>
<td>6.0</td>
<td>X</td>
</tr>
<tr>
<td>9</td>
<td>1.3</td>
<td>3.6</td>
<td>5.5</td>
<td>4.3</td>
<td>5.6</td>
<td>X</td>
</tr>
<tr>
<td>11</td>
<td>—</td>
<td>3.5</td>
<td>5.6</td>
<td>4.6</td>
<td>6.0</td>
<td>X</td>
</tr>
<tr>
<td>14</td>
<td>X</td>
<td>2.0</td>
<td>5.3</td>
<td>3.8</td>
<td>5.8</td>
<td>X</td>
</tr>
<tr>
<td>21</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>—</td>
<td>5.3</td>
<td>—</td>
</tr>
<tr>
<td>28</td>
<td>—</td>
<td>2.3</td>
<td>X</td>
<td>—</td>
<td>4.0</td>
<td>—</td>
</tr>
</tbody>
</table>

* Titers Are Shown as Median Embryo Lethal Dose Per Milliliter.

X Means Bluetongue Virus Was Isolated.

— Means NO Bluetongue Virus Was Isolated.

** Blood in OPG Samples Obtained on Day After Inoculation 28 for all 3 Cattle Were Negative for Isolation of the Virus.
# TABLE 4

Bluetongue in Goats: Viral Titers* in 3 Infected 2-Year-Old Goats For a Number of Selected Days After Inoculation From 3 Blood Fractions As Assayed By Intravascular Inoculation of Embryonating Chicken Eggs

<table>
<thead>
<tr>
<th>DAY AFTER INOCULATION</th>
<th>GOAT 67</th>
<th></th>
<th>GOAT 68</th>
<th></th>
<th>GOAT 69</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Buffy Coat</td>
<td>RBC's</td>
<td>Plasma</td>
<td>Buffy Coat</td>
<td>RBC's</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>2.5</td>
<td>X</td>
<td>2.5</td>
<td>3.6</td>
<td>X</td>
</tr>
<tr>
<td>5</td>
<td>X</td>
<td>3.6</td>
<td>4.3</td>
<td></td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>7</td>
<td>X</td>
<td>3.5</td>
<td>4.5</td>
<td>1.4</td>
<td>4.4</td>
<td>5.4</td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>3.4</td>
<td>4.5</td>
<td>X</td>
<td>4.3</td>
<td>5.3</td>
</tr>
<tr>
<td>11</td>
<td>X</td>
<td>4.0</td>
<td>4.4</td>
<td>X</td>
<td>4.5</td>
<td>4.4</td>
</tr>
<tr>
<td>14</td>
<td>X</td>
<td>3.4</td>
<td>3.8</td>
<td>X</td>
<td>2.8</td>
<td>4.0</td>
</tr>
<tr>
<td>21</td>
<td>—</td>
<td>—</td>
<td>2.0</td>
<td>X</td>
<td>X</td>
<td>2.3</td>
</tr>
<tr>
<td>28</td>
<td>—</td>
<td>—</td>
<td>2.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>28**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Titers Are Shown As Median Embryo Lethal Dose Per Milliliter.

X Means Bluetongue Virus Was Isolated.

— Means NO Bluetongue Virus Was Isolated.

** Blood in OPG Samples Obtained on Day After Inoculation 28 For 3 Goats Were Negative for Isolation of the Virus.
REFERENCES

REPORT OF THE COMMITTEE ON EVALUATION AND DEVELOPMENT OF STATE-FEDERAL PROGRAM

J. G. Milligan, Montogomery, Ala., Chairman

Ex-Officio Members:

U.S.A.H.A. Regional Representatives:

Federal:

Livestock Industry:
Cattlemen’s Assoc., Burton Eller, Denver, Colo.; Swine, J. Ralph Bishop, Tipton, Ind.; Livestock Conservation, Paul Zillman, Hinsdale, Ill.

The Committee on Evaluation and Development of State-Federal Programs is a new committee of the United States Animal Health Association.

President Wheeler in his address to this body last year brought out the needs for a most thorough study and evaluation of the present State-Federal Programs. With this thought in mind, he decided to enlarge the Committee on Veterinary Medical Resources and to assign a number of duties and responsibilities to this new committee.

Membership of the present committee is made up of the officers of this Association, one representative from the four geographical areas in the United States, members representing the regulatory programs with the U.S. Department of Agriculture and members representing the livestock interests throughout the United States.

Dr. Wheeler outlined to each member of the committee by letter his opinion of what the committee needed to do and suggested methods of how the committee could best reach the objectives and responsibilities that he assigned the committee. His outline was as follows:

Function of the Committee:
(1) Decide on programs that should demand the Committee’s immediate attention.
(2) Establish priorities for handling the numerous problems.
(3) Determine specific assistance desired from the various standing committees.
(4) Make the work assignments so that some information may be available to
the Evaluation and Development Committee Meeting in Philadelphia. Initial information could be gathered by making use of an indepth, comprehensive questionnaire with the various sections of the questionnaire prepared by the specific standing committee, such as brucellosis, tuberculosis, etc. Questionnaires should be sent to both State Veterinarians and Federal Veterinarians in Charge, to be completed independently of each other.

(5) Later, field evaluation teams should be designated and dispatched to make on-the-spot determinations, concentrating on those areas of deficiency suggested by the completed questionnaire. Teams should have an equal number of State and Federal representatives plus industry representation, and function on a regional basis.

(6) After the field study has been made, the Committee would then develop recommendations for individual states as well as national application.

Areas That Should Demand The Committee's Immediate Attention

(1) Lack of uniformity in the enforcement of Federal regulations from state to state is confusing the public when Federal requirements are enforced in some states and ignored in others. This should be corrected.

(2) There is a dramatic need to standardize the State-Federal Programs, especially the brucellosis, hog cholera and tuberculosis efforts. This lack of standardization has resulted in states losing confidence in the programs and in order to protect themselves imposing additional requirements on livestock moving from states of similar status — thus creating unnecessary barriers to livestock commerce.

(3) There is a great need for one set of health requirements governing the interstate movement of livestock. Instead there is one Federal and fifty additional and different state requirements. This has, in my opinion, grown out of the Animal Health Division's policy of establishing minimum or no requirements and depending upon the states to supply the "teeth". More uniformity has resulted recently in the brucellosis area when the Animal Health Division strengthened its interstate requirements. This forced the deficient states to bring their regulations up to the Federal standards. Perhaps the role the Federal Agency should play in interstate in the future should be one of leadership by establishing requirements at a level adequate to protect the national interest. The states would adjust to it. This would result in fifty one sets of regulations but all identical. I feel this would be preferable to Federal Government take-over of the interstate commerce field altogether.

(4) We must have a comprehensive, effective emergency disease program with all phases of the livestock industry being informed and actually participating in it. The hog cholera eradication program has certainly shown how poorly we have prepared the livestock industry, the practicing veterinarians and the auction operators, as well as our own personnel, for such an ordeal. These people must be convinced of the importance of Emergency
Programs and the roles they are expected to play in them. The industry should be prepared for the economic disturbances that are bound to occur during an actual outbreak. It may be too late already as the real thing may make its appearance before we've had time to adequately prepare the public — and ourselves.

(5) The agony of giving birth to State Meat Inspection Programs equivalent to the Federal meat services is evident in most states. Some state officials ask that the "Law of Reason" prevail as they feel state inspection can be equivalent without being identical. Certainly this is justified but if the "Law of Reason" is replaced by the "Law of Repeated Compromise" then state meat inspection would be neither identical or equivalent. It would, however, be bait for the "Nadar Raiders" who have already shown an interest in the Meat Inspection Program and their desire to have it placed under the Department of Health, Education and Welfare, or a Federal Consumer Protection Agency. Should this happen, meat inspection's great contribution to animal health programs in the future may not be forthcoming. Perhaps the United States Animal Health Association can be of assistance in overcoming some of the meat inspection problems.

(6) Some of the older programs need to be updated, for example tuberculosis eradication. In fact, in its 1969 Committee report in Milwaukee the Tuberculosis Committee recommended that field evaluation studies be conducted.

(7) The need for new programs, such as anaplasmosis, mastitis, equine infectious anemia and others should always be under consideration.

The Committee met in an organization session in Washington, D.C. when the State-Federal Relations Committee met, and organized the Committee at that time. It was decided at this meeting that we would take immediate steps to implement the programs assigned by President Wheeler. The Committee fully realized that the job ahead is a long and tedious task and that all areas of State and Federal programs cannot be studied and evaluated at one time. It was decided that we would concentrate on some of the major programs now being carried out. It was also decided that we would work through the Chairmen of the committees that were responsible for these programs. The Chairmen of the committees on tuberculosis, hog cholera, livestock commerce, and brucellosis were requested to present comprehensive questionnaires that would cover all phases of the individual program so that we would have a start in our undertaking. Questionnaires from each of these four chairmen have been returned to this committee.

Dr. Wixon of the Committee on Brucellosis sought the heads of the officials from Washington and prepared a most comprehensive questionnaire that we feel can be used as a model in preparing future questionnaires. Further study will be given this questionnaire by the Committee. We will then contact professional help within the A.R.S. in getting this questionnaire in order so that all answers can be computerized. If this pilot undertaking is a success, final steps will be taken in preparing questionnaires on other major problems. We have been assured of all
professional help needed through the services available within the U.S. Department of Agriculture.

Although the Committee cannot report any glowing progress during its first year, we do feel that progress has been made and that there is a bright future for the Committee provided it continues to function with the same enthusiasm that has been present during this first year. The Committee wishes to thank all of those responsible for the aid and assistance that the Committee received and we are looking forward to working with these people in the future.
REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF SHEEP AND GOATS

M. D. Mitchell, Pierre, South Dakota, Chairman, J. L. Hourrigan, Hyattsville, Maryland, Co-Chairman
C. C. Beck, Ann Arbor, Michigan; G. L. Crenshaw, Davis, California; E. S. Cox, Austin, Texas; Donald Baker, Albuquerque, New Mexico; W. J. Hadlow, Hamilton, Montana; W. A. Hickman, Pierre, South Dakota; J. B. Henderson, Austin, Texas; Blaine McGowan, Davis, California; R. E. Simmons, Boise, Idaho; T. B. Snodgress, Dallas, Texas; O. H. Timm, Dixon, California; Ward Van Horn, Buffalo, South Dakota.

During the past year and during our deliberation here at this meeting, your Committee considered several health problems of sheep and goats.

BLUETONGUE

Your Committee wishes to call the Association's attention to our recommendation of last year supporting additional bluetongue research. We believe that this disease will become of increasingly greater significance to our sheep and cattle industries and probably also involving wild ruminants.

Further scientific aspects of the bluetongue problem were discussed by Dr. A. J. Luedke. His paper has been submitted for inclusion in the proceedings of this meeting.

SHEEP FOOT ROT

During previous sessions we discussed sheep foot rot at length and made several recommendations. In order to evaluate the importance of foot rot and losses caused by this disease, questionnaires were sent to each state. The States of California, Georgia, Mississippi, New Jersey, Montana, Oregon, and Tennessee considered sheep foot rot of first importance. Alabama, Florida, Idaho, Indiana, Iowa, and Louisiana considered the disease second in importance; it was considered third in Washington, fourth in Nevada, and fifth in Utah and Nebraska. In Virginia about 12 percent of sheep flocks were considered infected. The remaining states did not indicate the importance of the disease or considered it as being not important.

The 13 states considering foot rot of first and second importance reported a 5-60 percent flock infection rate. The morbidity in infected flocks was 5-80 percent with an average of about 30 percent.

Monetary losses from foot rot include expense of attempting to control the disease, culling or death of infected ewes and reduced gains of infected lambs. In order to eliminate the problem the owner may go out of the sheep business and thus sheep production potential in many states are not realized. A strict control program will cost about 60 cents per year per ewe for labor and medication. In one
Oregon flock, returns to the grower were increased 5 dollars per year per ewe following elimination of the disease. If there are 500,000 infected sheep in the United States this represents an annual cost of considerably more than $1,000,000 to sheep producers.

**NEONATAL LAMB LOSSES, OVINE VIRAL ABORTION, RAM EPIDIDYMITIS AND VIBRIOSIS**

Your committee recognized that reducing losses resulting from these diseases and from foot rot involves not only developing more effective vaccines and/or treatments but also informational programs to enable owners to better appreciate the losses they are suffering and to encourage them to more effectively utilize veterinary resources in handling these problems.

Better solutions to these problems are needed. This requires research including development of improved biologics. The problem is further complicated due to the reluctance of commercial firms to develop and market products for which they do not anticipate sufficient sales volume.

We recommend this Association support additional research for these diseases and in regard to neonatal lamb losses a most significant factor in sheep production.

**VIRGINIA'S SHEEP HEALTH PROGRAM**

Dr. A. J. Roth of the Virginia Department of Agriculture and Commerce, reviewed a voluntary program in Virginia by which owners can establish and maintain flocks of sheep that are free of certain infections and contagious diseases. It includes recognition of such health status by official flock certification under the program.

The committee commends Virginia for initiating such a program and encourages them to continue in order that we may benefit from the experience and data they develop.

**SCRAPIE OUTBREAKS DURING FISCAL YEAR 1970**

Scrapie was reported in eight flocks in four states during fiscal year 1970, the same number of outbreaks as occurred during the previous year. The eight outbreaks involved seven Suffolk Flocks and one Hampshire-Suffolk crossbreed flock. The number of flocks under surveillance has risen to 312, up 16 flocks from last year. The increase in flocks under surveillance are the results of tracing sales from the disperal of a large Suffolk source flock in Oklahoma.

The eight outbreaks occurred, one each, in San Joaquin County, California; Greer County, Oklahoma; and Cooke County, Texas; two in Acadia County, Louisiana; and, three in Pottawatomie County, Oklahoma.

One of the outbreaks was reported by an Extension Service Veterinarian; two were reported by the flock owners; and five were reported when tracing bloodline
and/or exposed sheep.

All bloodline and nonbloodline exposed sheep in the eight infected flocks and their source flocks have been slaughtered with the exception 15 bloodline sheep from a Texas infected flock which were taken to Mission, Texas, for use in the Scrapie Field Trail.

All bloodline sheep sold from the infected and source flocks are being traced and slaughtered. Nonbloodline exposed sheep sold from the infected and source flocks in Louisiana, Oklahoma, and Texas are being traced and slaughtered. Nonbloodline exposed sheep sold from the infected and source flock in California have been traced and placed under 42 months surveillance.

SCRAPIE FIELD TRAIL — MISSION, TEXAS

The Scrapie Field Trail has been underway since November 1964 (70 months), during this period scrapie has been confirmed by histopathological examination in 97 animals. These 97 cases have occurred on infected premises No. 3 in the following categories of animals either taken to Mission or born on the premises.

a. Scrapie bloodline exposes sheep 76
b. Field suspects held for observation 14
c. Nonbloodline exposed sheep 2
d. Nonbloodline exposed goats 5

The Scrapie Field Trail has demonstrated that when bloodline exposed sheep are held under observation scrapie losses in these bloodlines are significant and can run as high as 27 percent per year and can affect as many as 40 to more than 60 percent in certain bloodlines.

CONTACT SPREAD OF SCRAPIE TO SHEEP AND TO GOATS

The first known case of natural scrapie to affect a goat in the United States occurred at Mission, Texas, in May 1968. The exposed mixed breed female dairy goat, five years of age, was born and reared in a Missouri flock where she was exposed to scrapir-affected Suffolk sheep until four years of age. She was taken to Mission and developed signs of scrapie one year later.

During the past year contact spread of scrapie has been confirmed in four additional goats and two sheep. These animals were born at Mission and reared since birth in contact at pasture with a series of Suffolk and Cheviot sheep and with goats affected with natural scrapie.

In June 1965 one male Nubian and three female Nubian and one female Toggenburg goats, four months of age, were taken to Mission and added to the flock of sheep affected with natural scrapie. These goats were mated in the fall of 1965. Seven kids were born in February and March 1966. One of these died at five months of age from lobar pneumonia. Of the remaining six, four showed clinical signs of scrapie. These were a Nubian-Toggerburg doe which died at 44 months of
age, a Nubian buck at 44 months, a Nubian doe at 45 months, and a Nubian buck at 47½ months.

The youngest goat had shown only signs of increased nervousness. The oldest goat showed typical signs of scrapie for two months and the remaining two showed clinical signs for only two weeks prior to death.

The affected Hampshire ram born at Mission in March 1967, first showed signs of scrapie in January 1970 and was destroyed in May 1970 at 37½ months of age. His sire and dam were born in February 1965 and taken to Mission in October of that year.

The affected Rambouillet ewe born at Mission in March 1967, first showed signs of scrapie in May 1970 and was destroyed in June 1970 at 38½ months of age. Her sire and dam were born in the spring of 1965 and taken to Mission in July of that year. Her dam died at Mission at 52 months of age from bluetongue complicated by pneumonia, her sire died at Mission at 58 months of age from congestive heart failure. Histopathological examination of the brain tissue of the sire and dam were negative for scrapie.

Histopathological studies in all six cases revealed vacuolation and astrocytosis confirming the clinical diagnosis of scrapie.

It is believed that these four goats and two sheep developed scrapie as a result of contact exposure to the natural disease at Mission. In each case the sire and dam of the affected animal was purchased from farms or ranches where it is believed scrapie had not occurred. The purchased sheep and goats were not of bloodlines in which scrapie has occurred. Twins of the purchased animals were left in the flocks in origin as controls. None of these have shown evidence of scrapie.

Although only the six animals have been confirmed cases of scrapie several additional sheep in the contact experiment are showing signs indicative of the disease.

SCRAPIE SYMPOSIUM
HYATTSVILLE, MARYLAND – MARCH 12, 1970

The Animal Health Division held a symposium attended by scientist working in the field of human and veterinary medical research and regulatory workers to discuss scrapie from the field aspects, observations at the Mission Field Trial, and the similarities of scrapie, transmissible mink encephalopathy (TME), kuru, Creutzfeldt-Jakob (C-J) disease, and multiple sclerosis. The possibility of a human health aspect form scrapie and TME was also discussed.

It was the consensus of the group that although scrapie, TME, kuru, and C-J disease have much in common and are caused by a unique group of infectious agents, it is not possible at this time to state how closely one of these agents may be related to the others.

The group recommended that the Scapie Eradication Program be continued and that the Mission study be continued and expanded in cooperation with research scientists to include mouse inoculation studies on diagnosis and pathogenesis of
scrapie, the feeding and inoculation of scrapie-affected sheep tissue to mink to learn the relationship of TME to scrapie; that active scrapie research continue as a model for the study of slow virus diseases of man; and that attention be given to comparing the host range of scrapie, TME, Kuru, and C-J disease particularly in primates.

The group believed that animals showing signs of scrapie or other communicable disease on antemortem inspection should not be slaughtered for human consumption. This represents no change from past and present practice. In the absence of definite research information indicating danger to human health from the consumption of exposed animals that are free from signs of scrapie, the group believed that we are not justified in withholding such animals from regular slaughter at this time.

RESOLUTIONS

Your committee had one resolution which along with the report we gave to the Executive Committee we recommended approved.

Scrapie

Whereas the State-Federal scrapie eradication program has greatly restricted the spread of scrapie in the United States and has held the disease to a minimum, and Whereas there have been efforts on the part of a limited number of Congressmen to amend and strike "scrapie and" from the basic act of May 24, 1884, and Whereas such an amendment would greatly restrict the action of animal disease regulatory agencies and defeat the purpose of the eradication program, and Whereas research has disclosed that there are certain similarities of scrapie of sheep and goats, transmissible mink encephalopathy of mink, and kuru of Creutzfeldt-Jakob disease of man,

Therefore be it resolved that research be continued to develop more information on these diseases including the possible human health aspects of scrapie and transmissible mink encephalopathy, and that the USAHA opposes bills in Congress which will make control of scrapie more difficult.
REPORT OF THE 1970 COMMITTEE ON
IMPORT – EXPORT

John F. Quinn, Lansing, Mich., Chairman
Sub-Committee on Domestic Animals and Birds:
Dr. C. L. Campbell, Tallahassee, Fla., Chairman
   Paul C. DeLay, Beltsville, Md.; Frank Harding, Geneva, Ill.; James R. Hay,
   Chicago, Ill.; James B. Henderson, Austin, Tex.; John R. Langridge, Hyattsville,
   Md.; Robert Rumler, Brattleboro, Vt.
Sub-Committee on Animal Products and Byproducts:
Dr. J. C. Shook, Harrisburg, Pa., Chairman
   E. L. Brower, Trenton, N.J.; J. J. Callis, Greenport, L.I., N.Y.; M. G. Hynes,
   Dublin, Ireland; M. L. Crandall, Detroit, Mich.; G. S. Kaley, Albany, N.Y.; P.
   D. Cazier, Lanham, Md.; W. W. McMichael, Hyattsville, Md.; A. R. Miller, Falls
   Church, Va.; C. H. Pals, Arlington, Va.; W. L. Sulzbacher, Beltsville, Md.
Sub-Committee on Wild and Endangered Species of Animals and Birds:
Dr. T. H. Reed, Washington, D.C., Chairman
   R. A. Bankowski, Davis, Calif.; G. E. Cottral, Greenport, L.I., N.Y.; L. J. Goss,
   Cleveland, Ohio; Gus Griswald, Philadelphia, Pa.; R. E. Omohundro, Hyattsville,
   Md.; John Richardson, Atlanta, Ga.; C. C. Shroeder, San Diego, Calif.; C.

The Import-Export Committee of the United States Animal Health Association
met in the Jefferson Room of the Warwick Hotel in Philadelphia on Wednesday,
October 21, 1970. On roll call by Wilson Powell, Secretary, the following members
were present: John F. Quinn, Chairman, C. L. Campbell, Paul C. DeLay, Frank
Harding, J. C. Shook, E. L. Brower, M. G. Hynes, Grant S. Kaley, W. W. McMichael,
C. H. Pals, and Claude A. Smith.

The Committee considered the following resolutions which were approved and
ordered to the Committee on Nominations and Resolutions:

RESOLUTION

WHEREAS, International trade in meat and poultry products is essential to the
national welfare; and

WHEREAS, consumers have a right to expect that all imported meat and
poultry products sold are wholesome and prepared under standards equal to the
United States standards; and

WHEREAS, there is no substitute for adequate veterinary antemortem and
postmortem inspection at the plant of origin by veterinarians and inspectors in that
country; THEREFORE,

BE IT RESOLVED that the Department of Agriculture be commended for its
plan to improve its foreign review program by assigning highly qualified
veterinarians to foreign posts.
BE IT FURTHER RESOLVED that the veterinarians so assigned should be of a rank commensurate with their responsibility for being the primary representative of the United States in the area of meat and poultry inspection in the foreign country.

RESOLUTION

WHEREAS, consumption of meat from animals and poultry containing biological residues (as defined in the Federal Meat Inspection Regulations) may be injurious to human health; and

WHEREAS, consumption of meat from animals and birds infected with zoonotic diseases may be inimical to human health; and

WHEREAS, legislative authority is not uniformly provided for the quarantine and disposition of animals and birds containing biological residues or infected with such zoonotic diseases, which are intended for slaughter; THEREFORE,

BE IT RESOLVED that the United States Health Association urge the Congress of the United States and those State legislatures which have not already done so to enact legislation to provide the mandatory quarantine and disposal of animals and poultry so affected.

A third Resolution forwarded to this Committee by the American Association of Zoo Veterinarians had to do with the development of a health certificate by the Association to be used for the movement of zoo animals. Your Committee wishes to commend the Association for their interest in animal health and urges them to have continued interest in bettering the circumstances from which animals are moved to provide proper inspection and certification.

The Committee wishes to commend the U.S. Department of Agriculture for its action in drawing up a proposal to be published in the federal register regulations which will preclude the entry of animals possibly affected with equine piroplasmosis and Dermacentor nitens ticks, including zebras, from these foreign countries.

The Committee recommends that the federal government promulgate regulations giving them more authority over the importation of animals which would possible carry diseases or viruses of diseases, endangering our livestock. However, it was brought out by Committee members from USDA that even if we had laws giving this authority, the present facilities are not sufficient to make such inspections and surveillance possible. It is recommended that more money be made available by USDA for the purchase of facilities to remedy this situation, including the purchase of land if necessary.

It was brought out in Committee discussion that there should be a study made on the various wild animals imported into the United States, the diseases with which they can be affected, and the organisms they may serve as possible carriers for, and finally in each case the tools of diagnosis and detection which we have available for the various diseases, and weaknesses and suggestions for improvements. One of the Committee members has volunteered to have someone in his organization make this study.
A matter of concern in the organization within the Agricultural Research Service has been brought to the attention of this Committee. It has to do with reorganization of the Plant Quarantine Division and the import-export functions of the Animal Health Division of ARS. Information has been received that these functions per se will be absorbed by the Plant Quarantine Division, which will have the responsibilities of import inspection and quarantine on animals and animal products as well as their plant responsibilities. It is the feeling of this Committee that such action would relegate the veterinary profession as subservient to supervision which is not knowledgeable in the field of animal diseases and organisms concerned therewith. Therefore, this Committee recommends that the U.S. Animal Health Association request of the Secretary of Agriculture an early meeting so that the Secretary may be made aware of the concern of this Association toward maintaining the high standards of inspection and surveillance over the importation of animals and animal by-products into this country.

It was brought out in Committee discussion again that the United States is the only country not charging so-called landing fees for people or products entering into this country. Previously this was suggested as a possible means of financing a more complete animal inspection and quarantine service on all imported animals and animal products. A Committee member was delegated to make an investigation of how this is done by other countries and of possible ways that such a policy could be inaugurated and be put into operation in this country. And, most important, how such a fee could be levied and then set aside for the express purpose of financing animal inspection and quarantine activities. This information to be brought before the Committee next year.
REPORT OF THE COMMITTEE ON
STATE-FEDERAL RELATIONS

M. D. Mitchell, Pierre, S.D., Chairman
W. L. Bendix, Richmond, Virginia; J. R. Hay, Chicago, Illinois; T. A. Ladson,
College Park, Maryland; M. D. Mitchell, Pierre, South Dakota; G. B. Rea,
Salem, Oregon; J. C. Shook, Harrisburg, Pennsylvania; W. C. Tobin, Denver,
Colorado; F. B. Wheeler, Baton Rouge, Louisiana

The State-Federal Relations Committee of the United States Animal Health
Association met in Washington, D.C. on March 16-20, 1970. At this meeting we
were in conference with United States Department of Agriculture Officials,
Directors, and staff of the Animal Health Division, Animal Disease and Parasite
Research Division, Veterinary Biologics Division, Consumer and Marketing Service,
Federal Extension Service, and the Bureau of Veterinary Medicine of the Food and
Drug Administration. The Committee wishes to express appreciation to all Federal
personnel who gave freely of their time and efforts at this meeting and we hope
that mutual benefits will result for all agencies and associations concerned. The
State-Federal Relations Committee presents the following statements for mutual
consideration and guidance.

ANIMAL HEALTH DIVISION

This committee has reviewed all the animal health programs, and is in complete
agreement with the concept of eradication of animal diseases rather than control.
This concept is sound and realistic in addition to being more effective and efficient
in controlling diseases of food producing animals. Disease losses can no longer be
afforded if we are going to provide the necessary food and fiber for our expanding
population. Coupled with these increased production demands is the decrease in
available lands for agricultural and specifically animal production.

Present animal health programs have proven their effectiveness, and we are on
the threshold of complete eradication of a number of diseases with which we are
presently concerned. Other programs have been delayed by lack of sufficient funds
to adequately administer phases of the programs essential to complete success. In
some instances some programs have found it necessary to borrow funds from other
program areas to meet existing everyday situations. A case in point is the need for
the Hog Cholera eradication program to ask for funds for indemnity payments
which had to be diverted from other program areas. In this particular program we
recommend the division make immediate moves to stop all salvage of exposed
swine, curtail the feeding of garbage to swine, and more closely control the
movement of swine. The cost of this program has never approached the predicted
sums; and the sooner we put all our forces together, the sooner the eradication
effort will succeed. We recommend the Department adequately support this
program financially to provide all their present needs and prevent the need of
diversion of funds from other programs which may tend to prolong the eradication of those diseases.

With emphasis on eradication our existing diseases, we are cognizant of the increasing danger of the introduction of foreign animal diseases. It is imperative that we exert more effort to control import-export animals and animal products. Changes are occurring rapidly in transportation methods and techniques of handling animals and animal products. We must not only meet these challenges, but make every effort to keep ahead of them sufficiently to prevent introduction of any foreign animal diseases into the United States. This will undoubtedly require more manpower and facilities. We must also be certain we have maximum capabilities of diagnosing and controlling these diseases, should they be introduced.

In the case of screwworm control through the use of a sterile fly barrier zone, this committee recommends that serious consideration be given to realistic negotiation with the Mexican Government to eradicate the insect from Mexico and establish a barrier zone in Southern Mexico. This would result in an ultimate savings of 4 million dollars annually.

The policy of assigning staff members in foreign countries to review disease programs in those areas and give us increased assurance that animals and animal products with a potential threat to our industry do not leave those countries is sound and should be continued and expanded as needed.

In all of our animal health programs we must consider the effectiveness of herd and flock depopulation in disease outbreaks. Our present techniques and policies have gotten us to our present point of success, but with more movement of livestock over greater distances in less time it is often more economical to depopulate than to quarantine and attempt to wrestle out of the situation with testing and vaccination. Brucellosis, Tuberculosis, and Hog Cholera are examples where depopulation of infected herds and flocks may be the final answer to eradication. This method may require changes in funding since the initial cost may be greater, but savings of many millions of dollars over the long period will result. These savings are reflected not only in decreased costs of administering a control program, but in the saving of livestock production losses from disease.

When we take these seemingly drastic measures we have got to be sure we have a foolproof identification system so that meat inspection and animal health can work hand in hand at getting to the source of infection quickly and with confidence. An effective identification system must be developed at the national level with the cooperation of all states. It must involve all species and not be limited to any specific disease.

The trend toward decentralization of certain program areas must be approached very carefully. While guidelines and funds may be provided to the states by the Federal Government, caution should be exercised to be sure proper coordination on a national basis remains and that we do not move too quickly through the transition phase that uniformity of effort is lost rather than enhanced. Political atmospheres in the various states vary greatly and caution must be taken to assure us that this atmospheric pollution does not cloud our thinking. Sometimes
we need someone looking over our shoulder to snap us out of these illusions.

This committee wishes to compliment the Animal Health Division for the effective manner in which they have been conducting all of the animal disease and related programs. We also express our gratitude for the assistance and guidance they have provided to the states in our cooperative programs. Jointly we are facing a challenge of greater magnitude than ever. We hope we do not fail to meet this challenge by being penny wise and pound foolish in our disease eradication efforts.

VETERINARY BIOLOGICS DIVISION

The Committee wishes to express appreciation for the cooperation extended to it by the Director and his staff in explaining the aims and goals, and the requirements of the Veterinary Biologics Division. The Veterinary Biologics Division cannot accomplish their objective without testing the products being produced commercially under Federal license, and they cannot test unless they have the test methods to do so. With existing funds, facilities, and people, the Veterinary Biologics Division is accomplishing 50% of its mission, and to this extent, a good job is being done.

The Veterinary Biologics Division has received some assistance in the form of additional Biometrical Service personnel, but additional help is still apparent in this field in order to cover all methods and to be sure they are statistically valid.

For this division to maintain its current status it becomes imperative that adequate test facilities be provided along with personnel and funds. Last year this committee suggested specific facilities that could be provided. At this time we are anticipating that some of these suggestions are still being considered. The committee recognizes that the efficiency of the Veterinary Biologics Division is being maintained at a high standard by the fact that since 1967 not a single dose of Poultry vaccine has been found contaminated with PPLO compared with 156 million contaminated doses the previous year.

Safety and efficacy of licensed veterinary biologics is the primary responsibility of the division. In order to accomplish its objectives, adequate testing methods are essential. A program to develop adequate tests for the efficiency of such products as:

- Equine Influenza vaccine for horses
- Coccidiosis vaccine for poultry
- TGE vaccine for swine
- Anaplasmosis vaccine for cattle
- Feline Pneumonitis vaccine

These are just a few of the important products we have mentioned that are being produced today.

It is still evident to this committee that contaminated dangerous and worthless products are still available for use within the livestock and poultry industries. For these reasons the committee recommends that proper field investigations of all complaints are essential and serve a useful purpose and that the division should
request sufficient funds to implement this program.

**ANIMAL DISEASE AND PARASITE RESEARCH DIVISION**

Reliable figures show that we have a livestock industry worth about 30 billion dollars. We are losing somewhere in the vicinity of 15% of that industry or 450 million dollars annually to diseases and parasites. We are spending about 35 million annually on research to reduce this loss. We are using the wrong amortization tables to finance research for animal diseases and parasites. When we consider the welfare of the citizens of this country we must not forget that reduction in the efficient production of food does not contribute to that welfare.

For the past two years the Animal Disease and Parasite Research Division appears to have been the unwanted child when it comes to budget support. The efforts of other agencies depends a great deal on the results of work done by the A.D.P.

How can our Animal Health specialists continue to reduce the inroads of disease without the necessary tools? When you design a new aircraft, you also have to provide new tools to produce that craft. When new diseases are found, you must develop new methods of combating them. The same is true when we find that certain parasites are carriers or vectors of disease.

There is a great need for the development of training procedures and facilities for the further education of diagnosticians of exotic diseases. The United States is today a sitting duck for these foreign invaders, and only through great diligence on the part of the few people working in this area, and also a lot of luck, that we haven’t already had a catastrophe such as England experienced a few years ago.

The A.D.P.’s request for improvement of Plum Island for further training of diagnosticians has gone unheeded for the past two budget years. This year they are resubmitting planning monies for the same facilities.

Blue tongue in Sheep and Cattle is being diagnosed more frequently and in ever increasing areas of the United States at an alarming rate. In Cattle especially we do not have satisfactory treatment. Several foreign countries (Ireland & Australia) will not accept livestock that cannot be certified as being free from exposure to this disease. These countries will dramatically increase in number as awareness of this situation becomes greater. A request for support of this work was denied last year. The ADP has again submitted budget requests to support additional facilities and staff for further research.

The General Services Administration is about to release a Toxicology and Insects Research Laboratory facility to the ADP. These buildings are adequate for 18 scientists and their back up staff, animals, and equipment. The division will require funds necessary to initiate staffing procedures immediately. Certainly with the already demonstrated need for control of environmental pollution, water, soil, man and animal, the search for new and better ways of control and, or eradicating insects — must receive top priority. When we control livestock insects we also control human pests.
Calf Scours and Enteric Diseases of cattle are causing an annual loss of 8 million dollars to the cattlemen of the United States. This loss does not include that due to reduced feed efficiency, infertility, and other allied maladies. Less than 400 thousand dollars is presently being spent to support research in this most important complex of diseases. It is estimated that it will take at least 50 scientific man years to develop the tools necessary to defend the industry against this group of diseases.

In Swine a similar problem known as Transmissible Gastroenteritis contributes losses up to 16 million dollars annually. Minimal research efforts are presently less than 300,000. Swine owners of the nation are confronted with a major catastrophe. It has been reported that this disease is actually affecting the cost of pork products to the consumer. Funds necessary to support 6 additional scientific man years will be necessary to develop research data for applications of control procedures.

(1 million dollars has been identified for respiratory, reproductive, and gastrointestinal research requested by the horse industry.)

CONSUMER AND MARKETING SERVICE

The United States Animal Health Association appreciated this opportunity to present some of its views and a recommendation or two with regard to the Meat and Poultry Inspection activities of the Consumer and Marketing Service. We primarily represent the several states through their animal health activities, but we have been drawn into your programs, as you have been drawn into ours by congressional mandate.

We would like to direct our remarks today to three principal themes. 1. The cooperative effort — 2. Animal Disease Control and 3. Talmadge-Aiken program funding. Other matters may come up during discussion, but these three represent our main concern at this time.

1. The Cooperative Effort

We have had a most agreeable and we feel production discussion with the staff people in meat inspection. We compliment the Department on the greatly changed attitude and the improved relationships we see emerging. Perhaps on our part there is less belligerence and resentment. Certainly there was none of the “take it or leave it” on the part of C&MS as seen in previous sessions. Some of the tensions seem to be gone on both sides — this is good.

a. Your system of assistance and guidance through the Federal State Cooperation Officers is very sound and should be continued. State Meat Inspection may eventually merge into one cooperative program, but the time for that is not yet and we urge the continuance of the present system.

b. We need new regulations. There are many areas in which clarification is needed. What is a retailer? What is permitted retailers? What will custom slaughter operations consist of? We are concerned about this so called “Cold Inspection” concept. Federal experience is mostly limited to the operation of large interstate plants. State experience is limited to the
smaller local operations — each has much to give the other. We urge the opportunity for this be provided. We all need definite written guidelines to follow that will not be lightly or surprisingly changed.

c. Your meat inspection cooperative officers along with the staff people here and in the field met last fall with the U.S. Animal Health Association at its Milwaukee meeting. Your people now serve on many of our committees and C&MS has official membership on our Executive Committee. We are extremely pleased at this and feel this is going to be a mutually profitable relationship. We urge that this relationship be continued and allowed to grow as it will.

2. Animal Disease Control

Whether the law makers on Capitol Hill realized it or not, or whether United States Department of Agriculture realized it or not, when Congress mandated total and equal meat and poultry inspection throughout the land, meat and poultry inspection joined the animal health team.

Where it existed State Meat Inspection has long been used to further animal disease control efforts. Federal meat inspection has held itself aloof and said that its job was to see that the diseased animal was removed from trade channels (and the consumer table) and it had no interest in what caused the disease or even reported it. Are we aware of the exception in reporting autopsy findings on Bovine Tuberculosis reactors and reporting suspicious Tuberculosis like lesions, but by and large this was the full extent of it. Times have changed. No longer do disease control officials control disease by checking every farm premise physically. They screen animals moving through concentration points and we must make slaughter centers a part of this effort. We are trying to eradicate Hog Cholera. Not just visibly sick swine, but tissue samples from slaughter centers for analysis. Bovine Brucellosis is being conquered, but slaughter surveillance will surely be necessary. The concept of total herd depopulation is developing to insure our final victory over Bovine Tuberculosis. It is reliably estimated that this alone will shorten the time it takes us by about 15 years and will reduce the cost by between 100 to 200 million dollars. Meat Inspection personnel will play a very important role in this program. The market cattle testing philosophy will undoubtedly be extended to other species, and other diseases as the need arises. Why? Its simple, it works, and its cheap.

Meat inspection must be a part of the team. This is another and vital reason that the meat and poultry inspection staff should maintain close and intimate association with U.S. Animal Health Association and fully participate in its deliberations. You want to remove discarded animal products from the food supply. We want to remove the cause of disease so that they are not offered for slaughter. We can do this as a team.

3. Talmadge-Aiken Program Funding

To project the total Federal and State effort through FY1971 — figures we have gotten indicate the following:
Poultry Inspection
   Interstate – 951 plants – $32,000,000 Federal
   Intrastate – 1839 plants – $1.8 million – Federal and $1.8 million – State

Meat Inspection
   Interstate – 3600 plants – $68,000,000 – Federal
   Intrastate – 1500 plants – $22.5 million each Federal and State.

This is the broad general breakdown. The other side to this triangle are the Talmadge-Aiken plants where state personnel are providing Federal inspections, using Federal stamps and the product is acceptable in interstate commerce. Federal funds provide 50% of this cost of this type of inspection. The question is being asked more and more frequently; Why are the states required to pay 50% of the cost of providing strictly Federal inspection? What is the answer?

This is not a large item. The record shows that there are only 161 T.A. meat plants and 4 T.A. poultry plants. Federal estimated costs for FY70 are $1,300,000 for the meat plants and $20,000 for the poultry plants. 100% Federal financing would only double this modest figure. Rumor has it that some states are getting a much better “deal”, than others up to and including better than 100% financing of their efforts.

May we have clarification and a full and open review of this whole matter? We are not in a position to make a firm recommendation at this time. There are two reasons – First, we don’t have all of the facts, and second, we don’t know the feelings of all the states. We do know the facts need to be brought forth, the Departments position needs to be known, and the matter needs to be settled. We ask that this be done.
RESULTS OF A THREE YEAR RESEARCH PROGRAM ON A SIMPLE SYSTEM OF CONTROLLING MASTITIS


This three year experiment is designed to measure the value of a simple hygiene method along with dry treatment of all quarters in reducing the mastitis infection level in herds under different management systems. Data are now available for the full 36 months of the experiment so that this is a preliminary report of the first three years of the project. This work involves the Animal Science Department of the New York State College of Agriculture and the New York State Mastitis Control Program of the New York State Veterinary College at Cornell University. In addition, we are indebted to, and are working in cooperation with, the mastitis research workers at the National Institute for Research in Dairying, Reading, England.

The work is being done in two counties in New York and was started with twenty-seven herds with just over 2,000 cows. The initial sampling was done on milking cows and quarter samples were obtained from over 1,756 cows. Two herds have been removed from the experiment so that we are at present working with 25 herds with about 200 cows less than at the start of the study. Fourteen herds are housed in free-stall units, at present, 11 herds are in stall barns.

The program compares two hygiene programs with the basic idea that the reduction in the spread of infection in the herd should be of value in a control program. All cows are dry treated after the last milking in order to reduce existing infection and reduce the occurrence of new infection during the dry period. Specifically, the two hygiene routines are:

1. Partial Hygiene
   - Milker wears smooth rubber gloves for milking.
   - Rinse gloved hands in disinfectant (600 ppm hypochlorite) before handling any cow.
   - Examine foremilk with cup or plate before udder washing.
   - Wash udder using paper towel and disinfectant or hose with water containing disinfectant from aspirator device or hose and water followed by paper towel and disinfectant solution.
   - When milking is completed, rinse hands in disinfectant and remove teat cups after minimum machine stripping.
   - Dip each teat in disinfectant (40,000 ppm available chlorine).
   - No treatment of teat cups between cows.

2. Teat Dip Only
   - Milker may wear rubber gloves to protect hands.
   - Examine foremilk with cup or plate before or after udder wash.
   - Use common cloth or sponge to wash and wipe.
   - Dip each teat in disinfectant (40,000 ppm available chlorine) at end of
milking.

No treatment of teat cups between cows.

The partial hygiene program is similar to one in use in England that reduced the new infection rate 44%. It is being compared to a program using teat end dipping only as its major component.

For dry cow therapy an experimental preparation of one million units of penicillin and one gram of streptomycin in three percent aluminum monostearate in peanut oil is used in each quarter. This is not the same material (Cloxacillin) as is used by the English workers but was shown to be efficacious in earlier trials by the workers at the New York State Mastitis Control Program. It is under study by the Food and Drug Administration but is not yet acceptable to them for dry cow therapy. This material is infused in each quarter after the last milking and no further treatment or milking is permitted until calving, unless there is an obvious clinical case of mastitis.

Milk samples for bacteriological examination are taken at the following times:
1. Two herd tests of all quarters of all milking cows, one week apart, at the start of the experiment.
2. A single annual herd test on all quarters of all milking cows.
3. Double samples are taken at drying off. The procedure used is to clean the teat end, sample, reclean the teat end, and resample immediately. The same procedure is used for cows culled from the herd.
4. Two samples taken one week apart from each quarter on the first and second weekly visit of the technician following the cow's calving date.
5. Double samples are taken by the milker before the treatment of clinical mastitis.
6. Two samples are taken 21 and 28 days after treatment of a clinical case.
7. A single sample is taken on any occasion when a repeat sample can be taken and is required to confirm a change in the state of infection.

The samples are, in general, taken by one of the three technicians who visit the herds each week for this purpose. With the possible exception of samples which cannot be retaken (drying off, clinical case, leaving herd) the same pathogens must be found in two consecutive samples in order to diagnose an infection. The farmers generally treat clinical cases with material provided by the project and are asked to treat a clinical case twice with a 24 hour interval between treatments. All bacteriological work is done by the laboratory of the Mastitis Control Program at the Veterinary College.

RESULTS

TABLE 1
Change in Levels of Infection over 36 Month Period

<table>
<thead>
<tr>
<th>MONTHS</th>
<th>0</th>
<th>12</th>
<th>24</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Quarters Infected</td>
<td>29.6</td>
<td>12.5</td>
<td>7.8</td>
<td>9.7</td>
</tr>
</tbody>
</table>
RESULT OF RESEARCH ON CONTROLLING MASTITIS

Table 1 shows the change in the percentage of infected quarters over the 36 month period. These percentages were taken at the time of the annual survey of every quarter of all milking cows and is based on two samples showing the same organism. It is noticeable that there was a considerable decrease over a two year period but that there has been a slight increase during the third year. These is no satisfactory explanation at present for this change.

During this time period the annual cow turnover rate was 23 percent which is exactly the same as that of tested herds in New York. Also, the initial surveys of the New York Mastitis Control Program indicated that there had been no change in the average percentage of infected quarters in the other surveyed herds over the same period of time. The trend in percentage of infected quarters is similar to the trend shown in the similar experiment carried out by the National Institute for Research in Dairying in England.

This reduction in overall infection level has been accompanied by some considerable changes in the proportion of the different organisms in the herds.

Table 2 shows how these have changed over the 36 month period.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Change in Percentage of Quarters Infected by Different Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months</td>
<td>H. Staph.</td>
</tr>
<tr>
<td>0</td>
<td>10.0</td>
</tr>
<tr>
<td>12</td>
<td>2.6</td>
</tr>
<tr>
<td>24</td>
<td>1.9</td>
</tr>
<tr>
<td>36</td>
<td>1.4</td>
</tr>
</tbody>
</table>

The average figures show that there have been considerable decreases in the importance of hemolytic Staphylococcus and *Streptococcus agalactiae* but that *Streptococcus dysgalactiae* and Others have decreased only slightly. This is presently being studied but no conclusions have been reached.

In a study of a large number of herds it is important to get some idea of the performance of individual herds. This is particularly true where a large part of the success of the method is dependent upon the continuing use of teat-dip at every milking. In Table 3 the number of infections has been broken down into categories and in the body of the table is shown the number of herds falling into each category for each organism and in total.
TABLE 3

Number of Herds with Different Numbers of Infections

<table>
<thead>
<tr>
<th>No. Infections</th>
<th>H. Staph.</th>
<th>Str. ag.</th>
<th>Str. dys.</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>13</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1- 4</td>
<td>13</td>
<td>8</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5- 9</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>10-14</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>15-19</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>20-29</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

This table shows that most of the herds are maintaining low levels of the common infections but that a few herds make up a large proportion of the total infections. Several of the herds with no infections of the common organisms have been able to maintain this position over considerable periods. One herd, which over the period of the experiment has expanded considerably in numbers, accounts for 16% of the "Others" category and 12% of the total infections. From this it is reasonable to assume that there have been other factors in this herd than obtained in the more successful herds.

No differences were found between the partial hygiene and the teat-dip only programs or between housing systems. This indicates that the differences between herds are probably due to differences between operators rather than between the hygiene systems or the housing methods.

Table 4 shows the new infections which appeared during the latest 13 week period broken down by organisms.

TABLE 4

New Infections in Cows in Milk (Latest 13 Week Period)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. Staph</td>
<td>31</td>
<td>6</td>
</tr>
<tr>
<td>Str. ag.</td>
<td>43</td>
<td>8</td>
</tr>
<tr>
<td>Str. dys.</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>S. uberis</td>
<td>89</td>
<td>17</td>
</tr>
<tr>
<td>Other</td>
<td>314</td>
<td>62</td>
</tr>
<tr>
<td>Total</td>
<td>501</td>
<td>100</td>
</tr>
</tbody>
</table>

This table again emphasized the effectiveness of the system in reducing new infections caused by the more common mastitis causing organisms, but shows again
RESULTS OF RESEARCH ON CONTROLLING MASTITIS

A proportional increase in infections caused by the "Others" category of organisms. Again, it was found that the occurrences were not divided equally among herds but that a few herds contributed to a high percentage of the total.

An important finding is that of 543 cows calving during the latest period only 87 new infections had occurred during the dry period. This tends to support the farmer reaction that the cows calve without the mastitis problems so often found at calving time.

This effectiveness of treatment of dry cows is shown very clearly by the information given in Table 5. Here is shown a comparison of the effectiveness of treatment at drying off with the results of treatment of the various causative organisms during lactation.

| TABLE 5 |
| Effect of Treatment on Dry and Lactating Cows |

<table>
<thead>
<tr>
<th>H. Staph.</th>
<th>Treated</th>
<th>Cured</th>
<th>% Age Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating</td>
<td>456</td>
<td>202</td>
<td>44</td>
</tr>
<tr>
<td>Dry</td>
<td>784</td>
<td>622</td>
<td>79</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Str. ag.</th>
<th>Treated</th>
<th>Cured</th>
<th>% Age Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating</td>
<td>317</td>
<td>232</td>
<td>73</td>
</tr>
<tr>
<td>Dry</td>
<td>1,248</td>
<td>1,077</td>
<td>86</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Str. dys.</th>
<th>Treated</th>
<th>Cured</th>
<th>% Age Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating</td>
<td>425</td>
<td>293</td>
<td>69</td>
</tr>
<tr>
<td>Dry</td>
<td>189</td>
<td>171</td>
<td>90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S. uberis</th>
<th>Treated</th>
<th>Cured</th>
<th>% Age Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating</td>
<td>917</td>
<td>556</td>
<td>61</td>
</tr>
<tr>
<td>Dry</td>
<td>1,202</td>
<td>824</td>
<td>81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Others</th>
<th>Treated</th>
<th>Cured</th>
<th>% Age Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating</td>
<td>1,743</td>
<td>1,035</td>
<td>59</td>
</tr>
<tr>
<td>Dry</td>
<td>705</td>
<td>564</td>
<td>80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>Treated</th>
<th>Cured</th>
<th>% Age Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating</td>
<td>3,858</td>
<td>2,318</td>
<td>60</td>
</tr>
<tr>
<td>Dry</td>
<td>3,946</td>
<td>3,258</td>
<td>83</td>
</tr>
</tbody>
</table>

This table clearly supports the use of treatment at time of drying off over the use of antibiotics during lactation for removal of infection. This has been strikingly demonstrated by the fact that overall herd infection level has not been reduced over
many years of antibiotic use during lactation. The treatment of dry cows also has the advantage of being done at a time when the milk is no longer being used as human food. This means that there is little danger of any antibiotic residues in the milk.

CONCLUSION

This study has clearly demonstrated the advantages of a control system including the treatment of cows at drying off. It also has indicated that a substantial decrease in new infection can be obtained by dipping the teats in an effective disinfectant immediately after milking. The combination of these two practices has brought about a considerable decrease in the infection levels in the 25 herds. The variation in the responses of the herds certainly indicates that further studies need to be carried out to pinpoint the weaknesses and strengths of the system. It is, therefore, very important to discover the specific reasons for such a wide range in the new infection rates of herds. Solving this problem is necessary to develop a major improvement in the control scheme.

Another major area of inquiry must be to accurately classify the organisms responsible for the category of “others”. The classification of these organisms in the present study was to broad so that more work is needed. The 25 herds are being continued on the system with some modification. They will be monitored for infection levels at six monthly intervals.

In addition, the fact that the Food and Drug Administration has not yet accepted the use of the high levels of penicillin and streptomycin used in this research means that other materials must be tested. Lack of research funds is causing problems in further studies but it is hoped that some testing of treatment materials will be continued until some acceptable, efficacious product may appear.

The results of this study and similar studies have shown that the combination of teat-dipping immediately after the last milking of lactation will bring about a reduction in infection level. This will improve milk quality and reduce the danger of antibiotic residues in milk due to lactation treatment. The problem remains of obtaining acceptance of this proven efficacious material or finding some other material which would be as effective and is acceptable to the Food and Drug Administration.

The program is economically sound and could benefit both the dairy industry and the consumer. Improvement of the program will follow further research but it should be recommended and applied Nationally.
REFERENCES


REPORT OF THE MASTITIS COMMITTEE

J. S. McDonald, Ames, Iowa; Chairman

The annual open meeting of the U.S.A.H.A. Mastitis Committee was held on October 21, 1970. Thirty-two interested persons, including 5 committee members and 27 guests were in attendance.

Three informal reports were presented. Dr. D. S. Postle gave an updated report on the three year hygiene-dry cow therapy approach to mastitis abatement in 25 herds involving over 2,000 cows. Methods included post milking teat dipping and dry cow medication without costly laboratory procedures. Results showed a reduction in udder quarter infections from 29.6 to 9.7%.

Dr. K. R. Hook presented a preliminary report on an area wide attempt to eradicate Streptococcus agalactiae from all herds shipping milk to one cooperative in Wisconsin. This project was initiated in January of 1970, and sufficient data has not been analyzed to be meaningful.

Dr. C. D. Van Houweling stated that Food and Drug Administration is requiring efficacy data on all mastitis medications now being used in lactating cows. This type of data has not been required in the past.

Several areas of discussion followed. Even though several mastitis control programs are in existence, additional correlation of the effect of these programs on the level of somatic cells in bulk milk from cooperating herds is needed. It is anticipated that considerable information in this area will soon become available.

The feasibility of a national mastitis control program was discussed. Even though technological procedures have been developed, have been proven to be effective, and are available for application, it was the unanimous consensus of the Committee that we are not ready for a national mastitis control program. Diversity of management, environment, marketing conditions, and insufficient laboratory capability preclude application of a uniform national control program.

Mastitis is primarily a condition involving economics and milk quality. The public health implications of this disease are limited. Therefore, the Committee feels that the individual dairyman should take immediate action to clean up his own house and should not rely on regulation to force necessary action. A better approach would be to initiate a monitary incentive program involving payment for a premium quality product.

As Streptococcus agalactiae is an infectious, contagious disease, the Committee feels that infected animals should not be permitted to move interstate. Therefore, the following resolution was unanimously adopted:

BE IT RESOLVED that the U.S.A.H.A. strongly recommends that the United Stated Department of Agriculture appoint a committee to devise; with the aid of
consultants from the U.S.A.H.A., National Mastitis Council, and A.V.M.A.; a protocol for controlling the interstate movement of *Streptococcus agalactiae* infected dairy cattle over 18 months of age.

The Committee again recommends that the U.S.A.H.A. maintain continuing membership in the National Mastitis Council.

This constitutes the report of the Mastitis Committee. I respectfully submit it for approval by the Executive Committee.

| John S. McDonald, Chairman | R. I. Hostetler |
| H. S. Bryan | E. J. Kersting |
| R. J. Farnsworth | K. J. Peterson |
| R. S. Guthrie | D. S. Postle |
| H. B. Haynes | R. J. Schroeder |
|                     | E. L. Henkel |
EXTENDING REGULATORY WORK — COOPERATIVELY

Robert C. Hammond, V.M.D.
College Park, Maryland

The Cooperative Extension Service can, and does, and will continue to support regulatory programs to control disease.

We should all be proud of the record of achievement so far. Diseases such as Foot and Mouth Disease, Vesicular Exanthema, Contagious Pleuro-Pneumonia have been eradicated from the United States. Other diseases such as Hog Cholera, Brucellosis, and Tuberculosis are on the run, and with a concentrated effort can be completely eradicated in the future. In time, we might anticipate programs to eradicate still other diseases — Anaplasmosis, Rabies, Mastitis, and Salmonellosis are good bets. We have prevented many diseases, prevalent in other parts of the world, from becoming established here; Rinderpest, African Horse Fever and African Swine Fever are only a few.

Only a small percentage of the population understands the broad and important role of animal disease control. We should never miss a chance to get public support for disease control and eradication programs by keeping them informed. We can point out that animal disease control safeguards their food, particularly concerning those diseases common to animals and man. It makes possible medical research; many treatments and cures for humans were developed in animals. Immunological discoveries made in animals have paved the way for preventative medicine of great importance in mankind, and it helps provide them with adequate supplies of protein at a price they can afford.

For example, in 1926, 1,200 children died in New York City from Tuberculosis, one-fourth of them from the bovine type. Now Tuberculosis sanitariums are closing and bovine Tuberculosis is almost unheard of in humans. Hunchback children are now a rarity.

Undulant fever has decreased in direct proportion to the incidence of Brucellosis in cattle.

Disease and parasites cost the livestock industry 2.5 billion dollars per year, or about 15 percent of the market value. Without disease control — from regulatory, extension and practicing veterinarians — the losses would be much more severe, and the cost of food higher.

Regulatory programs cannot be accomplished by regulations alone. To be effective, any disease control program must be preceded by an educational program. When all segments of the livestock industry are aware of the situation, action for eradication of the disease can begin.

The two groups of organized veterinary medicine that provide leadership in disease control work are regulatory veterinary medicine and the Extension Service. Both of these groups are independent, self-contained and capable of independent operation and potential success without interaction between them. While they can succeed without one another, the success of either is enhanced by cooperation between them.
The Extension Service through its contact with livestock producers and other segments of the livestock industry is best equipped for the organization and implementation of educational programs. Regulatory personnel are best equipped to interpret and enforce programs in disease control.

The Extension Service is engaged in the education of people, concerning all aspects of agriculture, home economics, rural development and 4-H extension.

Extension is a vast organization involving a University staff of specialists in many fields including Veterinary Medicine, and Animal, Dairy and Poultry Science. Many of the best friends of regulatory animal health programs are University livestock and dairy specialists. These specialists magnify their efforts many times through their contacts with livestock producers.

In addition to the specialists, the Extension Service has Agricultural Science Agents in almost every county. These agents know their area — they have contacts with radio and TV stations and newspaper editors. They know the agricultural leaders are and are quite adept at setting up educational meetings. They also have mailing lists of livestock owners in their counties.

It is not uncommon to find Extension organizations that are active in some areas and less active in others. It follows that unless the Extension Service in your state maintains one or more full-time extension veterinarians, the role of disease control and animal health can be completely overlooked, or be a hit-and-miss affair. Thirty-six states now have Extension veterinarians as specialists in animal health. It is their job to coordinate all of the animal health programs in their state’s Extension Service.

Extension Veterinary Medicine has a primary duty to bring animal health education programs to livestock producers, pet owners and the public. The veterinary specialists suggest ways and means of keeping animals healthy, growing the producing. We aim our programs at preventing disease. When animals become sick, we want the diagnosis and treatment referred to practitioners. Of course, we’ll help all we can.

We teach basic principles. For a disease to be contagious, there must be an organism present. For a disease to spread, the organism must leave the infected host, it must find a way to a new host and must be capable of causing the disease in the new host. A disease can be controlled if this cycle can be broken at any point. We also teach producers the Federal government’s concept of disease: If possible, keep it out; if it gets in, eradicate it; if eradication is impossible; control it. Federal veterinarians apply this on a federal level to keep diseases out of the country; state veterinarians do the same in their state, and it works well on every individual farm.

When these basics are understood, we can apply them to any specific disease eradication program.

Extension Veterinary Medicine looks at its educational program as serving another distinct need — that of continuing education of the veterinary profession. In furthering regulatory work, this could mean telling practicing veterinarians of changes of regulations — although this is often done by regulatory personnel — or it
could mean participating in continuing education sessions for regulatory veterinarians and livestock inspectors.

Veterinary Science Extension can also serve by keeping the lines of communication open between livestock producers and regulatory personnel — as well as vice-versa. Extension personnel have many contacts with farmers on a different basis than do regulatory personnel. Livestock owners are more willing to express their views to Extension veterinarians. The problems farmers have and the programs they need can be taken back to regulatory agencies and adapted to the needs of all.

I would like to comment a bit on the results of a questionnaire that I sent to all Extension veterinarians, to all state veterinarians and to all federal veterinarians-in-charge.

From the questionnaire answers, I have decided that the federal veterinarians-in-charge have the fewest problems. Virtually all of them are satisfied with Extension’s help to them. Whether or not their state has an extension veterinarian, they seem to have very few complaints and very few suggestions for the future. One commented, “We can recognize the assets one [Extension veterinarian] could be and we can also recognize the problems one could cause.” Another explained that he could think of no changes he would like in the future so he must be satisfied with the present. Three asked for help in obtaining extension veterinarians.

The state veterinarians were mostly inclined to hand out bouquets, but a few threw some brick bats and many had ideas for the future. In general, their comments made me feel good being an Extension veterinarian. Many gave specific examples of how Extension veterinarians helped them.

From the replies of Extension veterinarians, it is not hard to visualize them as dispensers of information, with ideas on what should be done and some ideas on how it should be accomplished. If you want an opinion on something, don’t ask your regulatory friends — ask your Extension veterinarian.

I am sure you would like to enjoy some of the comments with me. One commented that he was asked to help with the programs ultimately, but only after they had been bogged down.

Another said, “We have many projects that need work but have trouble getting regulatory people off their butts — to move on a new project.”

But, you know, nearly all Extension veterinarians say they are fortunate in the working relationships they have with regulatory personnel. At present, there is an excellent working relationship, but we all have to try to keep it that way. Dr. Charles Dobbins of Georgia said it this way, “We have a close working relationship with regulatory officials. We do not hesitate to call each other and express our ideas. If we differ — which we occasionally do — this is done privately, and our differences are worked or worked out so a united front can be advanced to the public.”

Dr. Guy Reynolds of Oregon comments, “I have been most impressed with the calibre of personnel used as regulatory officials in this state.”

Dr. David Ibsen of Arkansas summed up the subject very well: “As a former
regulatory official, I found that without the Extension Service and the leading farm organizations, no program could get off the ground.”

A combined State-Federal man best expressed what I've been trying to tell you. Dr. A. A. Erdmann of Wisconsin said, “Extension veterinarians serve on our advisory councils, serve on committees dealing with special problems, support all our programs at Extension meetings throughout the state and even at national meetings, provide assistance in producing educational material, set up special training courses and programs for our employees, call on our division for assistance when needed, and support other cooperative efforts too numerous to mention.”

The goal of all of us is disease control and eradication. We can do it best with livestock producers, public health people, veterinary practitioners, regulatory personnel and the Cooperative Extension Services working together. This is what we would call “Extending Regulatory Work – Cooperatively”.

The Committee on Professional Education and Extension met yesterday afternoon in the Green Room of this hotel. Actions resulting from the Committee are as follows:

1. The Committee recommends that the U.S.A.H.A. establish a five year cumulative index plan for the U.S.A.H.A. proceedings. It is a fact that much valuable material in our annual proceedings book is virtually inaccessible to those who cannot take the necessary hours of searching to find it. In previous years, this committee has recommended that the Agricultural Science Library be approached to do this indexing. In view of the apparent inaction on the part of the Agricultural Science Library, we recommend that this project be undertaken forthwith by the Association.

2. The Committee recommends that the Executive Committee review the committee organizational plan of the U.S.A.H.A. This should include a hard look at every committee function to ascertain whether or not it is actually needed. If so, every committee should be provided in writing with a specific duty, otherwise it should be disbanded.

3. The Committee recommends that the U.S.A.H.A. commend Livestock Conservation, Inc. for the excellent job they have been doing on public education of animal disease programs.

4. The Committee recommends that the U.S.A.H.A. further emphasize to the public the accomplishments and benefits of animal disease control as related to food products, public health and preventative medicine. There are many benefits to the public emanating from disease control that they do not associate with regulatory work. We feel that additional support would be forthcoming from a better informed public.

5. The Committee views with concern the long interval between the annual meeting and publication of the Proceedings. The committee recommends that the Executive Board take whatever action is necessary to shorten this interval. In the interim, it is recommended that provision be made at the registration desk for interested persons to indicate what committee reports or technical papers they would like copies of. Copies would then be mailed to them by the Executive Secretary immediately following the meeting.

Mr. Chairman, these recommendations and decisions constitute the actions of the Committee on Professional Education and Extension, and they are respectfully submitted. We recommend their adoption.
Mr. Jerry Weintraub, Entomologist at the Lethbridge Research Station, Alberta, Canada, was the guest speaker for the official meeting of the Committee. He spoke of the cattle grub research currently being carried out at the Lethbridge Research Station. Mr. Weintraub’s remarks were illustrated with excellent slides.

Considerable discussion followed Mr. Weintraub’s presentation. There was doubt expressed as to the feasibility of an area grub eradication program. It was the opinion of the Committee that further research and pilot programs be established in areas in which liniatuum bovis exist in order to determine that eradication can be accomplished.

Mr. Weintraub’s presentation will appear in the official proceedings of the meeting.

Under the subject of proposed cancellation of registration of pesticides — chlorinated hydrocarbons in particular, it was brought out there remains a serious problem to the livestock industry, as well as to the consuming public, if such products are removed from registration without provision for their replacement. DDT and toxaphene are among the pesticides to be canceled.

Concern by the Committee was expressed that chemical companies engaged in the manufacture of parasiticides will be reluctant to research and produce such products without assurance of continued registration of products.

The Committee presented for consideration a resolution directed to the Food and Drug Administration protesting the proposed cancellation of the registration of all chlorinated hydrocarbons. This resolution is included with this report.

Dr. James Hourrigan presented his report concerning cattle and sheep scabies, tick eradication and the screwworm program. This report appears as a portion of the Committee report.

SHEEP SCABIES ERADICATION

The sheep scabies eradication program during FY 1970 marked the first year the psoroptic sheep scabies was reported from only one state. Three flocks, with a total of 109 head, were involved in two series of outbreaks in Hunterdon and Gloucester counties in New Jersey. The last previous outbreak in the state occurred
in April 1969. The first outbreak series involving two flocks was reported in December 1969; and the second outbreaks series, with only one flock, was reported in January 1970.

The inspection of sheep has decreased from 14,563,649 (4,297,926 at public stockyards and 10,265,723 at other locations) in FY 1969 to 13,602,843 (3,833,231 at public stockyards and 9,769,612 at other locations) in FY 1970. A large part of this decrease was in the central states which are part of the area in which sheep scabies had remained to spread to other portions of the country prior to 1970.

During 1970, 77 sheep were dipped at public stockyards and 12,110 at other locations. In 1969, 20 sheep were dipped at public stockyards and 40,802 at other locations.

There were no reports of psorergatic sheep scabies. Chorioptic mites were collected from two Iowa sheep flocks and from one flock in California. Sarcoptic mites were collected from one sheep flock in Iowa and one in Illinois and from one herd of goats in New York. Psoroptic mites were collected from goats' ears in seven herds in Texas and one in Alabama. Psoroptic mites were collected from ears of one bighorn sheep in Nevada, two deer in Georgia, and one deer in South Carolina.

Your Committee urges that all states pursue an active scabies inspection and eradication program to continue until three years after the most recent case of psoroptic sheep scabies is found in this country so that the goal of complete sheep scabies eradication is fully reached.

CATTLE SCABIES

The cattle scabies eradication program during fiscal year 1970 was marked by a decline of inspections from 46,041,366 (16,615,776 at public stockyards and 29,425,590 at other locations) in FY 1969 to 44,303,196 (15,632,899 at public stockyards and 28,670,297 at other locations) and by a lesser number of reported outbreaks.

A single outbreak of psoroptic cattle scabies was reported. It followed the pattern of recent years as it involved a feedlot (in Castro County, Texas) and the origin was not determined although the feedlot, but not the cattle in it, was involved in one of the FY 1969 outbreaks.

During 1970, 2,408 cattle were dipped at public stockyards and 28,190 at other locations. During 1969, 43,672 cattle were dipped at public stockyards and 118,140 dipped at other locations.

There were no reports of psorergatic cattle scabies. Sarcoptic mites were collected from four herds, two in Vermont and one each in Iowa and Kansas. Two dippings of each herd were required. Sarcoptic mites were also collected from one herd of horses in Vermont, from Llamas in a show traveling in Texas and Louisiana (Chorioptic mites were also collected from the Llamas.) and from Llamas in California.

During FY 1970 chorioptic mites at livestock shows included the following:
PARASITIC DISEASES AND PARASITICIDES

Louisiana State University shows — collections from 26 lots of Louisiana cattle.
Northeast livestock show and Southern University show, Louisiana — one collection at each from Louisiana cattle.
Arizona National Livestock Show — Collections from 3 lots of Arizona cattle.
Fort Worth Fat Stockshow — Collection from one lot of Kansas cattle and 4 lots of Texas cattle.
International Cattlemen's Exposition, Las Vegas, Nevada — Collection from a lot of cattle from Colorado.
National Western Stockshow, Denver — Collections from 42 lots of cattle from Arizona, Colorado, Illinois, Indiana, Iowa, Kansas, Minnesota, Missouri, Montana, Nebraska, New Mexico, Oklahoma, South Dakota, and Texas, as well as from Canada.

TICK ERADICATION

In Texas efforts were continued to keep Boophilus spp. ticks from entering Texas from Mexico to prevent outbreaks from spreading and to eradicate the ticks from infested premises.

During fiscal year 1970, in the area under Federal quarantine in Texas 41,151 lots of 806,926 livestock were inspected for ticks and 14,482 lots of 71,695 livestock were dipped. Outside the quarantined area 19,857 lots of 558,057 livestock were inspected and 1,367 lots of 30,843 livestock were dipped.

A total of 51 tick-infested premises were found in Texas, 36 within the quarantined area and 15 outside the quarantined area. This includes collections of Boophilus spp. from Mexican livestock illegally entering the United States. Of 147 equidae caught, 20 were infested; and of 110 cattle caught, 20 were infested.

The series of infestations found outside the quarantined area, in Dimmit and Maverick Counties, were eradicated, and as of August 1970 there were no State quarantines outside the area under Federal quarantine. Of 671,824 livestock offered for importation from Mexico into Texas, 24,327 were rejected because of Boophilus spp. infestation.

Dioxanthion (Delnav), one of the two permitted organophosphate dips, was used with excellent results in handling the Dimmit County outbreaks. A total of more than 30,000 cattle and horses were dipped or sprayed. The other organophosphate dip permitted for ticks is coumaphos (Co-Ral). Permitted arsenic dip was used in most instances. A new arsenic dip, formulated without cresylic acid, was used. USDA has petitioned Food and Drug Administration, Department of Health, Education, and Welfare to grant a tolerance for arsenic in the tissues of dipped livestock. The petition has not been acted upon.

The most recent finding of Amblyomma variegatum ticks on St. Croix, U.S. Virgin Islands, was in August 1969, 17 months after the last previous finding in April 1968. Official dipping of livestock in the area under insular quarantine was discontinued in May 1970.
Inspections on St. Croix during fiscal year 1970 included 31,875 animals. 30,266 animals were dipped. An A. variegatum tick was collected from one bull B. microplus were collected from 145 cattle, 5 horses, 28 deer, 8 goats, 4 sheep and 1 mongoose. Dermacentor nitens ticks were collected from 14 cattle, 94 horses, 1 donkey, 12 deer, 3 goats, 1 dog and 2 sheep. Ornithodoros puertoricensus were collected from 10 mongooses.

During fiscal year 1970, exotic ticks were collected in the United States from animals recently imported or offered for importation as follows.

Collections from cattle: 141 collections of Boophilus spp. were made from cattle offered for importation from Mexico into Texas.

Collections from horses: Dermacentor nitens in Florida from Nicaragua. Haemaphysalis longicornis in New Jersey from Australia and New Zealand.

Collections from dogs: Rhipicephalus sanguineus in Florida from North Africa and in Oklahoma from South America. Ixodes sp. in New York from dog cage from Germany.

Miscellaneous collections: Amblyomma cajennense in Maryland from ferns from Guatemala and in Texas from plants from Mexico. Rhipicephalus sanguineus in Oregon from coir yarn from India.

Collections from zoo animals in Florida, New Jersey, or New York: Rhipicephalus evertsi from impalas. Rhipicephalus evertsi, R. pulchellus, and Amblyomma gemma from zebra from Kenya. Ixodes sp. in New Jersey from hedgehogs. Dermatobia hominis (human bot) in Washington from ocelot cat from Nicaragua.

Your Committee noted that zoo and other animals arrive in the United States infested with ticks. Many of which are more dangerous than the cattle fever tick and recommends that USDA regulations be strengthened to require all zoo animals and livestock to be freed of ticks in the country of origin and they be inspected upon arrival in the U.S. and if found tick-free be given a precautionary dipping and if found to be tick infested with any species of tick be held for two clean dippings.

It is further recommended that USDA prevail upon the Mexican government to eradicate cattle fever ticks from areas in Mexico adjacent to the U.S. thus lessening tick pressures against our borders.

SAFE USE OF PESTICIDES

The use of pesticides, with the attendant problems of exposure to applicators, environmental contamination, animal tissue residues, and disposal of pesticide and containers, is receiving an increasing amount of attention.

State and Federal research to develop safer pesticides and alternate means of pest control and disposal of pesticides and containers should be continued at a high level and adequately supplied with funds and personnel.

State and Federal regulatory personnel have a responsibility to provide leadership and set good examples to persons using pesticides on livestock. In order to carry out this responsibility personnel need to be adequately trained.
and guidelines must be constantly updated to keep pace with research developments.

**SCREWWWORM PROGRAM**

The operation of the screwworm sterile fly barrier zone in 1969 kept screwworm migrations from Mexico into the United States at the lowest level since records have been kept. There were only 219 laboratory-confirmed infestations in the United States. Mexico also had a very low number of infestations. Before 1969, the previously low year was 1964 when 403 infestations were found in the United States. Operation of the barrier zone during the first 6 months of 1970 continued to be successful in controlling screwworms in northern Mexico and hindering migrations from Mexico into the United States. There were only 29 laboratory-confirmed cases in the United States between January 1 and June 30, 1970.

Beginning in late June and extending into July 1970, an outbreak of screwworm occurred in the Pecos River region of Texas. By the end of July, the outbreak posed a threat to the rest of the Southwestern United States.

In July 1969, screwworms were identified for the first time in the U.S. Virgin Islands, and in the British Virgin Islands in January 1970.

During 1970, a total of 4,257 million sterile screwworm flies were released in Mexico and the United States. Northern Mexico received 82 percent of the total flies released.

Informal discussions with Mexican officials continue in an effort to develop a cooperative screwworm eradication program in that country. The objectives of such a program are to free Mexico from losses to their livestock, remove the threat of screwworms migrating into the United States, and establish a more economical sterile fly barrier zone at the Isthmus of Tehuantepec in southern Mexico.

**RESOLUTION**

WHEREAS, certain ectoparasites are being eradicated by use of Toxaphene, and

WHEREAS, Toxaphene does not create the persistent residue problems as chlorinated Hydrocarbons in general.

And further, since toxaphene is and has been the standard insecticide for the control of several extoparasites in livestock resulting in considerable economic benefits to the livestock industry, as well as contributing to the economic advantages to the meat consumers,

And further, since it has been proposed in the Federal Register that Toxaphene be included in a list of persistent chlorinated Hydrocarbons listed,

THEREFORE BE IT RESOLVED that the U.S.A.H.A. in its 74th Session at Philadelphia, Pennsylvania on October 17-23, 1970 advise the Food and Drug Administration that we oppose such action pertaining to Toxaphene and be it further resolved that this association offer to consult with FDA in developing sound, standard recommendations for the use of Toxaphene prior to such action being taken.
My talk this morning will focus on two recent developments in veterinary biological production that are making better vaccines possible. The first has to do with controlling potency of modified live virus vaccines by the seed lot principle. The second concerns the use of established cell lines as a uniform substrate for producing virus. Both of these new procedures emphasize quality control at the start, rather than at the end of manufacturing. Furthermore, these procedures relate the quality of one serial of vaccine to the next.

Biological production is still partly an art and partly a science. Contrary to popular belief, many of our methods are empirical. The historical procedure for biological production has been to produce a serial of vaccine and then by suitable control procedures determine if it is pure, safe and potent. This has not been conducive to standardization of production and has not served to relate the quality of one serial of vaccine to the next. The now discarded term “minimum test requirements” is descriptive of this type of testing.

Perhaps it would be interesting to consider for a moment the circumstances under which the first modified live virus vaccine was prepared. In 1796, Edward Jenner made his first vaccination against smallpox in a hut in the garden of the Vicarage in England. The hut, as shown in Figure 1, is there today looked after by the Jenner Trust. The vaccine was prepared by scraping pustules from cattle infected with cowpox. Consider his problems for a moment. He had no knowledge of micro-organisms let alone viruses. The procedure for collecting material for vaccination by necessity had to be entirely empirical. The safety and potency were measured by the results of vaccination in people. However, when we consider that smallpox at this time was the cause of death in one out of five naturally infected people, and it was common practice to use material from smallpox pustules to produce a controlled infection, even a risky procedure like Jenner’s looked good.

The concept of controlling the potency of modified live virus vaccines by measuring the amount of virus in them has been used for many years as a practical method of vaccine evaluation. The first proposal that this procedure be standardized for canine distemper vaccine and used to replace the existing canine potency test was made by Dr. George Sharpless of Lederle Laboratories in 1967 at a meeting of the Veterinary Biological Licensee’s Committee of the Animal Health Institute. The Standard Requirement then in effect, and only recently discontinued, required that the potency of each serial of vaccine be determined by testing in dogs. For a satisfactory test at least two vaccinated dogs had to survive a challenge dose of virulent virus which would produce typical distemper in at least two non-vaccinated control dogs. If the number of dogs was increased to five, then only 80% of the vaccinated dogs had to be protected and only 80% of the control dogs needed to show signs of distemper. Statistically, this test left much to be desired. By chance alone a poor vaccine could pass or a good vaccine could fail. One test by itself did
not permit a manufacturer to determine if a serial of vaccine was especially potent or barely getting by. Furthermore, it did not provide a procedure whereby a desired potency level for vaccine could be maintained. Study and discussion between members of the veterinary biological industry and the Veterinary Biologics Division over a period of two years resulted in the present procedure which does allow for a direct comparison of the potency of serials of vaccine.

The seed lot principal test is based on the concept that once a known amount of virus has been proven capable of producing a desired level of immunity then any serial of vaccine with this amount of virus or more should produce equally good immunity. To begin the procedure a pool of suitably modified virus is designated as master seed. It is stored in a biologically inactive state by freezing at temperatures down to -186° C. Under these conditions its biological properties will remain constant because there is no enzymatic activity and the possibility of mutations become virtually nil. It is then subjected to a comprehensive series of tests for purity and safety. However, since the master seed virus must be propagated to produce vaccine, there is a possibility that genetic mutation may occur after it has been selected. To reduce this risk, the number of passages allowed between master seed virus and the virus actually used for vaccine may not exceed five.

To begin an antigenicity test, a pool of virus prepared from the fifth passage is frozen away. Ten accurate titrations are made on this virus pool. Following this, based on the manufacturer's experience as to the amount of virus he wishes to have in his vaccine, the pool is suitably diluted for a definitive test in dogs which is designed to show that this amount of virus is truly potent. One-tenth of the minimum amount of virus to be in each dose of vaccine is used to vaccinate a statistically significant number of animals. For canine distemper this number happens to be 20 dogs. The dogs are allowed to develop immunity and then challenged with standard virulent virus supplied by the National Animal Disease Laboratory. For a satisfactory test, at least 19 of 20 dogs must be protected and the challenge virus must be shown to be adequately virulent by killing not less than four of five non-vaccinated dogs.

A virus pool which passes this rigid test has to be potent. A virus pool which would be 95% effective if tested in an infinite number of dogs has only a 74% chance of passing this test the first time. A pool with 80% effectiveness has only about a 7% chance of passing this test the first time. As you can understand from these figures, it is necessary to select an amount of virus which will be nearly 100% potent to make the test worth undertaking.

Upon satisfactory completion of this test, serials of vaccine may be released for sale when prepared from virus not more than five passages removed from the master seed virus and providing they have a virus titer at least 10 times greater than the amount used in the test. Since there is some deterioration of virus throughout the vaccine's dating period, the initial titer must, of necessity, be even greater for the company to release it. By careful control of the master seed it is possible to produce serials of vaccine of nearly identical potency for many years. This test procedure is now required for canine distemper and infectious canine hepatitis.
vaccines. July 1, 1971, it will be required for infectious bovine rhinotracheitis, bovine virus diarrhea, and parainfluenza type 3 vaccines for cattle. It is being considered for other modified live virus vaccines. The steps for establishing potency by this procedure are summarized in Table 1 and the requirements of a satisfactory potency by this procedure are summarized in Table 2.

Of equal importance with the standardization of the seed virus is standardization of the substrate on which it is produced. Viruses are only able to multiply within living cells. During the past year standard requirements have been issued requiring the use of well-characterized cells for tissue culture vaccine production. The cells have been defined as either primary cells, below the 10th tissue culture passage, or as cell lines, the 11th tissue culture passage or beyond. An established cell line is one that has been through a sufficient number of passages that it has the potential to be subcultured indefinitely in vitro.

It is my opinion that cell lines provide the maximum opportunity for achieving uniformity and safety of vaccine production. Theoretically, an established cell line contains only one type of cell and every cell is genetically related. Because of this, within the limits of biological variation each individual cell has the same biochemical characteristics, the same growth rate, the same viral susceptibility and when infected with a virus will respond the same way. If molecular biology has a message for veterinary biological producers it is that a virus-infected cell is a new biological entity. Starting with a well characterized seed virus and adequate control of cultural conditions, such as medium, pH, temperature and length of incubation, the results will not be predictable if the cells are not uniform. Attempting to grow viruses in a completely uncharacterized cell, whether it be in a living animal, an embryo- nated egg, or cell culture, will never lead to a uniformly predictable result. This concept is summarized in Figure 2. Well characterized cell lines provide the best guarantee of freedom from latent viruses and other intracellular organisms. No reasonable amount of in vitro or in vivo testing on a finished serial of vaccine can be as complete and thorough as the tests used to evaluate cell lines. The tissue culture literature contains many examples of primary cells being contaminated with viruses, some of which are listed in Table 3. Unless well characterized cells or a cell line are used, the manufacturing process must remain empirical.

The study of the standardization of cells by industry and the Veterinary Biologics Division has resulted in two standard requirements providing test procedures for characterizing and selecting primary cells and cell lines for vaccine production. The tests required include sophisticated methods for evaluating cells, such as preparation of karyotypes, fluorescent antibody techniques for detecting latent viruses, detection of tumor producing properties by inoculation of animals with increased susceptibility produced by immuno-suppressant drugs or irradiation, and serological methods for detecting species of origin. It is to be anticipated that some of these tests will be found more useful than others. New tests will undoubtedly be developed that are even more critical and some will be discarded. I anticipate that eventually concern about cellular nucleic acid-producing tumors will disappear and a wider selection of cells specifically tailored for improved
multiplication of viruses will become available.

From these two examples, it is obvious the trend in veterinary biological production is toward more uniform products. This is being achieved by eliminating purely empirical methods and replacing them with methods which can be standardized and controlled throughout the manufacturing process. As biological production becomes less of an art and more of a science there will be benefits for everyone.

Where should credit be given for this new approach to veterinary biological production? The credit for the concepts must go to the producers of veterinary biologicals. However, it is to the everlasting credit of scientists and administrators of the Veterinary Biologics Division that they have recognized the importance of these ideas and taken the initiative in making them regulations. I feel it is very appropriate that the United States should show leadership in this area.
TABLE 1
Steps for Establishing Potency of Modified Live Virus Vaccines by the Seed Lot Principle

1. Selection and testing master seed virus.
   a. Modification to desired level.
   b. Purity, safety, etc.
2. Establish optimum viral titer for vaccine.
3. Prepare fifth passage virus pool.
4. Titrate fifth passage virus pool.
5. Inoculate a statistically significant number of animals with 1/10 amount of virus required in vaccine.
6. Challenge animals with a standard virus supplied by National Animal Disease Laboratory.

TABLE 2
Controlling Potency of Modified Live Virus Vaccines by the Seed Lot Principle

1. Vaccine virus must not exceed five passages from master seed virus.
2. Vaccine must contain required amount of virus as determined by an in vitro titration procedure.

TABLE 3
Viral Contaminants Demonstrated in Live Virus Vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Contaminating Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallpox</td>
<td>Foot and mouth disease</td>
</tr>
<tr>
<td>Smallpox</td>
<td>Milker’s nodules</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>Serum hepatitis</td>
</tr>
<tr>
<td>Polio virus</td>
<td>SV40</td>
</tr>
<tr>
<td>Adenovirus 7</td>
<td>SV40</td>
</tr>
<tr>
<td>IBR</td>
<td>Bovine virus diarrhea</td>
</tr>
<tr>
<td>Canine distemper</td>
<td>Infectious canine hepatitis</td>
</tr>
</tbody>
</table>
Figure 1 – The hut where Edward Jenner made his first vaccination against smallpox. (Courtesy of the Jenner Trust, Berkeley, Glouster, England.)

Figure 2 – Comparison of two procedures for obtaining virus for vaccine production.
REPORT OF THE COMMITTEE ON VETERINARY BIOLOGICS

Carl J. Norden, Lincoln, Nebr., Chairman

Regulatory Activities on Biologics

During fiscal year 1970, the number of establishments licensed by the Veterinary Biologics Division dropped from 55 to 53. Product licenses dropped from 1,174 to 1,039.

In past years, the committee has devoted much of its effort to reviewing and reporting details of regulatory activities. Starting with an issue dated October-November 1969, the Veterinary Biologics Division has been circulating a bi-monthly newsletter, VET TOPICS, to state veterinarians, licensees, industry groups, and other interested in the Division's activities. The four-page newsletter contains a review of all licenses issued and deleted, regulatory actions taken, official notices issued, press releases published, official speeches delivered, major topics discussed at meetings, and the like. The committee concluded that it was not necessary to duplicate this information.

Research Areas for Biologics

A number of well recognized diseases are still imperfectly understood because of their lack of "glamour". Consequently, basic information that would indicate either the need or the possible effectiveness of immunizing agents has not been developed. These include:

- Foothill Abortion in cattle (referred to as virus abortion or Psittacosis Lymphogranuloma Abortion.)
- Footrot in sheep.
- Parainfluenza
- Scours in young animals (specifically the role of E. coli)
- Vibrionic dysentery in swine

The committee emphasizes the need for basic research on some of these problems. In all cases, support of diagnostic and disease reporting services is required to establish the need for immunizing agents.

Development of Methods of Evaluation

Work on the development of improved test methods has intensified as the needs become greater. Developmental studies are conducted by producers and the VBD. In addition, there were 18 projects being carried out on contract with the Veterinary Biologics Division at various research and academic institutions during the fiscal year. These contracts are administered by the laboratory staffs at Ames
and results are used to improve quality control procedures and laboratory standards for licensees and VBD testing laboratories.

**Diagnostic Reagents**

The committee considered the resolution by the Salmonella Committee, AAVLD, on diagnostic reagents. We believe the terms used are somewhat ambiguous. We agree in principle with the resolution in regard to biological diagnostic reagents. Antigens are now licensed by the VBD.

**Recommendation**

The Committee recommends that the Association reexamine the objectives of the Biologics Committee, in light of the rapid changes in this area. Through appropriate officers or a task force, the new responsibilities should be defined, if it is to continue to serve a useful purpose.
REGULATORY ACTION TO CONTROL VIOLATION OF TOXIC RESIDUES IN MEAT AND POULTRY

H. M. Trobash, D.V.M.
Washington, D.C.

How many times have we heard the statement, "What you don't know will never hurt you." However, this is not true today or in the future. It seems everyone is conscious of, or emotionally involved in, the way livestock and crop production has led to the general public concern over pesticides in the food supply.

The National Residue Monitoring Program is presently being conducted by the Slaughter Inspection Division, consumer protection program, in the consumer and marketing service. We want to protect the consumer from excessive residues found in the meat and poultry supply and also aid the grower or owner in preventing and correcting the problem that caused excessive residues in the food supply. The thought of, or the presence of, residues in the meat supply poses a threat to the population. As a result, control systems were established. Our program is just one ramification of this control program.

When the Slaughter Inspection Division determines that there is a detectable residue in meat or poultry from a known origin, the Agricultural Research Service is notified if it reflects a possible misuse of a pesticide and the Food and Drug Administration if it involves a drug or compound over which they have controls. To be exact, 20 notifications are dispersed from the Slaughter Inspection Division and the Agricultural Research Service, office of the administrator, to other agencies and individuals who may be involved with the residue problem or who are needed to complete a followup on a specific problem.

It will take the continuing combined efforts of the local, state and federal agricultural agencies to keep abreast of residue problems.

The Commissioner of Agriculture in the state where the residue originated is notified. The consumer and marketing service and the Agricultural Research Service do not have authority to take action for correcting a misuse of a pesticide or drug on a farm when tests reveal a residue violation. However, the Food and Drug Administration will, whenever possible, send a field man to the premise to determine the cause and assist the owner in making necessary changes to prevent future occurrences. When we are alerted to a specific instance of illegal residues, we must act to control the situation and prevent repetition. The states play an essential role in assuring that future shipments from the farm do not contain residues beyond the tolerance levels by going to the farm and:

1. Determining the cause and source of the residue.
2. Assisting the owner in preventing the residue by recommending corrective actions.
3. Testing suspect animals or poultry for future shipment to slaughter and to quarantine if necessary to prevent dispersement of the animals or poultry, and last but not least is
4. Education – Make the owner aware of chemical or drug problems to

To establish a functioning program for residue violations as related to the industries' handling of live animals and poultry, the following categories are listed with the action to be taken when test results reveal a sample:

1. To contain a detectable residue level more than 20 percent below the tolerance (Administrative or established tolerance level) level:
   No action taken.

2. To contain a detectable residue level between 20 percent below and the tolerance level:
   Owner will be notified that residues were detected which approach the violation level and future lots of animals or poultry will be monitored to assure that they do not exceed tolerance levels.

3. To contain a detectable residue level exceeding the tolerance level by no more than 20 percent:
   Owner will be notified that residues were detected in excess of tolerance levels. Future consignments to slaughter will be on a retained basis only unless the owner or grower certifies in writing to the consumer protection program officials that the lots are in compliance with tolerance levels when presented for ante-mortem inspection. In such cases, only selected animals or birds will be retained for monitoring purposes and the remainder of the animals will be slaughtered in a routine manner.

4. To contain a residue level exceeding the tolerance level by more than 20 percent:
   All future consignments of animals or poultry to slaughter by owners will be on a retained basis only until the residue problem has been corrected.

The following plan of action is established for the Slaughter Inspection Division field followup on a recorded residue finding:

1. When a residue approaches a violation (20 percent or less below the established tolerance), or exceeds established tolerance (20 percent or less above the established tolerance):
   a. Consumer protection program officials will make every effort to identify and notify the owner or grower that his herd or flock has been samples and has been found to contain residues that approach or exceed the violation level. In any event, a responsible plant management official is advised of the problem to convey the information to the owner or grower.
      The owner is encouraged to seek help from county and state officials to determine the source of the residue problem and a solution to prevent future financial losses.
   b. Consumer protection program officials will notify the owner or
grower that future lots will be samples when presented for ante-mortem inspection by selecting from the live animals or birds. Tissue samples will be taken after the selected animals or birds are slaughtered and will be submitted to the consumer protection program laboratory for analysis.

c. Consumer protection program officials will retain only the carcasses and parts from the selected animals or birds in such future lots if the owner grower certifies in writing to the consumer protection program that the lots are in compliance when presented for ante-mortem inspection.

d. Consumer protection program officials will retain any future lots if the samples taken after owner notification reveal residues that exceed the compliance level.

2. When a residue in a sample exceeds the established tolerance by more than 20 percent, one of the two following procedures must be successfully completed before animals or birds from the contaminated source are eligible for slaughter.

a. The owner or grower procedure. The owner or grower must present to the regional deputy director for slaughter a complete outline of the plan he has formulated to bring his herd or flock into compliance. The owner or grower compliance plan must include the following information and assume the following responsibilities:

1. He must list the total number of animals or birds that will be presented for slaughter.

2. The owner or grower must agree to withhold his animals or birds from slaughter on his premises, or other acceptable premises, until a consumer protection program official notifies him that the birds or animals may be slaughtered.

3. He must list the name and address of the laboratory he has selected to analyze samples from his herd or flock. He must have the laboratory notify the deputy director for slaughter of the results, to expedite shipments to slaughter after the analysis is completed.

4. The owner or grower must list the method of analysis to be used by the selected laboratory.

5. The owner or grower must submit duplicate samples of those submitted to the laboratory he selected to the consumer protection program laboratory. Information concerning the number of samples to be submitted can be obtained from the regional deputy director for slaughter.

6. The owner or grower will be notified by the deputy director for slaughter if submitted samples are in compliance. The remaining animals or birds may then be presented for slaughter at the preselected slaughtering establishment.
The animals or birds accepted for slaughter will be sampled by a consumer protection program employee in the plant. This sample will consist of 10 birds or animals. The carcasses and organs of the sampled birds or animals will be retained until the laboratory analysis of the samples is completed. The remainder of the lot will not be retained.

If the monitored bird or animal samples are in compliance, the owner or grower compliance plan will be accepted.

If the monitored bird or animal samples are not in compliance, the owner or grower compliance plan will be rejected and the following consumer protection program procedure will be used:

b. Consumer Protection Program Procedure. The owner or grower of the contaminated birds or animals must notify the veterinary medical officer at the slaughtering plant as to the time the birds or animals will arrive for ante-mortem inspection. When these birds or animals arrive, the veterinary medical officer will complete the following procedure:

1. He will select the appropriate samples required by the sample chart (see Appendix 1, Sample Size Selection) based on the size of the lot or flock.

2. He will permit the slaughter of the selected birds or animals under retention. He will retain the remainder of the live birds or animals in the lot depending on plant facilities. If so desired by the owner or grower, all birds or animals may be slaughtered and the carcass and organs retained.

3. He will collect and individually pack the samples from the birds and animals selected for testing.

4. The samples will be submitted to the appropriate consumer protection program laboratory accompanied by Form CP-515 marked selective phase.

5. He will promptly notify by telephone his regional deputy director for slaughter that the samples have been collected and mailed.

If animals or birds from the same owner or grower are presented for slaughter before his previous samples have been determined to be in compliance, they must again be sampled as outlined under the consumer protection program procedure.

Both plans require analyses on two successive herds or flocks produced from the premises to clear the premise for future shipments without retention.
APPENDIX 1
Sample Size Selection

Column A lists the number of animals or birds presented on ante-mortem inspection and Column B lists the number of animals required to be sampled.

1. If 10 or less animals are presented, sample all animals.
2. If any number over 250 animals are presented, sample 30 animals.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>8</td>
<td>50</td>
<td>21</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>60</td>
<td>22</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>70</td>
<td>23</td>
</tr>
<tr>
<td>14</td>
<td>11</td>
<td>80</td>
<td>24</td>
</tr>
<tr>
<td>15</td>
<td>11</td>
<td>90</td>
<td>24</td>
</tr>
<tr>
<td>16</td>
<td>12</td>
<td>100</td>
<td>24</td>
</tr>
<tr>
<td>17</td>
<td>13</td>
<td>110</td>
<td>24</td>
</tr>
<tr>
<td>18</td>
<td>14</td>
<td>120</td>
<td>25</td>
</tr>
<tr>
<td>19</td>
<td>15</td>
<td>130</td>
<td>25</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
<td>140</td>
<td>25</td>
</tr>
<tr>
<td>21</td>
<td>15</td>
<td>150</td>
<td>25</td>
</tr>
<tr>
<td>22</td>
<td>15</td>
<td>160</td>
<td>26</td>
</tr>
<tr>
<td>23</td>
<td>15</td>
<td>170</td>
<td>26</td>
</tr>
<tr>
<td>24</td>
<td>15</td>
<td>180</td>
<td>26</td>
</tr>
<tr>
<td>25</td>
<td>15</td>
<td>190</td>
<td>26</td>
</tr>
<tr>
<td>26</td>
<td>16</td>
<td>200</td>
<td>26</td>
</tr>
<tr>
<td>27</td>
<td>16</td>
<td>210</td>
<td>26</td>
</tr>
<tr>
<td>28</td>
<td>17</td>
<td>220</td>
<td>26</td>
</tr>
<tr>
<td>29</td>
<td>17</td>
<td>230</td>
<td>26</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>240</td>
<td>26</td>
</tr>
<tr>
<td>40</td>
<td>20</td>
<td>250</td>
<td>26</td>
</tr>
</tbody>
</table>
A LOOK AT THE POSITION OF PUBLIC HEALTH AND PREVENTIVE MEDICINE IN CURRENT AND FUTURE VETERINARY COLLEGE CURRICULA

Wm. E. Jennings, D.V.M.
School of Veterinary Medicine
Auburn University
Auburn, Alabama

The remarks presented herein are based on the author's experience in teaching in the present and previous four decades in three Veterinary Colleges, and from 15 years service as a member of the Council on Education of the American Veterinary Medical Association. The changes in veterinary professional activities and geographic locations, and contacts made, have hopefully prevented the author from becoming provincial and parochial. The evaluation of veterinary colleges in the United States and Canada as a Council member and subsequent recent visits thereto, have provided the author with invaluable experience in the procedures employed and the scope of instruction in veterinary public health. This experience has been augmented by active participation in international, national, and regional conferences and symposia on veterinary public health. In addition, extended visits have been made to the veterinary colleges in most European countries. From these visits it has become increasingly clear that the importance of community health increases in direct proportion with the affluence and cultural development of a population.

Sir William Osler said there is only one Medicine. Developments in human, animal, and dental medicine during the last decade or two would reinforce Osler's statement. The division of the medicines was arbitrary due in large part to a lack of understanding and lack of appreciation of benefits to be derived from total integration of medicine. Laboratory animal medicine, health-related research, and community medicine are in large part responsible for the "Oneness" of medicine as it is known today. One no longer asks: "Is there a veterinarian on the faculty of the School of Medicine?" but asks: "How many?"

The terms "Public Health", "Preventive Medicine", "Community Health" are used interchangeably in our American Society. Therefore, no attempt will be made to redefine or restrict their use in this paper.

Redesign of veterinary college curricula — in fact, curricula of all colleges — appears to be fashionable at this time. Is it change for change sake? Is it acquiescence to the whims and wishes of that vocal group of students who represent only a small, insignificant percentage of the student body but purportedly represent the majority?

The Faculty of a College of Veterinary Medicine in reevaluation and redesigning its curriculum should consider seriously the primary objective — to produce a Doctor of Veterinary Medicine. With this objective firmly in mind, the curriculum can be studied objectively and with the best interest of the Veterinary Profession and of total Veterinary Medicine. Graduates of each College of
Veterinary Medicine will be engaged in every type of veterinary professional endeavor — and will, of necessity, be communicating throughout their professional careers with their colleagues in all types of veterinary professional activities. Therefore, the veterinary professional curriculum should be so structured and designed to give the student a good broad, scientific foundation. Veterinary Medicine, as its sister professions is continuing to move from an art to a science.

The veterinary professional curriculum must be kept under constant evaluation and changed to meet current and forseeable veterinary professional requirements. Some changes in the Colleges of Veterinary Medicine are needed to modernize the instructional process. Retitling and restructuring of some courses will assist in modernization of the veterinary professional curriculum. The use of modern methods of instruction with increased use of visual teaching aids and autotutorial techniques will improve the learning process. Increased emphasis must be placed on challenging the student and developing his initiative. The student must be changed from the passive role to the active, participating state in his learning experience. Increased emphasis should be placed on the use of the biomedical literature and the various tools and indices to such literature. This will incalculate in the student the importance of keeping up-to-date. A reasonable amount of specialization should be encouraged. Many students, to include those with three, four, or more years of pre-veterinary education, are undecided even in their fourth year of the veterinary professional course, on the plan and expectations for their veterinary professional careers. Is there a trend to deemphasize the economics of livestock and birds, the source of valuable animal protein in the human diet? Is herd health — human and animal — receiving appropriate emphasis or is the instruction designed for the rapidly disappearing type practice “Rescue the Perishing”? Is overemphasis placed on patient care to the exclusion or minimizing instruction in prevention of disease? Emphasis placed on prevention will inculcate in the student’s thinking and planning for furnishing veterinary professional counseling for the large herds and flocks which are placed under terrific stress and housed and fed under unnatural conditions.

Moder public health requires utilization of the material learned in all the preclinical and clinical studies. Due to public interest and legislative support, the expansion within the fields of community health during the past few years has exceeded all expectations. Consequently, the community health picture is rapidly changing. It would appear to be an insurmountable task for one veterinary faculty member to monitor, supervise, teach, and provide dynamic leadership to the public health program in a College of Veterinary Medicine.

The teaching of public health and preventive medicine in many of the Colleges of Veterinary Medicine has not kept abreast with the developments in public and community health. It would appear that the graduates of colleges of veterinary medicine have not been properly apprised of the numerous, outstandingly significant contributions to public health that have and are being made by veterinarians. This failure to impress upon each student his role in community health is regrettable. For example, witness the attitude of Veterinary Medical
Faculties to the expanded role of the Veterinary Profession in the area of food hygiene and technology, resulting from the enactment by the Congress of the United States, of the Wholesome Meat Act of 1967 and the Wholesome Poultry Products Act of 1968. Attesting to this attitude are the following statements by Dr. G. H. Wise, Deputy Administrator, Consumer and Marketing Service of the United States Department of Agriculture at the 12th Annual Food Hygiene Seminar for Teachers of Food Hygiene in the Colleges of Veterinary Medicine, November 11 & 12, 1969, at Philadelphia, Pa., "If qualified veterinarians are not available, it is obvious that other professionals groups will be called upon. If it is not possible for veterinary education to provide time and support for this need, this will equally obviously increase the likelihood of looking to others."

The United States Animal Health Association at its 73rd Annual Meeting in Milwaukee, Wisconsin, October 12-17, 1969 passed the following resolution:

"WHEREAS, there has been a definite deemphasis placed on the teaching of food hygiene principles by some Veterinary Colleges in recent years, and

WHEREAS, there has been a definite increase in the need for veterinarians with this training, and

WHEREAS, the area of food hygiene should be considered as important an aspect of Veterinary practice as any other, and

WHEREAS, most livestock and poultry are used for human food,

THEREFORE BE IT RESOLVED that the United States Animal Health Association urges the Council on Education of the American Veterinary Medical Association to require the principles of food hygiene be emphasized to all students enrolled in the Veterinary Professional Course in the accredited Colleges of Veterinary Medicine."

The modern role of veterinary medicine in the total health program must be emphasized by each instructor, in each course, and at every opportunity in the veterinary professional curriculum. Graduates of Colleges of Veterinary Medicine should be taught to think in terms of community health. The faculty of each College of Veterinary Medicine should be urged to address itself to the problem and ask the question: "Are we teaching our graduates to make those contributions to human and animal health which our society rightfully expects?" The American Veterinary Medical Association, through its Council on Education, accrediting the Colleges of Veterinary Medicine, should also be urged to ask itself the following question: "Are the Colleges of Veterinary Medicine preparing their graduates to make a just return in terms of improved human and animal health for the tax dollars (Federal and State) budgeted for veterinary education and research?"

What should be included in the education in public health and preventive medicine in the veterinary professional curriculum? When and where in that curriculum should this instruction be given? Who should teach Community health? How should the material be presented? The faculties of the Colleges of Veterinary Medicine should give serious consideration to these questions of: WHAT, WHEN, WHERE, WHO, and HOW, of teaching public health. Then, and only then, will logical conclusions be reached and a workable, effective system be developed.
Instruction in public health and preventive medicine in the veterinary professional curriculum should embrace epidemiology and epizootiology, ecology, environmental health and hygiene, biostatistics, food hygiene, and technology, zoonotic diseases, and public health administration. The mode of living today and as projected, and the effects — for better and for worse — which man is making in his environment necessitates this broad scope of instruction. Urbanization, commercialization of the herds and flocks used for food purposes, the population explosion, and environmental contamination affect man directly or indirectly, adversely or beneficially. Many of these current changes and developments were either not present or were minor or insignificant in the previous decades. The consumer is beginning to receive through the various consumer protection programs the attention he rightfully merits. As an example, consider the current programs of industry and government at all levels, to safeguard our foods of animal and bird origin. The promiscuous use of antibiotics on food animals and birds and in products derived therefrom — has resulted in the establishment of effective controls to safeguard the consumer. Likewise, restrictions have been placed on the use of fungicides, rodenticides, defoliants, herbicides, insecticides, and the like. The Veterinary Profession is affected by these controls — so necessary for the protection of human health. It is incumbent upon the Colleges of Veterinary Medicine to instruct the student in the continuing changes and developments in the areas embraced in modern community health. Indoctrination in his role as a responsible member of a learned profession dedicated to the protection of animal and human health is the goal to be sought and hopefully achieved from the instruction.

When and Where should this instruction in public health be offered? Who should give this instruction? The broad scope of community health necessitates the involvement of every discipline in the Veterinary Professional Course. Teaching public health must not be restricted to microbiology as it unfortunately is in some institutions. Pathology, parasitology, anatomy, physiology, pharmacology, toxicology and clinical medicine are but a few of the disciplines involving community health. These disciplines would appear the logical places to integrate some of the instruction in public health. Each faculty member should strive to be knowledgable of modern community health. He should take every opportunity to show the public health implications of the subject under discussion. Each faculty member must recognize the importance of community health and indoctrinate the student in Veterinary Medicine’s role in the total medicine program. Involvement of each member of the faculty is the most effective means for teaching public health and preventive medicine. The courses in veterinary public health in the veterinary professional curriculum and at the graduate level, should be in charge of an experienced, highly motivated, professional staff whose responsibilities are restricted to teaching community health and conducting research in public health problems. Currently, the involvement of this staff in a variety of duties, only peripherally related to community health, jeopardizes the total public health program in the Colleges of Veterinary Medicine. This professional staff with
expertise in all aspects of community health must direct its efforts to a continuing
review and evaluation of all media pertinent public health. Such voluminous media
would include books, reports, regulations, standards, surveys, symposia, papers
published in the more than 2000 biomedical periodicals, films, film strips, and
other video and printed material. This professional staff would communicate
pertinent current information to the faculty of the College of Veterinary Medicine.

It is readily apparent that the faculty of the College of Veterinary Medicine
must have access to a modern library, well stocked with the current editions of
books and other printed material on public health. Papers on community health
topics are published in a large number of periodicals by personnel engaged in a wide
variety of public health activities on a worldwide basis. Therefore, the library must
maintain continuing subscriptions to a sizeable number of biomedical periodicals.
Too frequently this resource is found missing.

The ever changing and rapidly developing field of community health
necessitates the involvement of this professional staff on a total basis. Then, and
only then, will the offerings in all courses in public health, at the graduate and
undergraduate level, be kept abreast of modern trends, developments, and results of
research studies. The course offerings of this public health staff should augment
that given in the various courses in the veterinary professional curriculum. The
instruction in the public health courses will categorize the material in community
health previously received, place emphasis on those areas of greatest importance,
and include pertinent information on topics, diseases, subject areas, and problems
not previously covered in the other disciplines. To keep instruction in these public
courses current and meaningful, the syllabi require continuing revision — an
herculean task if properly accomplished. Concerned newspapers and magazines
presently carry long articles on environmental health and pollution. The public
interest in those topics has assumed phenomenal proportions, and will probably
continue to do so far into the present decade.

How should the material in public health be presented? What media and
methods would best serve the purpose and make the instruction most effective?
Maximum involvement of each student in the educational process should be the
goal. Lectures and distribution of much printed material appear to be the least
effective methods for the instruction in modern community health. Epidemiolog-
ical studies foster student participation and encourage orderly investigation of
disease processes. Such studies teach the student to use all the tools and evaluate
data properly for the formulation of his hypothesis. Slides, transparencies, films,
film strips, video tapes, and other visual material increase the effectiveness of the
instruction. Field trips properly planned, to selected county, state, regional and
federal public health laboratories, departments, and research facilities should be
conducted to augment the instruction. The importance of such trips cannot be
overemphasized. Clinical clerkship-type instruction in such laboratories depart-
ments, and facilities, and state and federal animal health departments and
organizations should be used to the maximum. The student thus becomes
conversant with the operational aspects of a total animal and human health
program in which medical, veterinary, and health-related personnel are routinely engaged. Instruction in the principles of food hygiene, sanitation, and technology can be augmented through assignments and preceptorships under federal and state veterinarians and key ancillary personnel in abattoirs and other plants processing foods of animal origin. Autotutorial instruction employed to the maximum has proven most helpful to augment the teaching of community medicine. The student must be required to use and evaluate current biomedical and other literature in the development and presentation of topics of his selection related to community health. Such instruction emphasizes the necessity for continuing education and daily commitment to self-improvement throughout his professional career.

What does the future hold for the Veterinary Profession in public health, preventive medicine, community health? This will depend in large part on the attitude of the faculties of the Colleges of Veterinary Medicine. These faculties may continue to de-emphasize and remain apathetic to community health. They may relegate the teaching, supervision, and organization of this discipline to the faculty member whose experience, education, and training does not enable him to see the total picture of public health. If these conditions continue, then veterinarians may either serve under or be replaced by, sanitarians and other paramedical personnel. Witness what is presently occurring in the Veterinary Profession in many European countries. For more than a century and a half, instruction in public health was restricted primarily to food hygiene. Utilization of veterinarians in community health activities was very limited. Gainful employment of the veterinarian was not realized in these health programs. The veterinary profession in these European countries now recognizes that modern veterinary medicine must play an expanded role in community medicine. They note with much interest and concern the many important contributions the veterinarian can make to community health. Consequently, the faculties of many European Veterinary Schools are reevaluating and strengthening their course offerings in public health.

Unless the trend in the Colleges of Veterinary Medicine in the United States, as expressed in the current and proposed veterinary professional curricula, is modified, the profession of Veterinary Medicine will forego the opportunity to serve and expand in an area of medicine for which it should be preeminently qualified, and for which the profession will be adequately compensated. Further, budgets for veterinary education and research may be subjected to additional scrutiny. The old adage continued to remain true: "The fundamental purpose of education is to prove that change is necessary and unavoidable."
Many private practicing veterinarians now find themselves faced with the task of doing meat inspection work for the first time. In looking at this task most of them realize that further training is necessary in order to do the proper job. In meeting these new training needs, it is quite necessary to design a program that will fit into the everyday busy schedule of the private practitioner. This means that a large block of time necessary to attend an existing training center is not possible and that the training will have to be broke up into small segments and taken out into the field where the private practitioner can fit it into his own schedule.

One other thing that is very important to keep in mind when designing this type of training is that these veterinarians will be working in very small locker plants for the most part and very few will be working in operations of any size. They have to stop and start their training in interrupted segments and there is very limited time available for concentrated study. These are some of the things that we took into account when we designed an auto-tutorial program for the contract veterinarians in Minnesota. These veterinarians realized that they needed further training and were quite anxious to take part when this training was offered to them.

The term “auto-tutorial” itself means self-teaching. That is facilities, equipment and materials are provided and the student or trainee sets his own pace to achieve mastery of the learning or to meet the objectives that have been set down for this training. We felt that the auto-tutorial approach would fit into the needs of these veterinarians best of any method we considered. The home study auto-tutorial approach was combined with two area meetings out in the state, one at the beginning and one at the end of the home training.

I started the preparation of this training by writing objectives and these were based on what the veterinarian would have to know and how he would have to use the information we were going to present. I used Mager’s book of “Preparing Instructional Objectives” as a guideline. As we were preparing the instructional objectives I realized that much of the information I had planned on presenting turned out to be unnecessary or really not needed by the veterinarian about to do meat inspection work. Mager’s idea is that unless we can write an objective for what we are going to teach, we have a hard time proving we have taught anything. Also, we have a hard time measuring what we have taught. I prepared two sets of objectives; one was the overall course objectives, and another one which was specific for the type of terminal behavior we wanted the veterinarian to demonstrate.

An example of one of the overall objectives is; “You will be able to recognize abnormal tissue and determine the proper disposition of this tissue as well as the
remainder of the carcass from which it was derived”. An example of a specific objective is one taken from our discussion on post mortem dispositions. That is; “Given the description of several abnormal beef or swine livers, you will be able to give the proper disposition for each”. Veterinarians were asked to read these objectives over before they started listening to the recorded discussions and again after they had finished a particular segment of study. In this way they could find out whether they had met the objectives.

The home study material consisted of outlines for each area of training. These outlines contained title pages for each of the six subjects covered. On this title page was the name of the subject and the objectives of what the trainee should be gaining from that particular subject area. The topical outline which followed, covered the essential points of the material presented on the tape recording.

Throughout the outline, periodic review questions would be presented. The trainee would be asked to turn the tape recorder off and answer the questions. After he had answered it he was to turn the recorder back on and the lecturer would answer the question so the answers could be compared. In this way, the trainee would find out right away whether he was on the right track or not and get instant reinforcement that he was meeting the objectives.

In the presentation on post mortem dispositions, there were a number of case studies presented. Several slides along with a description of the animals condition on ante-mortem and post-mortem were presented. The veterinarian was to come up with the proper diagnosis and the proper disposition. When completed, he could again turn on the tape player to learn the instructor’s diagnosis and disposition.

One of the greatest advantages of the auto-tutorial approach in following these outlines is that the learner can stay on the track and get instant reinforcement of his learning. If he strays from the materials or is distracted for a period of time, he can simply review by listening to the last few minutes over again.

Slides were used along with these outlines and there were approximately 270 used to illustrate the six subject areas. Each trainee was given a set of these slides. The sources of these slides included the Consumer & Marketing Service and the University of Minnesota. Some of the pictures had to be taken while others were done by artwork. This artwork was done both by the Training Group of the Consumer & Marketing Service and by the Agricultural Extension Service at the University of Minnesota. A real attempt was made to have the slides fit the needs of getting the material across rather than the material fitting into what slides were available. Once the master set of slides was compiled, we had 13 copies of each one reproduced. These were then placed in plastic sheets holding 20 slides each. The plastic sheets were then placed in a notebook so that each trainee could have a notebook containing all of the slides necessary to cover the six subject areas. We could have used a carousel tray or simply a slide box to transport these slides. After the experience we have had so far, I believe slide boxes would have been better and probably the most time saving for the trainee. Some of the trainees related that it took quite a good deal of time to take the slides out of the plastic sheets and replace them again.
The third part of the home study materials, in addition to the outlines and slides were the audio tapes themselves. The taped lecturers were prepared by "talking" from an outline and not by reading a fully written text. We found from past experience that reading and recording is simply deadly when used for large blocks of information. Having somebody read to him is of very little interest to the trainee and he will find his thought wandering very quickly. I believe the best method is to talk or carry on a conversation with the trainee. Upon completion of the master tapes, duplicates were prepared in both cassette and reel to reel types. This was necessary because there is such a variance in the type of tape players that are available in the veterinarians home or office.

The first of the six subject areas that I covered in our training program at Minnesota was Sanitation. This included the basic principles of plant construction, equipment and installation, cleaning and sanitizing, personnel hygiene, heat and cold in meat preservation and also rodent and insect control. The second subject area was Ante-Mortem and Humane Slaughter. Next was Sanitary Dressing Procedures; I discussed only cattle and swine in these dressing procedures. Fourth was Post Mortem Dispositions. Here I covered the basic principles of making a disposition and gave many examples of the types of conditions seen with more detailed discussions of the most common ones. As I mentioned previously, case studies, illustrated by using several slides and descriptions of the abnormal conditions, were used to test the veterinarian's grasp of these principles in making a disposition. Fifth was Edible Offal Control, and this included sanitation and handling and the preparation and inspection of edible offal. The sixth subject was the Control of Condemned and Inedible Products. This included a listing of edible products from each carcass and a listing of the inedible products from each carcass, even though they may be normal. Also included were the methods of destroying inedible and condemned products, approved agents and how they can be applied.

With each new class of trainees we held an area meeting in their part of the state. During this meeting we covered the inspection techniques by film and discussion. In addition to this, we held a discussion of the lymphatic system and how this was important to them as veterinary meat inspectors.

Another area that we covered during this first meeting was to find out what areas or topics of discussion the veterinarians would like to have covered in more detail. This was an attempt to design the course to their specific needs. This initial meeting was concluded by distributing and explaining how the home study material was to be used. The trainees then returned home to utilize this material and attempted to reach the objectives set down for them.

In eight days to two weeks, a second meeting was held. During this session we collected the materials and discussed questions that the veterinarians brought up as a result of going through this material. We also discussed materials or subjects that the trainees had raised at the first meeting concerning areas on which they wanted more discussion. One of the very common subjects was that of knife sharpening. Most veterinarians felt they had a real need for further training in how to sharpen a knife. After we had discussed the material the veterinarians had questions on, I
asked some questions.

These were situation problems for testing how well the trainees had met the original objectives. The questions we used concerned ante-mortem and post-mortem situations but the same type of situation problems could be utilized to cover all areas of the training program.

Carrying on a new program such as this was not without some problems. One of those we encountered was mechanical failures where there were blank spots in the tapes or whole blank tapes. Another problem was not having the veterinarian show up for the second meeting. That meant he had to be located in order for the home study materials to be picked up. This was necessary because they were to be utilized by another trainee the following day at another location in the state. Also, on occasion the veterinarian may have encountered difficulty with the tape or with the slide or with the written outline, but didn't notify me. This meant there may have been a material defect that I went ahead and passed out to another veterinarian in another location unknowingly. Another problem that became apparent after the materials were prepared was that the segments of the discussions were too long. In other words, the topics should have been broken down into 15 to 20 minute segments rather than going on for an hour and a half on Post Mortem for example.

There are many advantages to using the auto-tutorial approach in veterinary training. The most apparent one is that the veterinarian can learn when he has the time available. More important is that he can learn at his own rate. In the past we put material out in lecture form and assumed that everyone in the class learns at the same rate; when in fact they do not. This approach allows the trainee to review material, stop the material to look at the slide longer, and reinforce his knowledge by going over the material at a later date. He can utilize this material to suit his own learning abilities.

We learn best by using several of our senses simultaneously. We Remember:

- 10% of what we Read
- 20% of what we Hear
- 30% of what we See
- 50% of what we Hear and See
- 70% of what we Say
- 90% of what we Say and we Do

With auto-tutorial, several of the trainees' senses are utilized and that we should be getting up into this higher range of the memory percentage.

Several educators have projected that maybe they will work themselves out of a job if everything they know is down on tape. Actually, with auto-tutorial the instructor has more time to discuss the questions and the problems that the trainee is having and does not have to spend his time in putting out the basic material over and over again to different audiences. Veterinarians who have taken this training so far relate that they like this method of continuing education. Students who have utilized auto-tutorial also say they want more of their material presented in this form.
There are many possible future uses for auto-tutorial as a teaching method such as more continuing education courses that can be presented this way. This is especially important to the veterinarian who has to fit his continuing education into a busy schedule. Also, if the library of this material can be built up, the veterinarian can come into a college or other central point and review several different areas of information, specifically of interest and need to him.

The auto-tutorial approach should be used more by the Consumer & Marketing Service and by State Meat Inspection Programs for training full-time employees as well as part-time employees. The trainer will not have to spend near as much time training in the plant as he is presently doing. Getting in-plant training seems to be increasingly difficult now anyway. On the job training can be accomplished in the veterinarians' own plant or in his home station, rather than going to a central point to receive this training. The trainer will not have to repeat basic lectures and can spend more of his time problem solving.

With auto-tutorial or structured teaching a training program can simulate most any type of training situation. At the present time, much of the meat inspection training consists of simulating some of the situations and hoping that the rest will be seen during the time spent in the plant. With all of the apparent advantages and few disadvantages to this type of training approach, I believe that the meat inspection programs in this country should look closely at using more and more auto-tutorial.

RESOLUTIONS

WHEREAS, consumption of meat from animals and poultry, containing biological residues (as defined in the Federal Meat Inspection Regulations), may be injurious to human health, and
WHEREAS, consumption of meat from animals and birds, infected with zoonotic diseases, may be inimical to human health, and
WHEREAS, legislative authority is not uniformly provided for the quarantine and disposal of animals and birds containing biological residues or infected with such zoonotic diseases.
THEREFORE BE IT RESOLVED that the United States Animal Health Association urge the Congress of the United States and those State legislatures, which have not already done so, to enact legislation to provide the mandatory quarantine and disposal of animals and poultry so affected.

RESOLUTIONS

WHEREAS, International trade in meat and poultry products is essential to the National Welfare, and
WHEREAS, consumers have a right to expect that all imported meat and poultry products sold are wholesome and prepared under standards equal to the U.S. Standards, and
WHEREAS, there is no substitute for adequate veterinary antemortem and postmortem inspection at the plant of origin by veterinarians and inspectors in that country,

THEREFORE BE IT RESOLVED that the Department of Agriculture be commended for its plan to improve its foreign review program by assigning highly qualified veterinarians to foreign posts and,

BE IT FURTHER RESOLVED that the veterinarians so assigned should be of a rank commensurate with their responsibility for being the primary representative of the United States in the area of meat and poultry inspection in the foreign country.
REPORT OF THE COMMITTEE ON
MEAT AND POULTRY HYGIENE

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

Your Committee reviewed its report of the 73rd Annual Meeting of the United States Animal Health Association, Milwaukee, Wisconsin. A continuing study of all major topics covered in that report has been conducted during the past year.

Active liaison has been maintained with those committees of the United States Animal Health Association, the activities of which are of concern directly or indirectly to your committee on Meat and Poultry Hygiene.

Your Committee has been organized into ten subcommittees to permit a continuing, in depth study of the ten respective areas which your Committee recognizes as its major responsibilities.

I. Residues in Meat and Poultry Products
Your Committee recommends that the United States Animal Health Association support the new National Residue Monitoring Program which determines the presence of biological residues (as defined in the Federal Meat Inspection Regulations) in animals and poultry at the time of slaughter, prescribes and implements regulatory control actions, and assists in corrective procedures to prevent the adulteration from recurring. This monitoring program functions through the cooperative efforts of the United States Department of Agriculture, Food and Drug Administration, and responsible state agencies, the livestock and poultry industries, and the livestock and poultry producers. Your Committee recommends that the United States Animal Health Association recommend the establishment of a national system of gathering, evaluating, and rapidly disseminating data from residue monitoring programs of all agencies (State and Federal) to enable the regulatory control procedures of the national residue monitoring
program to be used to its fullest extent for consumer protection.

A resolution urging the Congress of the United States and state legislatures to enact legislation to provide for the mandatory quarantine and disposal of animals and poultry, containing biological residues or affected with zoonotic diseases, has been submitted by your Committee, to the Executive Committee of the United States Animal Health Association.

II. Research in Meat and Poultry Hygiene

Your Committee recommends that the Agriculture Research Service of the United States Department of Agriculture sponsor basic research to develop the time and temperature requirements for assuring destruction of *Cysticercus bovis* to prevent human infection. This information is necessary to determine the adequacy of present regulations concerning carcass affected with cysticercosis.

Your Committee recommends that the Agricultural Research Service of the United States Department of Agriculture affect a research project to determine the distribution of tuberculosis bacilli in the musculature, organs, blood, and lymphatics in food animals when tuberculosis lesions are found. This information is necessary to determine the adequacy of present regulations concerning the disposition of carcasses affected with tuberculosis.

III. Coordinated Efforts to Bring Together Needs and Resources for Research in Meat and Poultry Hygiene

Your Committee recommends that a coordinated effort be made to provide technical information necessary to improve both the efficiency and quality of state and federal meat and poultry inspection programs. Such an effort could result in the orderly application of resources to solving operational problems common to many inspection programs. A standing sub-committee of the Committee on Meat and Poultry Hygiene with Dr. J. A. Libby as its chairman, has been appointed to serve as a medium for identifying problems, setting priorities, locating problem-solving resources (funds, facilities, personnel etc.), and disseminating the findings.

IV. Control of Bovine Cysticercosis

Since man is natural host of *Taenia Saginata*, the control of its intermediate stage *Cysticercus bovis* is a problem involving both human and veterinary medicine. Your Committee recommends that findings of cysticercosis in all slaughtering establishments should be reported to state animal health officials and public health officials for appropriate action.

V. Inspection of Poultry Broth

It is clearly the intent of the Congress of the Unites States, as expressed in the passage of the Wholesome Poultry Products Act of 1968,
that all poultry and poultry products offered to consumers be processed under an acceptable system of official inspection. In the orderly implementation of such systems and to alleviate some hardships as clearly spelled out in the law, certain exemptions are allowed. At the present time the Federal Regulations for poultry products exempt certain of these products from inspection. Among them is poultry broth. At present, poultry broth prepared at an unofficial establishment may enter a Federally inspected poultry plant and be included as an ingredient of product. To correct this omission in the inspection of poultry products, your Committee recommends and supports amending the poultry inspection regulations to include a standard for poultry broth and the deletion of it from the list of non-amendable products thus requiring all poultry broth to be produced under official inspection.

VI. Meat and Meat Products

Handling and transporting of exposed meat and poultry products account for varying degrees of product contamination. Your Committee recommends that present Federal and State inspection regulations be amended to require all exposed product be protected by an effective covering or container before being transported, loaded, and unloaded.

Consumer protection in its broadest sense cannot be fully achieved by meat and poultry inspection programs until surveillance includes transporting, storing, and handling of products outside of the inspected plant, including the retail store. Therefore, your Committee requests appropriate state and Federal groups cooperate in establishing general guidelines for such handling; and that implementation be encouraged. Technology that would detect thawing or other product mishandling should be thoroughly considered.

The generally accepted commercial practices involved in poultry evisceration, animal slaughter, dressing, or processing activities results in considerable quantities of waste and other potential pollutants. Your Committee, therefore, requests full support of the United States Animal Health Association toward anti-pollution legislation that may affect meat and poultry inspection activities.

VII. Trichinae Eradication Program

Your Committee has followed with interest the efforts of the swine industry, the packing industry, and the United States Department of Agriculture in developing the pooled-sample, artificial digestion technique for trichinae detection. This test, which was reported by Dr. John S. Andrews in the Proceedings of the 73rd Annual Meeting of the United States Animal Health Association, has proved to be highly accurate and practical. A Program Planning and Budget Model which is based on this test, and combines a consumer protection plan with a national eradication plan, has
been presented to the Secretary of Agriculture.

The resolution in support of this program, which resolution was adopted by the United States Animal Health Association at its 73rd Annual Meeting in October 1969, follows:

"WHEREAS, trichinae in our pork supply constitutes a public health hazard and has resulted in discrimination against United States pork in foreign trade and has had an adverse affect on domestic pork consumption, and

WHEREAS, the present low level incidence of trichinae in United States swine makes its eradication practical and opportune, and

WHEREAS, an accurate, practical test has been developed with the cooperation and support of the swine industry, the packing industry, and the United States Department of Agriculture, and

WHEREAS, a plan for utilizing this test in a combined consumer protection and national eradication program has been prepared for presentation to the Secretary of Agriculture,

THEREFORE BE IT RESOLVED that the United States Animal Health Association supports this national program for the eradication of trichinae."

VIII. Shipping Containers

At present, meat and poultry plants under Federal and state inspection are permitted to use a variety of new and second hand shipping containers. Use of some of these result in contamination of product. Your Committee recommends that appropriate action be taken to insure the use of only those shipping containers which will not contaminate product with microorganisms, pathogenic bacteria, and other contaminants.

IX. Export and Import of Meat and Poultry Products

International trade in meat and poultry products is necessary to assure a balanced supply of these important foods. The United States has need for lean beef and mutton imports to permit an adequate supply of processing meat to mix with our surplus fat beef trimmings. At the same time a large surplus of products such as livers, tongues, kidneys and edible rendered fat must be exported. Imported canned pork products have been well accepted. Last year over one billion pounds meat was consumed in the United States than was produced.

Each receiving country has the right to impose restrictions on imports designed to give its consumers protection equal to that provided for domestic production. This principle was expressed in the Wholesome Meat Act of 1967. It established the principle that imported meat should be inspected under standards equal to those applied in the United States. Fourteen veterinarians have been assigned to review foreign systems of inspection and to check regularly on the manner in which the inspection is applied in every plant identified as being eligible to export to the United
States. Systems and plants found not to be in compliance are removed from the eligible list.

For seven years the Service has recognized the desirability of stationing veterinarians in the countries where they will be working. Your Committee is pleased to report that the Department of Agriculture has now received authority to station eight veterinarians, with their families, in overseas locations. The Department of State and the Department of Agriculture are to be commended for this important accomplishment.

Each of the countries authorized to export to the United States has been found to have a National System of inspection equal to that of the United States. Each plant certified for exports has been examined by U.S. veterinarians to assure that the inspection is applied on an equal basis. No system of destination inspection can replace a knowledge that the meat or poultry has received adequate Veterinary antemortem and postmortem inspection by employees of the exporting country.

Restrictions imposed on exports and imports should be based on well recognized hygiene principles. The current embargo in Sweden and Italy debars products from animals that have received estrogenic hormones at any time. This emphasizes the need for controls so that U.S. veterinarians can be assured that no residue remains in the product. The Department of Agriculture should take a position of leadership in International organizations to gain wide acceptance of standards of inspection that will assure that only wholesome products will move in International trade. The United States representatives on review work and on the establishment of standards should be veterinarians with high qualifications and standing in food hygiene.

A resolution commending the Department of State and the Department of Agriculture for their actions has been prepared and submitted by your Committee, to the Executive Committee of the United States Animal Health Association.

X. Cooperative State-Federal Cross-Servicing Laboratories for Meat and Poultry Inspection

Your Committee in its consideration of this proposal recommends that cooperative effort be made to explore all aspects for establishing a working policy for cross-servicing for each type of laboratory service needed to carry out the regulation requirements under the Wholesome Meat and Wholesome Poultry Products Acts.

In addition, there should be a standardized fee list published for each such service.

XI. State-Federal Reporting System

The efficient conduct of an inspection program requires a reporting system to complement the control, direction, and planning efforts of a total
inspection program.

Your Committee has concluded it is reasonable to expect that under the cooperative inspection effort provided by the Wholesome Meat and Poultry Products Act, the participating programs should report their activities by using uniform methods of reporting.

The United States Department of Agriculture and the states are faced with the difficult task of integrating into the Federal Inspection Program Report System, the large volume of data being accumulated by the state programs.

Your Committee recommends that appropriate review and study be undertaken to determine data that should be included in developing a National Inspection Report System. This data should be identified relative to management, operational and budgetary functions. Subsequent to this determination, your Committee urges that individual states and the United States Department of Agriculture examine their reporting systems to ascertain, first of all, any lesser or greater needs. Secondly, consideration should be given to the adaptability of existing federal or state forms to satisfy both national and state requirements.

XII. 13th Annual Seminar for Teachers of Food Hygiene

Your Committee recommends that the United States Department of Agriculture's Consumer and Marketing Service again sponsor the Annual Food Hygiene Seminar for Teachers of Food Hygiene in the colleges of Veterinary Medicine in the United States and Canada. Your Committee further recommends that this seminar be held at the Continuing Education Center, The Ohio State University, Columbus, Ohio.

XIII. Instruction in Food Hygiene in Colleges of Veterinary Medicine

Attention is invited to the following resolution which was adopted by the United States Animal Health Association at its 73rd Annual Meeting in Milwaukee, Wisconsin, October 12-17, 1969.

"WHEREAS, there has been a definite deemphasis placed on the teaching of food hygiene principles by some Veterinary Colleges in recent years, and
WHEREAS, there has been a definite increase in the need for veterinarians with this training, and
WHEREAS, the area of food hygiene should be considered as important an aspect of Veterinary practice as any other, and
WHEREAS, most livestock and poultry are used for human food,
THEREFORE BE IT RESOLVED that the United States Animal Health Association urges the Council on Education of the American Veterinary Medical Association to require the principles of food hygiene be emphasized to all students enrolled in the Veterinary Professional Course in the accredited Colleges of Veterinary
Your Committee again indorses this resolution and urges appropriate action by the Council on Education of the American Veterinary Medical Association.

Your Committee strongly recommends that immediate attention be given to the curriculums studies and program changes being conducted at Colleges of Veterinary Medicine in the United States which would deemphasize the instruction in food hygiene and environmental health. The Wholesome Meat and Poultry Products Acts necessitates the direct utilization of many more veterinarians in contract and program positions than have been used previously.

Your Committee recommends that accreditation by the American Veterinary Medical Association include a requirement that a specific course in Food Hygiene be continued in the curriculum as well as an indoctrination on the principles involved in the various applicable courses.

It is further recommended that the United States Department of Agriculture’s Consumer and Marketing Service be encouraged to make available a Consumer Protection Program’s veterinarian on a part-time basis to assist in the training of undergraduate students in the Colleges of Veterinary Medicine. It is suggested that the veterinarian provided be permitted to pursue post doctoral training in the college to which he is assigned.

XIV. Post Doctoral Training of Veterinarians in Food Hygiene

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

XV. Intermittent Employment of Veterinary Students in Food Hygiene

Your Committee recommends that intermittent employment of Veterinary students be encouraged as summer employment between their fourth and fifth professional years as well as the summer between the fifth and sixth professional years.

Such employment would be the United States Department of Agriculture’s Consumer and Marketing Service. It is recommended the
assignment would be at one of the five Consumer Protection Program Training Centers which are located at St. Paul, Omaha, Los Angeles, Fort Worth, and Gainsville, Georgia.

XVI. *Continuing Education in Food Hygiene for Veterinarians (Post Doctoral Training)*

Your Committee recommends that the Colleges of Veterinary Medicine provide *continuing education* for practicing and inspection program Veterinarians so that they may assume their responsibilities in the conduct of state meat inspection.

Your Committee further recommends that the United States Department of Agriculture, Consumer and Marketing Service provide a Consumer Protection Program's Veterinarian to fulfill its responsibility to states for the training, as required by the Wholesome Meat and Poultry Products Acts.

Dr. James A. Libby, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, presented an excellent paper on "Auto-tutorial Meat Inspection Training for Contract Veterinarians," to your Committee. His presentation demonstrated a method that can be used effectively to provide continuing education to practicing veterinarians who are or will be employed as contract veterinarians in state meat inspection programs.

XVII. *Inclusion of Food Hygiene Subjects in Programs of Veterinary Association Meetings*

To keep the profession abreast and alert to the position of the Veterinarian in meat and poultry inspection programs, it is necessary that papers be given on the subject at professional meetings. Full support of local, state, regional, and national associations is solicited by this Committee to allow equivalent time to Environmental Health including Food Hygiene, as is given to other aspects of the profession.

XVIII. *Training of Meat and Poultry Inspectors*

Your Committee recommends that recognition be given to college-trained non-veterinary inspectors presently being graduated. Further your Committee recommends a continued expansion to other colleges and universities of such courses for inspectors.

XIX. *Distribution of Committee Reports*

In the interest of gaining the greatest results from the recommendations of the Committee of Meat and Poultry Hygiene of the United States Animal Health Association, it is urged that copies of the Committee's report be distributed as needed to the following, immediately upon approval by the Executive Committee of the United States Animal Health Association:
1. The National Food Inspection Advisory Committee.
2. Appropriate Congressional Committees.
3. State Departments and Agency Directors having meat and poultry inspection responsibilities.
4. The American Veterinary Medical Association.
5. Industry representatives and industry related organizations including trade publications.
6. Secretary of Agriculture.
7. Colleges of Veterinary Medicine.
THE HAEMOPHILUS SOMNUS COMPLEX

L. N. Brown, D.V.M., Ph.D.
R. C. Dillman, D.V.M., Ph.D.
Iowa Veterinary Diagnostic Laboratory
R. E. Dierks, D.V.M., Ph.D.
Veterinary Medical Research Institute
Iowa State University
Ames, Iowa 50010

Introduction and Review of the Literature

Infectious thromboembolic meningoencephalomyelitis (TEME) of cattle was first reported by Griner et al. of Colorado, in 1956. In 1960, Kennedy and his California co-workers further characterized the pathology of this disease, and reported its successful transmission with what they termed a Haemophilus-like microorganism. Subsequently, the rather wide distribution of this infection has been confirmed by the reports of veterinarians in a number of states and. Nor can we ignore the numerous reports of strikingly similar Haemophilus spp. infections of sheep observed in New Zealand, Australia and the United States. Independently, Shigidi and Hoerlein of Colorado, and Bailie at Kansas State University have studied the bacteriological properties of the etiological agent of TEME. Bailie has suggested the new name of Haemophilus somnus for this microorganism.

Panciera et al., after observation of field cases in Oklahoma and Texas, were among the first to call attention to the acute respiratory manifestations and the chronic joint and muscle involvement in cattle suffering from infection with Haemophilus somnus. Brown, Dierks and Dillman have noted the similarity of lesions found in Haemophilus somnus septicemias with those of shipping fever and clostridial infections. One of these authors (Dierks) has experimentally produced lesions resembling those seen in typical field cases by either intravenous or intratracheal administration of Haemophilus somnus to susceptible calves. He has also demonstrated the value of complement fixation test serology as an aid to diagnosis and epidemiological study of this disease.

Dillman has reported a polypoid tracheitis syndrome occurring in chronically coughing feedlot cattle which has now been associated with chronic Haemophilus somnus and Pasteurella multocida infection.

The purpose of this presentation is fourfold, and it has been divided into four sections as follows:

Section I. Epidemiology and Pathogenesis

This outline will indicate how primary Haemophilus somnus infection, coupled with invasion by secondary bacterial pathogens, may well be the cause of a wide range of costly bovine diseases — the "Haemophilus somnus complex". (Brown)
Section II. Gross and Histopathological Lesions
Lesions observed in field cases submitted to the Iowa Veterinary Diagnostic Laboratory will be compared with those produced in experimental *Haemophilus somnus* infections at the Veterinary Medical Research Institute, Iowa State University. These observations strongly support the hypothesis stated in Section I.

(Dillman)

Section III. Serological Procedures
The procedures for application of the complement fixation test to diagnosis and study of this problem will be described. Preliminary test results tend to confirm other field and research observations.

(Dierks)

Section IV. Differential Diagnosis
Suggested methods for the better differential diagnosis of *Haemophilus somnus* infections by both the practicing veterinarian and diagnostic laboratory personnel will be outlined. Cooperative efforts by all groups within the profession will be required to answer many of the questions posed by this presentation.

(Brown, Dillman and Dierks)

I. EPIDEMIOLOGY AND PATHOGENESIS OF HAEMOPHILUS SOMNUS INFECTIONS

L. N. Brown, D.V.M., Ph.D.
Iowa Veterinary Diagnostic Laboratory
Iowa State University
Ames, Iowa 50010

The working hypothesis outlined here is a summation and extension of the previously published descriptions of *Haemophilus somnus* infections of cattle by Kennedy *et al.* and by Panciera *et al.* Recent research and diagnostic findings at Iowa State University strongly support both this hypothesis and the findings of earlier investigators.

A. Epidemiology
1. Source of Infection:
   Acutely or chronically infected *carrier cattle*.
2. Mode of Transmission:
   Likely *respiratory*.
3. Incubation Period:
   Experimentally: As short as 2 days.
   Field Cases: Usually 2 to 7 days. Often as long as 10 days between onset of respiratory form and occurrence of septicemia.
4. Signs of Illness:
   At first, *respiratory*, with high morbidity.
Subsequent septicemia, much lower morbidity.
Complexity of clinical forms explained by pathogenesis.

B. Pathogenesis

1. Respiratory Transmission
2. Respiratory Infection
3. Septicemia
4. Injury to Vascular Endothelium
5. Thrombosis
6. Hemorrhage
   - Disrupted Function
   - Ischemia
   - Anaerobiosis
   - Necrosis
7. Secondary Infection
   - Pasteurella
   - Corynebacterium
   - Spherophorous
   - Clostridium
   - Moraxella

C. Respiratory Infection

1. Acute Pneumonia
   Must be included in the shipping fever complex. Probably a more important role than PI 3 virus. Pasteurella spp. still important secondary opportunists.
2. Acute Laryngitis
   Very probably the primary agent of calf diphtheria. Pasteurella, Corynebacterium, and Spherophorous spp. are secondary opportunists.
3. Chronic Tracheitis
   Chronic cough or polypoid tracheitis syndrome. Very good evidence for involvement.
4. Fibrinous Pleuritis
   Only agent consistently isolated.

D. Septicemia and resulting Thrombosis

1. Brain
   Thromboembolic meningoencephalomyelitis. (TEME)
2. Heart
   Myocardial infarcts. (Apparent heart attack)
3. Skeletal Muscle
   Dramatic hemorrhage. Impaired circulation probably results in lactic acid accumulation, stiffness and pain. Anaerobiosis paves the way for secondary Clostridium spp. infection. So-called Clostridium sordellii syndrome.
4. Gastrointestinal Tract
   Hemorrhages resemble enterotoxemia. May initiate enterotoxemia by
impaired oxygenation and impaired motility. Might be associated with chronic bloat.

5. Liver
   Could there be any association with increased incidence of liver abscesses in some feedlots?

6. Kidneys
   Could renal lesions cause a clinical manifestation which could be misdiagnosed as acute leptospirosis?

7. Joints
   Excessive joint fluid accumulation commonly observed. Fibrinous material clots following collection. Chronic arthritis is a common aftermath of acute infection.

8. Extremities
   Could this infection be a primary cause of footrot? Is it yet another cause of laminitis?

9. Reproductive Tract
   Could circulatory disturbance to placenta initiate abortion? Is the fetus ever infected? What about the bull?

10. Conjunctiva
    Excessive lacrimation accompanies acute infection. Relationship to pink eye needs investigation.

11. Udder
    Mastitis. Why not?

II. GROSS AND HISTOPATHOLOGICAL LESIONS OF HAEMOPHILUS SOMNUS INFECTIONS

R. C. Dillman, D.V.M., Ph.D.
Iowa Veterinary Diagnostic Laboratory
Iowa State University
Ames, Iowa 50010

INTRODUCTION

As with most infectious processes, the more acute the course of the disease the more predominant are the vascular and exudative pathological alterations. A subacute and chronic form of this disease syndrome exists; and as one would expect, the lesions associated with these forms are characterized by proliferative changes including hyperplasia, fibrosis, resolving necrosis, and abscessations. The disease syndrome experimentally reproduced was of an acute nature and, hence, the lesions were characterized by vascular and exudative alterations. The acute, and usually fatal, course of the experimental disease, in this initial study, is believed to have resulted from the experimental animals receiving a larger dose of microorganisms than they would have been exposed to under natural field conditions.

_Peipheral Lymph Nodes_ — In acutely affected field cases, the peripheral
lymph nodes are enlarged and hemorrhagic. All peripheral lymph nodes may be involved or only those regional lymph nodes draining tissues manifesting gross lesions. The same is true of the experimentally produced syndrome.

Microscopically, a lymphadenitis is present in acutely affected field cases and in experimental animals. Edema, congestion, and hemorrhage predominate with an occasional focus of fibrin formation and infiltration of neutrophils (microabscess) being observed. Thrombi have not been seen in lymph nodes.

Peripheral lymph node involvement has not been observed in those field cases manifesting the chronic form of the syndrome.

Tongue — As in other muscles of acutely affected cattle, intraglossal hemorrhage is often observed. The hemorrhages are focal and may be petechial or ecchymotic and tend to occur more commonly near the base of the tongue. The glossal lesions were not consistently observed in the experimentally reproduced disease.

Histopathological examination of the intramuscular tongue lesions reveals a necrotizing vasculitis and an occasional thrombus. Perivascular emigration of neutrophils is sometimes seen. Zenker’s necrosis of the muscle fibers is common.

Glossal lesions have not been observed in chronically affected animals.

Larynx — Hyperemia, hemorrhage, and ulceration of the laryngeal epithelium are observed in the acute phase of the disease in both the naturally occurring and experimentally reproduced disease. The hemorrhages range from petechial to ecchymotic and tend to be bilaterally symmetrical, fostering the hypothesis that the laryngeal blood-vascular system is primarily affected and the epithelium only secondarily involved. Indeed, the symmetry of the laryngeal ulcers suggests that they are the result of ischemic necrosis subsequent to infarction.

Subacute to chronically infected field cases have a high incidence of necrotic laryngitis from which Spherophorus necrophorus can be isolated.

The gross laryngeal lesions are easily confirmed microscopically. A vasculitis and associated foci of hemorrhage, with infiltration of neutrophils, and epithelial necrosis are consistent findings. No thrombosed laryngeal vessels have been seen to date.

Trachea — Hyperemia and hemorrhage are present within the trachea of acutely affected cattle from field cases and animals experimentally inoculated. The hemorrhages range from petechial through ecchymotic to diffuse throughout the length of the trachea. Chronically affected individuals from naturally occurring field cases are commonly “coughers”, and the clinical signs are reflected within the tracheas of these individuals by the presence of a roughened, thickened, tracheal mucosa; the lesions are often focal and have a hyperemic margin. Examination with a hand lens or under a dissecting scope reveals the presence of polyps and the condition has been previously classified as a “polypoid tracheitis” by one of the investigators.6

Microscopic examination of the tracheas from acutely affected cattle only confirms the gross observations of hemorrhage and hyperemia. Focal sloughing of respiratory epithelium is seen on occasion and an associated infiltration of
neutrophils into the submucosa. The experimental tracheitis is characterized by a submucosal vasculitis with thrombus formation and diffuse infiltration of neutrophils. Hyperemia and hemorrhage are also observed. The respiratory epithelium remained intact in those experimental animals examined.

The polypoid tracheitis associated with naturally occurring "coughers" has been previously described in detail. Briefly, the lesions consist of hyperplastic respiratory epithelium forming polyps with loss of cilia and squamous metaplasia of the pseudostratified ciliated columnar epithelium. Plasma cells and lymphocytes form a dense submucosal leukocytic infiltrate.

The lesion is not considered pathognomonic for the chronic form of the Haemophilus somnus disease complex, but the organism has now been isolated from the tracheas of two animals with polypoid tracheitis. The two animals represented two separate feedlots with herd histories suggesting primary Haemophilus somnus infections.

Heart — A fibrinous epicarditis-pericarditis is present in both field cases and experimental animals, although the latter manifest more severe lesions. Fibrous adhesions between the parietal and visceral pericardium have been observed in chronically affected cattle. Myocardial infarcts have been observed only in experimental animals, but one field case of coronary occlusion with myocardial infarction and death may have represented the sequel to a Haemophilus somnus infection, as suggested by the herd history.

Histopathologic examination of bovine hearts confirms the fibrinothrombotic epicarditis and myocarditis in acutely infected field specimens and experimental animals. Zenker's necrosis of myocardial fibers occurs within sharply demarcated infarcts. Vasculitis and emigration of neutrophils is commonly observed. Only one thrombosed coronary vessel has been observed, and it was present in the unconfirmed Haemophilus somnus suspect mentioned in the previous paragraph.

Lung — The gross pulmonary lesions, by themselves, cannot be differentiated from pneumonic pasteurellosis. The acute respiratory infection results in an anterior-ventral fibrinohemorrhagic bronchopneumonia in naturally occurring infections and a fibrinopurulent bronchopneumonia in experimental animals when the latter are inoculated intratracheally. Intravenous inoculations produce an acute fibrinothrombotic pneumonia with a uniform hematogenous distribution in pulmonary tissue. A chronic anterior-ventral bronchopneumonia apparently does not result from Haemophilus somnus infection alone but requires secondary bacterial opportunists.

The naturally occurring and experimentally produced pneumonias are easily confirmed by histopathologic examination. No specific changes are observed in field cases, and the lesions range from fibrin-filled alveoli and capillary congestion to a fibrinopurulent pneumonia. Vasculitis and thrombosis of pulmonary vessels have been observed in one experimental animal that was inoculated intratracheally.

Anterior and Ventral Skeletal Muscles — Intramuscular hemorrhage is a common lesion in acutely affected animals. Animals from both naturally occurring field infections and those experimentally infected have intramuscular hemorrhages
in the anterior and ventral skeletal muscles. The hemorrhages may be ecchymotic, but we most frequently observed massive, diffuse intramuscular hemorrhages within the muscle groups of the ventral neck and brisket. The subscapular and suprascapular muscles are less commonly involved. The hemorrhages are not observed in chronically affected cattle from field cases.

Coagulation of sarcoplasm with loss of striations and slight sarcolemmal proliferation can be recognized microscopically. Vascular changes consist of a necrotizing vasculitis with hemorrhage, infiltration of neutrophils, and thrombus formation.

Liver — Macroscopic or microscopic liver lesions have not been observed in naturally or experimentally infected cattle.

Kidney — Gross lesions within the kidneys of naturally infected animals have not been observed; however, renal infarcts have been experimentally produced by intratracheal and intravenous inoculation.

The microscopic occurrence of a focal, suppurative, interstitial nephritis in naturally infected animals has been observed in rare instances; but the lesions are inconsistently observed, lack specificity, and are described here only in consideration of the experimentally produced renal lesions. Focal areas of vasculitis, hemorrhage, infiltration of neutrophils, and tubular necrosis have been observed in experimental animals inoculated intravenously and intratracheally. The experimentally produced lesions vary from focal interstitial reactions to obvious renal infarcts. Thrombi have not been seen.

Spleen — No specific gross or microscopic lesions have been observed in field cases or experimental infections. Vasculitis and thrombosis have not been seen at this time.

Forestomachs — No specific gross or microscopic lesions have been observed in field cases or experimental infections. Numerous case histories suggest that a tendency to bloat may be one of the chronic manifestations following the acute phase of the infection. Consideration has been given to the possible impairment of the gastric circulation, but this has not been substantiated.

Small Intestines — Macroscopic intestinal lesions in acutely affected field cases are sometimes seen. They consist of an acute focal hemorrhagic enteritis involving segments of the small intestines in the range of 15.0 to 25.0 cm. in length. The ileum is most commonly involved when the lesion is present. The lesion has been produced experimentally by both intravenous and intratracheal inoculations. The intestinal involvement has not been observed in chronically affected animals from field cases.

Microscopic examination of the intestinal segments confirms the gross observations of hyperemia and hemorrhage. Vasculitis or thrombosis of the submucosal or serosal vessels has not been observed.

Large Intestines — No consistent gross or microscopic lesions have been observed in naturally or experimentally infected cattle.

Synovial Fluid and Joints — A fibrinopurulent arthritis is a common lesion in acutely affected field cases and those experimentally infected by intratracheal or
intravenous inoculation. Periarticular petechial and ecchymotic hemorrhages are associated with the acute arthritis. Lameness is one of the chronic manifestations observed in naturally infected animals surviving the initial acute infection; and a fibrinopurulent arthritis, without associated hemorrhages, is also a common lesion in these animals. All joints of the appendicular skeleton can be affected, but the stifles joints are most frequently involved. The joint fluid clots subsequent to aspiration.

Histopathological observations on the joints are incomplete at this time. Cerebrospinal Fluid — The cerebrospinal fluid clots subsequent to collection from acutely affected individuals. Clinical pathological evaluation of the cerebrospinal fluid is difficult because of the clotting.

Brain — The focal areas of hemorrhage and malacia, scattered at random throughout the brain and brain stem, have long been observed and described in detail as the feedlot encephalitis recognized as thromboembolic meningoencephalomyelitis. The gross and microscopic lesions of an embolic meningoencephalitis have been observed in all naturally and experimentally infected animals discussed here. In addition, microscopic lesions of a chronic embolic meningoencephalitis have been observed in a field specimen with permanent central nervous system impairment. Several foci of malacia containing large numbers of lipid-laden macrophages, marked capillary proliferation, and organizing thrombi were observed microscopically.

SUMMARY

The lesions produced by intravenous or intratracheal inoculation of cultures of Haemophilus somnus were generally the same in all organs and tissues except the lungs. A hematogenous pneumonia of uniform distribution throughout the pulmonary parenchyma resulted from intravenous inoculation as opposed to an anterior-ventral bronchopneumonia that resulted from intratracheal inoculation or aerogenous portal of entry.

The experimentally produced lesions were of the same quality as those seen in naturally infected animals, but within some tissues exceeded the lesions in field specimens in quantity; i.e., they were more dramatic.

The necropsy lesions observed in cattle infected with Haemophilus somnus, whether naturally or experimentally infected, can easily be confused with a number of diseases. Indeed, even the clinical signs add to the confusion.

The acute hemorrhagic tracheitis and fibrinohemorrhagic to fibrinopurulent bronchopneumonia with associated fibinous pericarditis is readily confused with acute infectious bovine rhinotracheitis and secondary pneumonic pasteurellosis. The massive intramuscular hemorrhages in the anterior and ventral skeletal muscles cannot, by themselves, be differentiated from a Clostridium spp. infection. The focal segments of hemorrhagic enteritis within the small intestines readily lend themselves to a diagnosis of enterotoxemia, particularly when the animal has died suddenly from an acute embolic meningoencephalitis and the brain is not examined. The lameness manifested in some of the animals surviving the acute
respiratory disease and possible encephalitis is easily mistaken for the laminitis associated with founder or a vitamin-mineral imbalance.

III SEROLOGICAL STUDIES OF
HAEMOPHILUS SOMNUS INFECTIONS

R. E. Dierks, D.V.M., Ph.D.
Veterinary Medical Research Institute
Iowa State University
Ames, Iowa 50010

A complement fixation test, utilizing the standard microtiter system, has been developed for serological studies of *Haemophilus somnus* infections in cattle. A number of different media for growth of the organisms and methods for preparation of the complement fixing (CF) antigens were tried but only the presently used method will be described. The *H. somnus* isolates were grown under microaerophilic conditions on a serum enriched bovine blood agar media. The cultures were then washed, sonicated and gently treated with trifluorotrichloroethane for use as CF antigens. Five different *H. somnus* cultures, isolated over a period of 4 years, were selected and prepared as antigens. Rabbits were immunized against whole washed cultures of each of the isolates and homologous-heterologous block titrations were completed. Block titrations of each isolate against homologous and heterologous antisera indicated that there were no or only very minor antigenic variations among the different cultures as determined by the CF test. A number of recent isolates have also been titrated against selected antisera with results similar to those obtained with the homologous antigen-antibody systems.

One of the major problems encountered were anticomplementary factors in bovine sera. In order to eliminate these factors from many of the bovine sera, it was necessary to use a kaolin adsorption procedure. A slurry of acid washed kaolin was prepared in borate saline buffer pH 9.0 and one volume of serum treated with 9 volumes kaolin borate saline mixture.

As part of a series of pathogenicity studies pre and post inoculation sera from experimentally infected calves and older cattle were taken for serological testing. It was found that there was a conversion to positive status by 4 to 6 days post infection with *H. somnus*.

In order to further evaluate the test for potential diagnostic value a series of both individual and paired acute and convalescent sera were obtained as coded samples from the Veterinary Diagnostic Laboratories. These sera were from cattle from which *H. somnus* had been isolated or a diagnosis of the disease had been established histopathologically as well as from cattle with other disease syndromes. An excellent correlation was obtained between the serological results and laboratory diagnosis of *H. somnus* infection. A rise in titer was often seen in animals from which paired serum samples were obtained.
With the assistance of the Veterinary Ambulatory Clinic a herd in which \textit{H. somnus} infection, with several acute deaths, had occurred was followed by periodic bleeding over a two month period. It was found that for the 35 cattle studied there was a positive serological response in 100\% of the animals indicating that this is a disease of very high morbidity but of low mortality. This has been confirmed by obtaining positive titers from normal appearing animals in several other herds in which acute deaths caused by \textit{H. somnus} have occurred.

In order to obtain further evidence of the occurrence and distribution of this disease in Iowa approximately 2000 serum samples were randomly selected from the State-Federal Brucellosis Testing Laboratory. These sera represented 267 herds distributed rather uniformly throughout the state. Eighty-eight of the 267 herds (33\%) contained reactor animals in the complement fixation test. In approximately 1/2 of the positive herds the number of reactor animals within a herd was close to or at the 100\% level. Surprisingly, however, there were a number of herds with only 10\% to 20\% serologically positive animals. These could possibly represent older infections where the antibody titer of many animals had fallen below the level readily detectable by the complement fixation test.

On the basis of epidemiological evidence of a very rapid spread of the disease to nearly all of the animals within a herd it is hypothesized that \textit{H. somnus} infections are transmitted primarily via the respiratory tract but produce a generalized septicemia upon infection of an individual animal. The organism is then capable of producing a variety of lesions through damage to the blood vascular system as well as by direct invasiveness of tissues. The hypothesis of respiratory transmission was recently strengthened when one of the authors (Brown) was able to isolate \textit{H. somnus} via nasal swabs from 6 of 10 animals sampled within an infected herd of approximately 250 animals.

\textbf{DIFFERENTIAL DIAGNOSIS OF HAEMOPHILUS SOMNUS INFECTIONS*}

L. N. Brown, D.V.M., Ph.D.
R. C. Dillman, D.V.M., Ph.D.
Iowa Veterinary Diagnostic Laboratory
R. E. Dierks, D.V.M., Ph.D.
Veterinary Medical Research Institute
Iowa State University
Ames, Iowa 50010

In a study recently completed at Kansas State University, Bailie\textsuperscript{1} has characterized many of the bacteriological properties of the agent which causes

\textsuperscript{*}Adapted From: Communications in Continuing Education. Veterinary Medical Extension. Iowa State University, Ames, Iowa. No. 122. (July, 1970) John B. Herrick, Editor
thromboembolic meningoencephalomyelitis (TEME) in feedlot cattle. He has proposed the name *Haemophilus somnus* for this microorganism, which has sometimes also been referred to as an Actinobacillus-like agent. It has the following properties:

- Gram-negative rod
- Pleomorphic on initial isolation
- Requires reduced oxygen tension, increased CO₂
- Produces small nondescript colonies on blood agar
- Slower growth than many contaminants
- Hard to isolate from mailed-in tissues, or antibiotic-treated cattle
- Antibiotic sensitivity pattern similar to *Pasteurella spp.*

*Haemophilus somnus* can cause respiratory disease in feedlot cattle, in addition to the more widely recognized “thrombo” condition, according to Panciera *et al.*\(^\text{15}\) of Oklahoma State University. Their description of feedlot disease syndromes caused by *Haemophilus somnus* can be outlined as follows: (with several additions by the current authors)

### PERACUTE INFECTION

**Clinical signs:**
- Fever
- Prostration
- Stiffness
- CNS disturbance
- Sudden death

**Post Mortem Lesions:**
- Multiple foci of hemorrhage and necrosis throughout brain
- Cloudy fluid in stifle joint
- Hemorrhages at random in:
  - muscles
  - respiratory tract
  - intestine
  - kidney

### ACUTE INFECTION

**Clinical signs:**
- Fever
- Depression
- Dyspnea
- Dry hacking cough
- Morbidity near 90%
- Stiffness, soreness
- Excessive lacrimation
- Nasal discharge

**Post Mortem Lesions:**
- Laryngitis
- Peritracheal hemorrhages
- Acute pneumonia with reddened heavy consolidated lungs
- Hemorrhages at random in:
  - muscles
  - respiratory tract
  - intestine
  - kidney
  - brain

### CHRONIC INFECTION

**Clinical signs:**
- Lameness

**Post Mortem Lesions:**
- Mortality low except for indivi-
**THE HAEMOPHILUS SOMNUS COMPLEX**

Stiffness  
Reluctance to move  
Dry hacking cough  
Poor performance  
Possibly foot rot  
Knuckling at fetlock  

duels with complications  
History may indicate *Haemophilus somnus* infection as a possible primary etiology with secondary infection by *Pasteurella*, *Clostridium* or *Spherothrix* spp.

**RESULTS AT IOWA STATE UNIVERSITY**

At the Veterinary Diagnostic Laboratory, *Haemophilus somnus* is being isolated with increasing frequency from bovine specimens submitted by Iowa veterinarians. These isolations, coupled with case histories, clinical manifestations, and a characterization of gross and histopathological lesions, strongly support the Kansas and Oklahoma reports. *Haemophilus somnus* infection has been diagnosed in cases of acute pneumonia, severe pleuritis, acute laryngitis, chronic tracheitis, suspected episodes of enterotoxemia, cases of suspected *Clostridium sordellii* infection, and others in addition to typical "thrombo" cases. *Haemophilus somnus* has been isolated from nasal swabs collected from feedlot cattle with acute upper respiratory disease.

At the Veterinary Medical Research Institute, field isolates from the Veterinary Diagnostic Laboratory have been used in the successful reproduction of typical lesions of this disease in susceptible calves and cows. A complement fixation test has been developed for the detection of specific antibody. Preliminary evidence suggests that antibody titers measured by this test may be a reliable indication of past infection and level of immunity to this agent.

The increased awareness of this problem has stemmed from cooperative efforts by clients, practitioners, animal scientists, clinicians, diagnosticians, and research veterinarians. Hopefully, these combined efforts will continue to further our awareness and understanding of this costly disease complex.

**SIGNIFICANCE FOR PRACTICING VETERINARIANS**

*Haemophilus somnus* has now been recognized as a probable cause of a number of bovine infectious disease problems in addition to "thrombo". It could very well be an important part of the shipping fever complex. *Haemophilus somnus* might prove to be the primary infectious agent in necrotic laryngitis (calf diphtheria). It may prove to be associated with persistent chronic cough in feedlot cattle. It is possibly the primary cause of hemorrhagic lesions in muscles or intestinal tract from which various *Clostridium* spp. (*sordellii*, *septicum*, or *perfringens*) are frequently isolated.

The actual frequency of *Haemophilus somnus* infection in cattle is yet to be determined. Also in need of confirmation is the possible role of *Haemophilus somnus* in a wider range of clinical manifestations. Although respiratory trans-
mission of this agent is a good possibility, epidemiological studies will be required to confirm this fact. No immunizing agents are currently available for routine use in attempts to prevent this infection. Accurate diagnosis of *Haemophilus somnus* infection is the key which will open the door to an improved understanding of this recently recognized disease complex. Try the procedures suggested here to confirm the frequency of *Haemophilus somnus* infections in your practice area. Your interest and cooperation will be appreciated.

**DIAGNOSIS OF HAEMOPHILUS SOMNUS INFECTIONS**

I. *Sudden Death:*
   A. Consider other likely causes:
      1. Bloat
      2. Overload
      3. Lightning
      4. Blackleg
      5. Toxicity
   B. Perform complete necropsy examination:
      1. Conduct post mortem as soon as possible.
      2. Do *not* stop after observing muscular hemorrhages suggestive of *Clostridium spp.* infection.
      3. Do *not* stop after observing lung lesions suggestive of acute pneumonia.
      4. *Do* proceed to look for excessive cloudy joint fluid.
      5. *Do* look for cerebrospinal fluid of abnormal turbidity.
      6. *Do* examine the brain for hemorrhagic foci.
   C. When desirable, collect tissues for laboratory confirmation.
      1. Collect small portion of lung.
      2. Collect any hemorrhagic lymph nodes.
      3. Collect samples of hemorrhagic muscle tissue.
      4. Save the brain.
      5. Joint fluid or cerebrospinal fluid may be collected in a sterile disposable syringe.
      6. Refrigerate all tissues as soon as possible.
      7. Formalin fix a 1/4" slice of lung, muscle, and brain containing typical lesions.
   D. If possible, have the owner transport specimens (or even the intact carcass) to the laboratory. Success in isolation rapidly diminishes in proportion to time specimens are in transit.
   E. Include herd history. Mention the following:
      1. Morbidity data.
      2. Probable source of infection.
      3. Apparent incubation period.
      4. Herd incidence of respiratory disease, cough, lameness, knuckling,
III. Chronic Disease Processes:

The possible etiological relationship of *Haemophilus somnus* to chronic cough, chronic arthritis or lameness, or chronic central nervous system disorder will be more difficult to determine. Collection of herd histories will be especially important in attempts to relate primary *Haemophilus somnus* infection to later occurrences of abortion, foot rot, liver abscessation and pinkeye. Serological test
results will be almost impossible to interpret realistically once these syndromes have appeared unless, in problem herds, earlier reference sera are available.

Another recourse may be to arrange for observation of gross lesions and collection of tissue specimens for histopathological examination at the time of slaughter.

REFERENCES


REPORT OF THE COMMITTEE ON
INFECTIOUS DISEASES OF CATTLE

John B. Herrick, Ames, Iowa, Chairman

1. A report of a sub-committee on artificial insemination of cattle was received, discussed, and accepted. This report is too detailed and lengthy for presentation before this body. It will be submitted to be reproduced in full in the proceedings of this meeting.

In short summary, it has been requested by this committee that in recognition of the complex disease transmission potential of AI that U.S.D.A.'s Agricultural Research Service, Health of Animals Division, give early and comprehensive consideration to establishing practical and sound regulations governing the collection, processing, storage, and distribution of bovine semen as well as its interstate movement and importation to and exportation from the U.S.A. This recommendation is in recognition of the facts that:

(a) More than half of dairy cattle are bred by AI and almost 3 percent of beef cattle are bred by AI, totalling more than 12,000,000 animal contracts per year. It is noted that a few bulls may each year produce semen used for as many as 75 to 100,000 inseminations in any or all of the 50 states extended over several years.

(b) The technology of semen freezing has made possible the preservation of bovine sperm as well as, potentially, pathogens for an indeterminate period of years before their deposition into heifers and cows.

(c) Voluntary procedures effectively followed by many but not all semen processors are no longer deemed adequate for the health security of the national cattle herds as developments increasingly attract the technologically upsophisticated into "custom freezing" of semen from bulls located on farms and ranches.

This Committee intends to supply a sub-committee to constructively assist U.S.D.A., as requested, in the formulation of proposed regulations for AI.

2. This Committee has considered the problem of the distressingly high incidence of bovine abortions seemingly associated with causes not clearly elucidated and urges renewed efforts and new funding for research on the causes of bovine abortions.
REPORT OF THE COMMITTEE

3. This Committee has considered and recommends to USAHA the establishment of a new and broadly based committee to study and develop a position paper for this organization dealing with livestock and poultry wastes and by-products as they involve human and animal health.

4. This Committee has considered and recognizes the need for renewed emphasis and continued funding for research upon the neo-natal diseases of cattle.

5. This Committee intends during the forthcoming year to give special consideration to losses resulting from infectious keratitis of the bovine.

6. This Committee after re-deliberation of the subject restates its conviction that new and comprehensive systems of cattle identification are fundamental to the diverse dairy and beef cattle improvement programs of this day and of the future and urges USAHA to assume leadership in encouraging research, development, and implementation of innovation methods and programs for cattle identification.

7. The Committee on Infectious Diseases of Cattle of the USAHA recommends a "position statement" on the use of antibiotics for livestock production. "There is no documentation evidencing human disease caused by drug resistant organisms which have resulted from antibiotics in animal feeds. Further, antibiotics play a vital role in livestock production and the curtailment of their use, all or in part, vitally affects livestock production particularly in the control of disease. Therefore, it is recommended that all antibiotics be allowed to be used in livestock production under proper supervision and with proper withdrawal period prior to slaughter if needed." The Committee recommends that this position statement when adapted by the USAHA be forwarded to the Food and Drug Administration.

REPORT OF SUBCOMMITTEE: 1969-70

The Subcommittee on AI of the Committee on Infectious Diseases of Cattle, USAHA, has given consideration to the multifaceted problem of the potential for disease transmission incident to artificial insemination of cattle and the unique problem of storage, frozen, of pathogens along with sperm cells for indeterminate periods of time, a few months to several years. A copy of this Committee's correspondence and the literature reviewed, etc., is attached. Additional copies can be made available.

This Subcommittee is of the opinion that it has been more than amply demonstrated that important infectious diseases of cattle can be, have been, and will be spread by artificial insemination, except when essential and effective preventive measures are scrupulously employed. It has been clearly demonstrated that most infectious agents survive the processes and procedures routinely employed in the preservation and storage of bovine semen. It follows, therefore, that it is in the interest of the dairy and beef cattle industries that bulls used as sources of semen be as free from the etiological agents of bovine diseases as is consistent with contemporary veterinary science, with the economics of the livestock industry, and the pragmatic realities of getting cattle pregnant by A.I. year after year.
While accepting that a potential exists for disease transmission through AI when irresponsibly implemented, it should be recognized that AI has and will continue to offer tremendous advantages over natural service in bovine disease control. With natural service, for breeding purposes, hundreds of thousands of bulls are moved country-wide between herds each year with all attendant risks in respect to disease. With AI, each bull can receive individual attention and be maintained under technically ideal conditions and rigid veterinary surveillance while replacing 500 to 5,000 bulls in natural service, which can receive only perfunctory consideration as to their disease status.

In 1970 in the USA 7,000,000 or 50% of dairy cattle and 1,000,000 or approximately 3% of beef cattle are being inseminated artificially. This means approximately 12,000,000 animal contacts by AI per year.

There is no regulation of interstate movement of bovine semen. Only 5 states: Mississippi, Montana, Virginia, Washington, and Wisconsin, presently regulate intrastate movement of bovine semen. Their individual regualtions are consistent with those recommended by USLSA in 1962. Only evidence of negative tests for tuberculosis and brucellosis (blood serum) are presently required by USDA for semen entering USA from foot and mouth disease-free countries.

This Committee recognizes that in the past, voluntary programs followed within the organized AI industry that were variously developed and recommended by AVMA, NAAB, and USLSA, and the regulations of the few regulating states, appear to have been quite effective in control of the diseases potentially associated with AI. However, it should be presently recognized that AI organizations are getting fewer and larger and that almost every AI organization distributes some semen country-wide – almost world wide.

Particularly as result of the rapid growth within the past decade of AI for breeding commercial beef cattle – from essentially zero to a rate of more than a million head per year – and, also, incident to the practice of speculatively proving young dairy sires through their early use in AI, the practice of on-the-farm and on-the-ranch collection of occasional ejaculates of semen from privately owned herd sires by itinerant “custom freezers” has become more common. Many AI organizations that maintain their own bulls and own premises concomitantly offer the service of “custom collection”, storage, and distribution of frozen semen from on-the-farm and on-the-ranch bulls. In such instances no responsibility is assumed for the health of the bull by the semen processor.

This Subcommittee is of the opinion that the time is rapidly passing when voluntary compliance with a Code, self-imposed by a majority of those in the business of collecting, processing, and storing semen, may rationally be considered adequate for the protection of the cattle population of the U.S.A. against transmission of diseases through AI.

Therefore, this Committee respectfully recommends to the Animal Health Division, Agricultural Research Service, U.S. Department of Agriculture, the early and comprehensive consideration of practical and sound regulations governing the collection, processing, storage, and distribution fo bovine semen, its interstate
movement, and importation to and exportation from the U.S.A.

Regulations should include especially the following considerations:

1. Individuals who collect, process, freeze, store, and/or distribute bovine semen should be known to USDA and/or veterinary officials of the indicated state. They should keep records and hold them available as to bulls and their owners, date and location of semen collections, quantity of ampules, semen quality as determined by customary laboratory examinations, and disposition of the semen.

   It should be recognized that individual collectors and processors of semen used in the breeding of purebred dairy cattle have for more than the past 20 years been licensed by the Purebred Dairy Cattle Association and that purebred beef associations variously require the keeping of records and the reporting of "semen transfers" to the respective association.

   Individuals engaged in the collecting and processing of semen should be adequately qualified as determined by USDA. In respect to matters of bull health, participation of a veterinarian with executive authority is an essential.

2. Ideally, bulls from which semen is collected for use in AI should be maintained on premises used exclusively for producing semen for use in AI and held separate from other livestock.

   Bulls, on-the-farm or on-the-ranch should be restricted from natural service, especially during periods when health tests are being made and semen is being collected.

3. Bovine semen is presently employed in artificial insemination in three forms: (1) processed to be held at 38°F in the liquid state and used within 2-4 days (2) processed to be held at ambient temperatures in the liquid state and used within a week (3) processed to be held at -320°F in the frozen state indefinintely and used over several years.

   In the USA 99% of bovine semen presently used interherd has been preserved by freezing.

   Any semen, especially if frozen, (a) which is intended to be transported interstate or, in the instance of those states regulating intrastate movement of semen, (b) which is intended to be used in a herd other than wherein collected, shall be so packaged as to be readily identifiable as semen qualified to be so moved and used. It is a reasonable assumption that a portion of all bovine semen that is preserved by freezing will be stored for several years, will be used in more than one herd, and will move interstate.

4. All semen packages should be distinctly and permanently marked so that the processor, bull, and date of collection can be determined. It is recognized that limitations of space may necessitate some use of codes.

   For semen used in insemination of purebred dairy and beef cattle from which offspring are to be registered the Purebred Dairy Cattle Association and the individual purebred beef associations have already prescribed such rules.
5. In the documentation of test and other health data for bulls whose semen is preserved by freezing, the essential principle should be recognized that testing and examination dates of the bulls and semen collection dates must be contemporaneous to be meaningful test and examination data. Conversely, it should be recognized that test and examination dates from same or different premises which post date collection of semen by several months or years have neither pertinence or meaning. Test and examination dates which relate to shipping dates of frozen semen several years old are less than meaningless. With frozen semen it should be recognized that the practical and highly desirable possibility exists of documenting the status of the health of a bull from which a given semen ejaculate originates both pre and post collection.

6. Because of the demonstrated ready survival in processed semen and/or transmissability through AI of the etiological agents of bovine tuberculosis, bovine paratuberculosis, bovine brucellosis, bovine venereal trichomoniasis, bovine genital vibriosis, and bovine leptospirosis, particular and precise consideration of these diseases is essential for bulls whose semen is used in AI. Frequency of testing bulls used in AI should be greater than for cattle under routine epizootiological surveillance because of the far greater frequency and the wider nature of the potential exposures from bulls in AI as compared with individuals in ordinary herds of cattle. Some bulls in AI in a year produce semen used in the insemination of in excess of 100,000 heifers and cows which may be located in any or all of the 50 states and several foreign countries.

For minimum security:
bovine tuberculosis requires a negative test ante dating first semen collection by not more than sixty days and semi-annually thereafter.
bovine paratuberculosis requires an annual negative fecal culture test.*
bovine brucellosis requires a negative blood serum and semen plasma test ante dating first semen collection and semi-annually thereafter. The relative lesser diagnostic significance of blood serum test results for brucellosis in bulls whose semen is used in AI should be recognized.
bovine venereal trichomoniasis requires a series of negative tests ante dating first semen collection and semi-annual tests thereafter.
bovine genital vibriosis requires a series of negative tests ante dating first semen collection and semi-annually thereafter.**

---

*It is urged that the counsel of the USAHA Committee on Tuberculosis and Paratuberculosis, and especially Dr. Audrey B. Larsen, NADL, be solicited in respect to adequate diagnostic procedures for paratuberculosis.
**It is urged that the counsel of the diagnostic laboratories of the States of New York and Wisconsin be solicited in respect to routine diagnostic procedures for Vibrio fetus var. venerealis.
bovine leptospirosis requires a serological test ante dating first semen collection and semi-annually thereafter.

7. As evidence of minimum fertility quality standards the collector and/or processor of bovine semen should be qualified to attest to and should record that the appearance of the penile and prepuce membrane is normal and healthy, that the preponderance of sperm cells are morphologically normal, and that the semen is essentially free of leucocytes. Per cent progressive motility of sperm cells pre and post freezing should be recorded. Antibiotics should be added to the semen at levels customary in the trade and their specific names and quantities recorded.

8. USDA's Agricultural Research Service should be cognizant of the existing limitations of knowledge in veterinary science as to diagnosis and maintenance of a disease-free population of bulls in respect to common viruses, i.e., bovine virus diarrhea, infectious bovine rhinotracheitis infectious bovine pustular vulvo-vaginitis, parainfluenza III and organisms of presently uncertain implication as Mycoplasma and Chlamydia. Solution oriented research, beyond the capabilities of the AI industry, is urgently needed.

9. USDA's import surveillance services should upgrade their regulations for semen entering the USA so as to achieve a health security level equivalent to that common to the AI industry of the USA, which respects and is consistent with the requirements of the five regulating states, and is consistent with the recommendations of the USAHA in respect to bovine semen used in AI. The potential for exotic virus, especially that of foot and mouth disease, to enter the USA in stored frozen semen or contaminated liquid nitrogen should be recognized by an administrative priority of the highest order of magnitude.

10. Scrupulous effort should be made to avoid unnecessary requirements and/or procedures or inclusion of disease considerations not established as pertinent in bovine AI. Specific requirements should be highly objective rather than subjective and include only those elements essential for the protection of bovine health, avoiding creation of trade barriers and national or organizational advantages or disadvantages.

11. Animal species other than bovine are outside the province of this Subcommittee on AI of the Committee on Infectious Diseases of Cattle, USAHA. Nevertheless, it is pointed out that both porcine and equine semen are being shipped in interstate commerce.
NEONATAL ISOERYTHROLYSIS OF THE BOVINE

J. S. Wilson, B.A. and J. C. Trace, D.V.M.*

Neonatal isoerythrolysis (N.I.) is a hemolytic anemia of the newborn — also known as immunohemolytic anemia. It is analogous to human erythroblastosis caused by the Rh blood factor. The hemolysis in the newborn animal is generally due to a maternal antibody being absorbed from the colostrum. These antibodies can be stimulated in the dam by blood antigens, feedstuffs1,2, microorganisms3, drugs4, or parasites5. Erythrocytes may also share common antigens with other cells or secretions of the body.1 Blood group antigens of one species may also be present in the tissue cells of an unrelated species. For example, the human erythrocyte antigen A is immunoantigenically related to human salivary antigen A, the somatic and serum antigen J of cattle, and the A antigen of the rabbit kidney epithelium.6

The pathogenesis of N.I. is bivalent, that is, genetic and environmental. For example, the dam produces an erythrocyte antibody following exposure to an antigen foreign to her own blood type. The calf must inherit this same blood type from its sire. The dam's antibody, which is concentrated in the colostrum, must then be absorbed into the blood stream of the nursing calf. The stage is then set for the antigen antibody reaction on the surface of the erythrocyte, resulting in its destruction and lysis.

Until recently N.I. was considered to occur in the various animal species with the notable exception of the bovine. Investigators have repeatedly failed to produce N.I. experimentally in cattle.7,8,9 They have successfully elicited erythrocyte antibody in the dams with injections of sire's blood. This antibody, in turn, was absorbed by the calf from the colostrum but produced no anemia. However, recently, N.I. has been diagnosed in cattle in Australia circumstantially implicating a piroplasmosis laden whole blood10 and in the U.S. with the anaplasmosis vaccine which is derived from blood.11 Death usually occurred by the third to the fifth day; rarely calves died as from shock within hours following the first nursing. Infrequently animals recovered without treatment.

There have been reports fitting this clinical syndrome in approximately 500 calves in some 60 U.S. herds during the past two year period. Most of the affected calves have been from Louisiana, Texas and adjoining states. Last year U.S.D.A. and A.R.S. personnel conducted a field investigation in 23 herds reporting early calf loss — averaging 10%. They diagnosed possible neonatal isoerythrolysis in about half of these herds and considered anaplasmosis vaccine as a potential contributing factor. The basis for this was the fact that all 23 herds had been vaccinated with anaplasmosis vaccine and the clinical pattern was virtually the same as N.I. in swine and horses and dogs in which vaccines of blood or tissue origin has been implicated.12,13

*From Research and Development, Fort Dodge Laboratories, Fort Dodge, Iowa 50501
REPORT OF THE COMMITTEE

MATERIALS AND METHODS

We conducted serological studies in 21 herds in which N.I. was clinically diagnosed with 2 normal herds serving as controls. Seventeen of the N.I. herds had received anaplasmosis vaccine and probably other vaccines as well, including leptospirosis. Four N.I. herds had been vaccinated with leptospirosis but not anaplasmosis vaccine. The tests and procedures used to aid in a diagnosis of N.I. were as follows:

Hemagglutination Test\(^{14}\)

The hemagglutination tests were performed using the dam's serum and the sire's red cells. The blood samples were centrifuged, serum withdrawn and inactivated for 30 minutes at 56\(^{\circ}\) C, then frozen at -60\(^{\circ}\) C, until used. The sire's erythrocytes were washed three times and resuspended to 2% in physiological saline. The test consists of adding .1 ml. of the dam's serum to .05 ml. of the sire's 2% red cell suspension in a 12 x 75 mm. test tube. A control for the test was .1 ml. of the saline and .05 ml. of the 2% sire's red cell suspension. These were mixed and allowed to incubate at room temperature for ten minutes. The tubes were centrifuged at 1,000 rpm for 1 minute. After centrifugation the tubes were gently shaken to dislodge the cell button. The degree of agglutination was judged macroscopically based on the number and size of the clumps. The reactions were classified from no agglutination (negative) to complete agglutination (4+).

Hemolytic Test\(^{9}\)

The hemolytic test was performed in a similar manner as the hemagglutination test. In addition to the .1 ml. of dam's serum and .05 ml. of a 2% red cell suspension of the sire, .05 ml. of complement (fresh rabbit serum) was added to complete the test. The controls used in this test included: (1) .1 ml. of saline, .05 ml. of a 2% suspension of the sire's red cells and .05 ml. of complement. (2) .15 ml. of saline, .05 ml. of a 2% suspension of the sire's red cells and no complement. The test was then allowed to incubate at room temperature with observations made at 30 minutes, 1\(\frac{1}{2}\) hours and 3 hours. The hemolysis of the red cells were graded from no lysis (negative) to complete lysis (4+).

Coombs Test\(^{15}\)

Direct: The direct Coombs test demonstrates the incomplete antibody on the surface of the calves' red cells. The calves' red cells were washed three to four times in saline and made into a 2% suspension. Two drops of the 2% suspension and 2 drops of goat or rabbit anti-bovine globulin\(^*\) were added to a 12 x 75 mm. tube. It was mixed and allowed to stand a few minutes. This was centrifuged for 15-30 seconds at 1,000 rpm. The tubes were gently shaken and the degree of agglutination was read macroscopically.

\(^*\)Colorado Serum and Microbiological Associates.
Indirect: The indirect test demonstrates the incomplete antibody in the dam's serum. The sire’s red cells were washed three times in saline and resuspended to 2% concentration. Two drops of the red cell suspension were added to two drops of the dam's serum. This was incubated for 1 hour at 37°C. The cells were then washed three times in saline and then suspended in one drop of saline. One drop of goat or rabbit anti-bovine globulin was added. It was mixed and allowed to stand a few minutes before centrifuging at 1,000 rpm for 30 minutes. The button was gently shaken and the degree of agglutination was read macroscopically.

Serological Results
An agglutinating antibody was detected in only 25% and 8% of the two vaccinated groups and not in either of the unvaccinated herds (Table I). The hemolytic antibody was not in evidence in the unvaccinated herd but the lysis incidence was 88% and 24% with the anaplasmosis and leptospirosis vaccines respectively.

The Coombs antibody was frequently observed in both vaccinated groups — 76% (direct) and 94% (indirect) in the anaplasmosis vaccinates and 50% (indirect) in the leptospirosis vaccinates. It presumably would not be present in the non-vaccinates, but was not checked.

DISCUSSION
Our serological findings in anaplasmosis vaccinated and unvaccinated herds is in general agreement with those of Dennis, et al\(^{11}\). Principal divergence was in the agglutination test results of the anaplasmosis vaccinated group. This can be explained by Dennis' addition of rabbit serum in the test procedure. This increases the sensitivity and would detect weak agglutinins. However, the N.I. antibody apparently is a hemolytic antibody and we conducted our agglutination test primarily for correlative interest. Most reliance for diagnosis of this non-specific antibody should be placed in the hemolytic test and the Coombs test.

Also of interest is Dennis' detection of a hemolytic antibody for more than 16 months after anaplasmosis vaccination. This was presumed to be persistent antibody. However, this would be the first such antibody to persist for such a long time. For example, C.F. antibody, elicited by anaplasmosis vaccine usually persists for 1-2 months — within the expected range for immunoglobulins. Van Der Walt and Osterhoff\(^ {16}\) produced hemolytic isoantibodies in 41 of 55 cattle by blood transfusions. The strong isohemolysins persisted for approximately 4 months., the weak hemolysins for only 2 months. Golemenov\(^ {17}\) detected natural isoantibody in newborn calves after consuming colostrum. It steadily declined and disappeared within 2 months. Therefore, perhaps Dennis' “persisting” antibody actually is a secondary antibody response to antigen from a different source. Reimmunization is the rule in women and could occur in certain pregnant cows via the uterus with non-intact fetal membranes. Secondary response, or even primary response, resulting in N.I. calves could be due to the variety of other non-vaccinal sources.
previously mentioned. A different vaccine, such as leptospirosis vaccine, might also be suspect.

Our laboratory results indicate that leptospirosis is capable of eliciting the incomplete Coombs antibody and the hemolytic antibody. Others have also reported this18,19,20. A component of the Leptospira cell causes hemolysis20 and leptospiral antibodies also play a role in the anemia19. Thus the hemolysis could be due to (1) leptospirosis infection, (2) leptospira antigen (3) media components of the vaccine, or (4) from the antibody response from vaccination. In several instances, calves one to five days of age diagnosed as typical N.I. have had high antibody titers to leptospirosis. From the information available, these calves, as well as all other N.I. calves were from dams which had been vaccinated repeatedly with leptospirosis vaccines.

In addition to Leptospira, other vaccines are replenished with sera, all of which could contain blood group antigens corresponding to the donor.6 Another disconcerting aspect is that cattle J. antigen may be detected on cells of a rabbit kidney cell line after as many as 200 subcultures.6 Kidney epithelial cells are still able to bind blood group antigen after 8 months of cultivation even though unable to grow and divide.21 Most tissue culture vaccines today are of primary culture rather than cell line and those that are of cell lines are likely much lower than the 200 passage level. Humans receiving vaccines and toxoids more often have detectable “erythrocyte” antibody than do other members of the population.6

Since the genetic aspect plays a vital role in N.I., an extensive study of blood types has been conducted in one N.I. herd. The objective was to hopefully incriminate a single blood antigen and subsequently avoid it accordingly. Dimmock and Bell10 identified 3 such antigens in matching sire:dam blood types and related this to two N.I. calves. However, our studies indicated that all blood types in 8 N.I. calves also occurred in 17 normal calves within the same herd. Both analyses may be valid since clinical erythroblastosis occurs in only 5% of the Rh incompatible parentages22.

An obvious question is, if N.I. has been produced experimentally in cattle or is not necessarily confined to anaplasmosis vaccinated cattle, why is it being diagnosed of late? Anaplasmosis vaccine has been available since 1965 and leptospirosis vaccines more than 10 years prior to that. There may be a number of factors involved other than misdiagnosis:

1. Breeding programs have changed.
   a. Inbreeding has become a rarity rather than commonplace — regardless of breed, different blood types introduced via bull increase chance of cow-calf blood incompatibility.
   b. Much greater frequency of artificial insemination and differing blood types from the cow’s — same results.
   c. Regular trend toward cross-breeding — same results.
   d. Influx of new “exotic” breeds mated to our English “domestic” breeds — same results.
2. Enhanced possibility of dam “immunization” with fetal (sire) incom-
patible blood (similar to Rh erythroblastosis in women).

a. Recent trend toward breeding heifers as yearlings instead of 2-year-olds could result in more dystocias and an imperfect placental barrier at next pregnancy.

b. Current trend of using large bulls to obtain bigger calves which frequently results in dystocias — same results.

c. Recent influx of cross breeding with “exotic” breeds resulting in larger calves — same results.

d. Pregnancy exams are now commonly performed, conceivable resulting in fetal blood spillage into maternal circulation.

This is not to imply that N.I. cannot result solely from anaplasmosis vaccine coupled with dam:sire blood type incompatibility. Experimental trials in progress may help clarify this point in one or two years. Regardless of the outcome, investigative studies should encompass the role that all other vaccines, feedstuffs, parasites and drugs might hold as a principal or contributing source of antigen.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Vaccination</th>
<th>Neonatal Isoerythrolysis</th>
<th>Hemagglutination</th>
<th>Hemolytic</th>
<th>Coombs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Direct</td>
<td>Indirect</td>
<td>Direct</td>
<td>Indirect</td>
</tr>
<tr>
<td>Anaplasmosis</td>
<td>Yes</td>
<td>25% (24/95)</td>
<td>88% (30/34)</td>
<td>76% (19/25)</td>
<td>94% (15/16)</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>Yes</td>
<td>8% (3/37)</td>
<td>24% (10/41)</td>
<td>100% (1/1)</td>
<td>50% (17/34)</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>No</td>
<td>0% (0/48)</td>
<td>0% (0/48)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
NEONATAL ISOERYTHOLYSIS

BIBLIOGRAPHY

22. Charles and Friedman, Rh Isoimmunization and Erythroblastosis Fetalis, Meredith, New York 1969, p. 3.
PROGRESS IN THE CONTROL OF BOVINE ANAPLASMOSIS

T. O. Roby, T. E. Amerault and B. R. McCallon
National Animal Parasite Laboratory
Veterinary Sciences Research Division
Agricultural Research Service
U.S. Department of Agriculture
Beltsville, Maryland 28705

Tetracycline antibiotics have been administered in different ways to cattle infected with *Anaplasma marginale* to control this hemotropic parasite. Since the testing and treatment approach for the control of bovine anaplasmosis is increasing in popularity among owners of infected herds, it is desirable at this time to review the previous reports on the most effective treatment methods. This review will be followed by a status report concerning results obtained from several trials now in progress.

REVIEW

Bearson et al. (1957) reported that the most effective treatment schedule for eliminating the carrier state of anaplasmosis was a daily dose of 5 mg/lb of tetracycline, given either intramuscularly or intravenously, for 10 consecutive days. Shorter periods of treatment were also effective in some cattle, but the authors indicated that borderline results could be expected when fewer than 10 daily inoculations were made. Among 10 Anaplasma-carrier cattle treated successfully, as shown by failure to transmit the parasite to splenectomized calves using blood, there was 1 that retained a positive complement-fixation (CF) reaction for 237 days post-treatment. While daily inoculation of anaplasmacidal drug is an optimal way to assure that the proper dose is given, this method of administration has the disadvantage of requiring the daily handling and inoculation of the cattle, and will not appeal to owner or veterinarian as a practical way for treating large groups of cattle.

Later, Brock et al. (1958) reported that chlortetracycline given orally at a dose level of 1.5, 2.5, or 5 mg/lb daily for 60 days would eliminate Anaplasma-carrier infections. In these experiments, the drug was in the form of the more economical Aureofac-10**. It was mixed with cottonseed meal so that each three pounds of meal contained the amount required for each animal daily. All of the cattle showed some anorexia and diarrhea during the first week after they were given the medicated feed. Inoculations of splenectomized calves using blood from the treated cattle were negative for Anaplasma at both 60 and 120 days following treatment.

Mention of products used in this work does not constitute endorsement by the U.S. Department of Agriculture.

*Dr. McCallon's address is Animal Health Division, ARS, F.C.B., U.S.D.A., Hyattsville, Md.

**American Cyanamid Company, Princeton, N.J. 08540.
The CF titers became negative within 8 weeks after the start of treatment. When the 5 mg/lb dose rate was applied under ranching conditions for 60 days to 18 CF-reactor cattle, 15 had a CF negative reaction 4 months after treatment, whereas 3 were still positive to the test. At the time this work was carried out, the cost of the drug was about $60.00 per head for adult cattle. This cost limited the use of the material for anaplasmosis. In a study using a shorter feeding period, Twiehaus (1962) reported that 26 CF reactor cattle were successfully treated by a 30-day feeding of chlortetracycline at 5 mg/lb/day. In this study, the drug, in the form of Aureomycin Crumbles,* was mixed with corn silage. There was no digestive upset reported as a result of the medicated feed. Twenty-one of the 26 cattle had CF-negative reactions when tested 35 days post-treatment. Blood from 1, which had a persistent 2-plus CF reaction at 10 months post-treatment, was inoculated into splenectomized calves with no evidence of A. marginale observed. The remainder of the herd was tested annually for the next 2 years and had CF-negative reactions on each test. In 1962, the Anaplasmosis Committee of the United States Livestock Sanitary Association recommended that infected cattle be given one of the tetracyclines in the feed at a rate of 3 to 5 mg/lb/day for 60 days. Some application of this method of control has been reported. For example, Ryff et al. (1964), in Wyoming, reported that an infected herd, which had a CF-reactor incidence of 3.5%, experienced very little new transmission after a two-year control program. They used routine vector control, sanitary surgical practices, and segregation of CF reactors and suspects from the CF-negative cattle. Also, the more valuable cattle, which had serologic evidence of anaplasmosis, were treated with chlortetracycline. The drug was fed in a pelleted ration so that each animal received 2.5 mg/lb/day for 60 days. In the group of 21 cows and bulls so treated, 8 were CF-negative and 11 were CF-positive when tested 5-8 months after treatment. Ryff et al. (1964) indicated that the treatment, along with the other control measures, reduced the losses from anaplasmosis in the herd. Other feeding studies have been carried out by Franklin et al. (1965). They reported that after infected cows were fed chlortetracycline for either 30 or 60 days at a rate of 5 mg/lb/day, the CF titers persisted for as long as 5 months after treatment. However, the blood from the cows was not infective when inoculated into test calves. In a later report, Franklin et al. (1966) observed that there was better agreement between CF and capillary agglutination (CA) tests when chlortetracycline was fed at a rate of 5 mg/lb for 60 days, than with lower rates for shorter periods. The cost of tetracyclines has decreased since the earlier reports; Garlick (1967) estimated that the cost for treating an adult cow at 5 mg/lb/day for 30 days was $7.50 to $9.00. When chlortetracycline is obtained in the form of AUROVET Crumbles* the cost is somewhat greater. However, in addition to being very palatable, this product will provide the high levels of drug required for anaplasmosis treatment. It is readily consumed by cattle and has not resulted in digestive disturbances.

The results reviewed thus far indicate that a high percentage of success can be

*American Cyanamid Company, Princeton, N.J. 08540.
expected in eliminating *A. marginale* from groups of cattle when tetracycline is fed at a rate of 5 mg/lb/day for 30 or 60 days. The rate of decline of serum antibody CF titers following drug treatment varied in different experiments. Factors which may influence the rate of antibody decline are (1) the stage of infection, (2) the level of drug which is administered, and (3) the length of time that effective blood levels of drug are maintained. One of the most apparent variables in feeding high levels of tetracycline to cattle on a free-choice basis is that of irregular drug intake. For consistent results, it is most important to feed a palatable form of the drug.

Amerault *et al.* (1968) reported that a herd of cattle in Arkansas had been fed Aureomycin-50 at 5 mg/lb in cottonseed meal for 30 days and that 2 of 10 splenectomized calves became infected after inoculation of blood from 10 of the treated cattle. The owner observed that one of the infected cows, which was not freed of *Anaplasma*, had been very reluctant to eat the medicated ration. Based on the reports in the literature, it was hoped that a 30-day feeding of 5 mg/lb of chlortetracycline would be uniformly effective in eliminating anaplasmosis. Data from several trials carried out in the past year are reported herein.

**CURRENT TESTING AND TREATMENT TRIALS**

The objective of these trials was to determine if bovine anaplasmosis could be successfully controlled under practical ranching conditions using the current diagnostic and treatment methods. The carrier state of *A. marginale* infection was determined principally by serologic means using the complement-fixation (CF) and the rapid card agglutination (CT) tests. When possible, confirmation of the serologic results were made by inoculation of blood from reactor animals into splenectomized calves.

1. Seven adult dairy cows were fed AUROVET Crumbles during the nonlactating period; each animal received 5 mg/lb/day of chlortetracycline for 30 days. The medicated ration was fed by the owner. All cows had positive reactions to the serologic tests. The presence of *A. marginale* in their blood stream had been confirmed by the inoculation of 50 ml. of citrated blood into splenectomized calves before treatment. The inoculation of blood into test calves was repeated 2 months after treatment to determine whether the parasite was still present. Table 1 lists the results obtained. Six cows did not harbor *A. marginale* in their blood stream following treatment, whereas all 7 had been infected before treatment. One cow remained infected. There was no indication of digestive disturbance from feeding the high level of tetracycline and all cows calved successfully. In 6 cows, the CT test was negative at the time of calf inoculation after treatment, whereas 5 still had CF reactions at this time. The cow that remained infected with *Anaplasma* following treatment could not be distinguished serologically from the 6 cows that were cured by treatment. The results indicated that complement-fixation tests will give unreliable results for several months following treatment because not enough time has elapsed for the serum antibody to disappear. Also, the results suggest that a 30-day feeding of chlortetracycline may not be a long enough period for
eliminating *Anaplasma* from all animals.

2. In 1958, the beef herd at the Iberia Livestock Station, Jeanerette, Louisiana, had a 28% reactor rate to the CF test for anaplasmosis. There were 630 animals in the herd at that time. Anaplasmosis is enzootic in this area and usually there are clinical cases of the disease each year. The herd was not tested again until January, 1969. At this time, both the CT and CF tests were conducted, and an incidence of 41% was found in 705 cattle. Reactors to both tests were fed tetracycline at 5 mg/lb/day for 30 days and all nonreactors were vaccinated with killed anaplasmosis vaccine (ANAPLAZ*) to help prevent clinical cases of the disease. When the herd was tested approximately 10 months after treatment and vaccination, 13% of the cattle reacted to both tests. These reactors were treated in the spring of 1970 and the herd will be retested later this year. Other herds in Louisiana have followed a similar program under the auspices of the State Livestock Health Officials. In some herds, the decrease in number of reactors has been similar to that observed in the herd at the Iberia Station, while in others the decrease was less marked. The reduced palatability of some of the locally mixed medicated rations has been a factor in the poor consumption of drug reported in some herds.

3. A large beef herd in Arkansas has been placed on a program of testing and treating reactors as revealed by the CT and CF tests. Two groups of test reactors have been fed AUROVET Crumbles; one received the medicated feed for 30 days and the second group received it for 45 days. The drug, which was readily consumed by the cattle, was given at a rate of 5 mg/lb/day. Table 2 shows the numbers of serologic reactors in each group before and after feeding the medicated ration. The retest was carried out 4 months following treatment. There were 37 CT and CF reactors in the 30-day feeding trial. When retested, 22 of these still showed some serologic reaction. In the 21 cattle that received the medicated ration for 45 days, there were 2 that remained reactors upon retest. It is anticipated that new reactors, which may develop from herd additions or from further transmission within the herd, will be treated with the medicated ration for a 45-day period. Clinical anaplasmosis has not occurred in the herd since the program was begun.

**SUMMARY**

The feeding of high levels of chlortetracycline (5 mg/lb/day) to cattle infected with *Anaplasma marginale* is an effective method for reducing the incidence and transmission of bovine anaplasmosis. A 45-day feeding period appears to be more effective than one conducted for 30 days. It is very important that the drug be mixed in a highly palatable feed supplement in order that all cattle obtain the needed medication by free-choice feeding. When chlortetracycline was fed to cattle in the form of AUROVET Crumbles no noticeable digestive disturbances were observed.

*Fort Dodge Laboratories, Fort Dodge, Iowa 50501.*
Table 1

Results of the *Anaplasma marginale* rapid card agglutination (CT) and complement-fixation (CF) tests and splenectomized calf inoculation tests on adult cows before and after treatment with AUROVET Crumbles.*

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>SEROLOGIC TESTS Before Treatment</th>
<th>2 mo. after Treatment</th>
<th>SPLENECTOMIZED CALF INOC. TEST Before Treatment</th>
<th>2 mo. after Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N.T. + 1:20</td>
<td>N.T. -</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+ + 1:20</td>
<td>- + 1:10</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+ + 1:5</td>
<td>- -</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+ + 1:20</td>
<td>- + 1:10</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+ + 1:40</td>
<td>- + 1:5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+ + 1:20</td>
<td>- + 1:5</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+ + 1:5</td>
<td>+ + 1:5</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

N.T. - not tested.

*Chlortetracycline 5mg/lb/day
Table 2

Number of cattle with serologic reactions to the *Anaplasma marginale* rapid card agglutinations (CT) and complement fixation (CF) tests before and after treatment with AUROVET Crumbles* for 30 and 45 days.

<table>
<thead>
<tr>
<th>Tests To Which Cattle Reacted</th>
<th>30 day treatment</th>
<th>45 day treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>4 mo. after</td>
</tr>
<tr>
<td>CT &amp; CF</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>CT</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>CF</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>22</td>
</tr>
</tbody>
</table>

*Chlortetracycline 5 mg/lb/day
ACKNOWLEDGEMENT

The authors gratefully acknowledge the cooperative support and assistance of the following Federal and State Livestock Sanitary Officials who have made this study possible: Dr. E. A. Schilf, ANH, ARS, USDA, Hyattsville, Md.; Dr. A. J. Roth, Assistant State Veterinarian, Richmond, Va.; Dr. Frank B. Wheeler, State Veterinarian, Baton Rouge, La.; and Dr. Paul C. Becton, ANH, ARS, USDA, Little Rock, Ark.

REFERENCES

CONTROL OF ANAPLASMOSIS UNDER WYOMING CONDITIONS

G. M. Thomas,* M. S., J. F. Ryff, D.V.M.,**
and H. A. Hancock,** D.V.M.

The problem of anaplasmosis has been of interest and concern to Wyoming for a number of years. A prevalence study in 1958 showed a rather high incidence of the disease. Studies which continued to 1965 indicated that anaplasmosis was widespread and was found in all counties in the state. The disease may be considered enzootic in at least one-half of the state, and moderate to slightly moderate in the remaining one-half, Figure 1. In Carbon county, located in the south central part of the state, 5000 cattle were CF tested representing 83 herds. Nine-hundred-eighty-three cattle (20%) from 58 herds (70%) reacted to the complement-fixation (CF) test. The owner of a Carbon county herd, because of clinical cases of anaplasmosis became interested in a control program in 1961. On CF testing of 940 cattle, 10 suspects and 33 reactor cattle (3.5%) were found. Subsequent annual CF tests performed indicated a minimum of spread when precautions were taken to preclude transfer by unsanitary techniques, practical arthropod control measures were carried out, and cattle reacting to the test were removed from the herd. Since several of the original reactor cattle were valuable, some were temporarily retained but segregated from anaplasmosis-free cattle during the summer months. Affected cattle were fed chlortetracycline, at the rate of 2.5 grams per animal daily for 60 days. Of 21 treated cattle, 7 became negative on the CF test within 5 to 8 months. At the time of this experiment there was very little information on lower level antibiotic feeding. It has been shown that the CA test is probably more accurate in determining the negative or carrier status of treated animals. In view of this more current information it is very probable that the carriers had been eliminated; however, they were handled as reactor animals.

With an annual CF test over the following and past 10 years, limiting additions to the herd of only CF negative cattle, no clinical cases of anaplasmosis have been observed. Yearly CF test results covering a period of 10 years are shown in Table 1.

The question may be raised as to whether or not there are wild game animals on the ranch which perhaps may serve as reservoirs of anaplasmosis. Both the mule deer (Odocoileus hemionus hemionus), and the pronghorn antelope (Antilocapra americana americana) can be seen on the ranch meadows and summer pasture lands throughout the year. Since the ranch is located near the Medicine Bow National Forest and Snowy Range Mountains, the deer population on the ranch is increased during periods of heavy snowfall which drive the deer to seek refuge at lower elevations.

**Wyoming State Veterinary Laboratory, Laramie, Wyoming.
The technical assistance of Mrs. Ella Nelson is greatly appreciated.
There are primarily two potential tick vectors of anaplasmosis in Wyoming, the Rocky Mountain woodtick, *Dermacentor andersoni*, and the winter tick, *Dermacentor albipictus* both of which are found in this area. *D. albipictus* is probably not extensively involved in the transmission of anaplasmosis since it is only active during the winter months, October to March. Clinically, bovine anaplasmosis can be seen in Wyoming from June to September. This perhaps corresponds to the period of greatest tick activity of the adult *D. andersoni*, which attacks its host in the spring (April to June) and to a lesser extent during the summer.

Numerous reports in literature indicate that there is definitely a host-parasite relationship between *A. marginale* and the black-tailed deer (*Odocoileus hemionus columbianus*) of California. A recent study reports the prevalence rate at 92% in adult deer. At the present time it appears that the Wyoming mule deer may be a less serious disease reservoir hazard than reported for California deer. The fact that this large herd of cattle has remained free of anaplasmosis in the midst of an enzootic area, and numerous deer suggests that either the deer do not harbor the disease in nature, or perhaps the difference is associated with the low transmission rate obtained with the Rocky Mountain wood tick in Wyoming. The apparent high transmission rate of Pacific Coast ticks probably accounts for the deer infection in California.

Other data collected in Wyoming supports the premise that the mule deer do not pose a threat in bringing anaplasmosis under control in Wyoming.

In Uinta county in the western part of the state, an earlier project revealed considerable background information. Uinta county had a 48.3% infection rate. The herd studied had a long history of anaplasmosis and clinical cases were commonly observed. On the initial herd test of all the approximately 2000 cattle, 51% infection was found. Seven species of ticks were collected in the area. *Dermacentor andersoni* was the most abundant and the only one normally infesting cattle. It was also found on a number of other animals including mule deer. Seventeen species of mosquitoes, horn flies, several species of horse flies and deer flies, and three other genera of blood sucking Diptera were collected in the experimental area. Under these conditions one would logically expect transfer of anaplasmosis between game animals and cattle.

At intervals over three years, mule deer were shot in the test areas. Samples were taken from deer for each of the calendar months in order that any transient seasonal infection might better be detected. An average of 143 ml. of blood from each deer was inoculated into a suitable observed splenectomized calf without demonstration of anaplasmosis. These calves were subsequently challenged and found susceptible. In addition, blood from a known anaplasmosis cow at the ranch handled similarly to the deer blood samples produced anaplasmosis when inoculated into a test calf. Of 14 deer used in this study, 2 reacted positively to the CF test, 8 were negative, 1 anticomplementary and 3 were not tested.
CONTROL OF ANAPLASMOSIS UNDER WYOMING CONDITIONS

Probably the most important and most practical considerations in bringing anaplasmosis under control in Wyoming and other Rocky Mountain states are: (1) the desire of the owner to want to do something about the disease; He should have an intelligent understanding of the various aspects of anaplasmosis; (2) prevent spread thru unsanitary ranching and veterinary procedures; (3) ectoparasite control, breaking this link in an infection chain and much will be accomplished in controlling the disease; (4) limit herd additions to cattle tested negative with one of the approved tests; (5) application of yearly tests — treatment of carrier animals, and/or removal of reactor animals; and last, there must be complete cooperation.

With these steps or procedures applied vigorously, a great deal can be accomplished in bringing anaplasmosis under control in Wyoming and other areas.

TABLE 1
Anaplasmosis Complement Fixation Test Results
Covering A Ten Year Period

<table>
<thead>
<tr>
<th>YEAR</th>
<th>NUMBER TESTED</th>
<th>REACTORS</th>
<th>SUSPECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1961</td>
<td>940</td>
<td>33</td>
<td>10</td>
</tr>
<tr>
<td>1962</td>
<td>1053</td>
<td>26</td>
<td>2</td>
</tr>
<tr>
<td>1963</td>
<td>1124</td>
<td>5*</td>
<td>15*</td>
</tr>
<tr>
<td>1964</td>
<td>1157</td>
<td>1**</td>
<td>2**</td>
</tr>
<tr>
<td>1965</td>
<td>1156</td>
<td>3**</td>
<td>1**</td>
</tr>
<tr>
<td>1966</td>
<td>23***</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1967</td>
<td>1082</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1968</td>
<td>882</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1969</td>
<td>916</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1970</td>
<td>945</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Reacting animals carried over from initial testing were disposed of.
** Reacting animals separated; tested negative on retest.
*** Aged animals 10-12 years which were sold. No complete herd test because of a change from late winter to spring testing of cattle.
REFERENCES

Fig. 1 - Anaplasmosis Survey

Wyoming

Number of cattle tested: 27,500
Number of heads tested: 900
REPORT OF THE COMMITTEE ON ANAPLASMOSIS

T. F. Zweigart, Raleigh, N.C., Chairman

Research:

State-Federal Regulatory:

Biologics and Pharmaceuticals:
J. C. Trace, Ft. Dodge, Iowa; Dreyfus Froe, Terre Haute, Ind.; Charles Phizer, Terre Haute, Ind.

Livestock Industry:

It is the consensus of the Committee that elimination of the infection should be the ultimate goal with Anaplasmosis. Before this becomes practical, certain tools must be available. These include a practical means of accurate diagnosis, an effective way to treat or dispose of reactors, and a method for eliminating or neutralizing reservoirs of infection. A premature attempt to free an area of the disease is undesirable in that disappointment with the results may make future attempts more difficult. Current restrictions on the interstate and international movement of cattle imposed because of Anaplasmosis, and the probability that more will follow, make the goal of eliminating Anaplasmosis on a herd, area and finally nationwide basis increasingly attractive.

Field trials conducted by various investigators over the past several years, and reported in the U.S.A.H.A. proceedings, indicate that infected herds in the South Atlantic and Gulf Coast States can be freed of Anaplasmosis and maintained free by employing currently used tests, plus adequate treatment of reactors with one of the tetracyclines. Other drugs for treating the disease are under development. Members of the Committee urge that research be continued in this area. The development of a safe, effective, one injection treatment that could be used with a rapid test, such as the Card Test, would solve many of our problems.

The Committee recognizes that the components of the Anaplasmosis problem may differ from area to area. With that in mind, a resolution supporting sponsorship by the U.S.D.A. of research hopefully leading to the development and maintenance of Anaplasmosis-free herds in several regions where the disease is endemic has been passed and sent to the Committee on Nominations, Resolutions and Internal Affairs.

The Committee believes that those owners who exert the effort necessary to develop and maintain Anaplasmosis-free herds should receive proper recognition. Accordingly, a sub-committee has been appointed to draw up a set of standards.
for Anaplasmosis-free herds. The sub-committee will report its findings at our 1971 meeting.

The problem of Isoerythrolysis in calves from cows vaccinated with an inactivated Anaplasmosis vaccine was discussed. It was emphasized that the condition is not confined to any particular breed of cattle. Recognizing that this type vaccine will continue to play an important role in reducing losses to owners of infected herds, the Committee urges that attempts be made to remove any undesirable antigenic material present from the vaccine.

Papers supporting the feasibility of establishing and maintaining Anaplasmosis-free herds were presented as follows:


They will be published in the proceedings.
PROGRESS OF THE STATE-FEDERAL BRUCELLOSIS ERADICATION PROGRAM

by
H. C. King, D.V.M.¹, E. A. Schilf, D.V.M.²

For several years we have been giving a report on the status of the Cooperative State-Federal Brucellosis Eradication Program. However, as there have been changes in program procedures to keep up with industry changes and new information, it may be well to change the title of the report as well. This year we will report on the progress of the program.

Progress is defined as moving forward or onward, a forward course; development or improvement; advance toward perfection or to a higher state. All of these definitions can be applied to the Cooperative State-Federal Brucellosis Eradication Program during fiscal year 1970. We did "move forward". As measured by the increased number of Certified Brucellosis Areas, we followed a "forward course of development." "Improvements" were made which will help advance the program to perfection — the eradication of brucellosis from the Nation. Some of the progress has been minimal and leaves much to be desired insofar as the rate of progress is concerned. We can expect that the rate of progress will depend on the total effort expended and the commitment of each of us that are involved in the program.

The progress can be measured in several ways. As an example, three States — Louisiana, Hawaii, and Nebraska — qualified as Modified Certified Brucellosis Areas. However, one State — Oklahoma — lost its status because of the failure of a few herd owners in one county to cooperate in followup herd tests. At the end of the fiscal year, there were four States — Florida, South Dakota, Texas, and Oklahoma — which had not achieved the intermediate goal of a modified certified status.

The attainment of a Certified Brucellosis-Free Area status by three States — Delaware, Virginia, West Virginia — and the reinstatement of Utah during the year is also an indicator of progress. Minnesota qualified since the end of the fiscal year so currently 20 complete States plus the Virgin Islands are Certified Brucellosis-Free. (Figure 1)

Progress in qualifying the remaining noncertified counties was satisfactory in most areas. In Florida legal action caused a long delay in progress; however, favorable court decisions have permitted the activity to again get underway. Since reactivation of the program, six counties have achieved modified certified status and only three counties remain noncertified at this time.

¹ Chief Staff Veterinarian, Brucellosis Eradication, Cattle Diseases, Animal Health Division, Agricultural Research Service, United States Department of Agriculture, Hyattsville, Maryland.
² Senior Staff Veterinarian, Cattle Diseases, Animal Health Division, Agricultural Research Service, United States Department of Agriculture, Hyattsville, Maryland.
Thirty-five counties qualified in Texas, seven in South Dakota, six in Nebraska, 20 in Louisiana, and one in Hawaii for a total of 76 counties reaching a Modified Certified Brucellosis Area status during the fiscal year. However, 10 counties in four States lost modified certified status during the year because of failure to adhere to the provisions of the Uniform Methods and Rules. At the end of fiscal year 1970, there were 1,606 modified certified brucellosis counties in 27 states.

Efforts to qualify counties as certified free were successful in 173 counties. This is not as good as the 195 which qualified in 1969. A total of 1,498 counties had reached a free status at the end of fiscal year 1970. At that time, over 98 percent of all counties were certified with over 47 percent certified free. Currently this is over 48 percent. With the exception of three counties in Texas, all remaining counties are conducting area work. (Figure 2)

Another measurement of progress is the number of infected herds found in relation to the certification status of the states. There has been a decline each year since 1966 in the total number of infected herds found. Last year 15,390 infected herds were found with almost 60 percent of these found in the four noncertified States. There was an encouraging decrease in the number found in modified certified areas recognizing that among the new certified States were some with very high initial infection. In the certified free States, only 184 herds were found with reactors — an average of less than 10 infected herds per State. Eleven of the 19 free States had five or less infected herds during the year. (Figure 2)

One State — Texas — in which an all-out eradication program is now underway accounted for over 45 percent of all infected herds found. Another three States — Louisiana, Mississippi, Oklahoma — accounted for over 24 percent. Nearly 70 percent of the infected herds were found in four contiguous States. In spite of a diligent effort to locate infected herds in these three modified certified States, the total infected herds there decreased by almost 40 percent compared with fiscal year 1969.

Fourteen States accounted for less than 11 percent of the infected herds and 28 States for 2.0 percent of the infected herds. (Figure 4)

Total blood tests conducted declined for the second consecutive year from 10.8 million in 1969 to 10.3 million in 1970. This decline was primarily in cattle tested on farms and ranches with the number tested by MCT remaining at about the 5 million level.

The total number of reactors found dropped below 120,000 for the first time since the inception of the brucellosis eradication program in 1935. This is significant, particularly in view of the concentration on testing herds most likely to be infected. This is an indication of real progress. In the last 2 years, the number of reactors found has been reduced by approximately 20 percent.

The market cattle testing program is one of the two critically important programs for detecting brucellosis-infected herds. Because of the reliance that has been placed on the procedure, the coverage of the population must be at a sufficient level to provide a high probability of identifying infected herds. Unfortunately, this level is yet to be attained. Coverage under the MCT program has
not increased during the past 5 years. Total blood samples collected at slaughter plants and markets from cows remains under 5 million. Less than 3 million of these are collected at packing plants which represents less than one-third of all of the cows slaughtered.

Over 10,000 herd tests were made as a result of finding 25,620 MCT reactors. Of these, 3,662 herds were infected containing 21,860 reactors on the initial test. This is 13.3 percent of the cattle in the infected herds. This rate of infection is almost four times that of dairy herds found infected as a result of followup blood tests of BRT and indicates a failure of the MCT program to locate infected herds until the infection has spread considerably within the herd, further indication of inadequate coverage by the MCT program. It is vitally essential that the MCT program be expanded if it is to serve the purpose for which it was designed. (Figure 5)

The BRT program has proven to be the single most important program procedure for locating infected dairy herds. The test is conducted at least three times a year with a majority of States conducting it four times a year. Last year over 1.25 million ring tests were conducted with about 0.4 percent being suspicious for the third consecutive year.

There were almost 5,500 suspicious BRT's and, as a result, 4,384 initial followup herd tests were made. Of these, 1,107 or 25 percent of these herds were found to be infected. Last year about one in five followup herd tests were found to be infected. (Figure 6)

The importance of maintaining close supervision over the BRT procedures was again demonstrated this past year. A new method of determining the butterfat content of milk has been developed and is being adopted by many States in place of the older Babcock test. This is the "milko-tester" which is a turbidmetric method of measuring the butterfat content of milk. The preservative recommended by the company manufacturing the equipment is potassium dichromate, which when used at the recommended concentrations, may result in false negative tests. Unless a preservative satisfactory to the dairy processing industry using this equipment can be found which will not interfere with obtaining accurate BRT results, it will be necessary to use only fresh milk samples.

Calf vaccinations declined by almost 480,000 during the past year — the sixth consecutive year of decline. The total 4,842,120 vaccinations compares with the 1964 peak of 7,255,030. (Figure 7)

There continues to be little relationship between the need for continued vaccinations and its use. Over 26 percent of the calves vaccinated last year were in Certified Brucellosis-Free States. Vaccination in five of these States increased during the year. Continued use of a live vaccine in areas free or relatively free of brucellosis is not compatible with the goal of final eradication.

Unfortunately, pressures of other programs and lack of personnel allowed little effort in the swine eradication program. This is most unfortunate because if the goal of eradication by 1978 is to be reached, the swine program must be greatly expanded without further delay.
The number of swine tested was approximately 1,000 less than last year. The percent of infected lots fell to 1.1 percent. (Figure 8)

There was little change in areas validated — an increase of six from 183 to 189 counties. California has only one county remaining not yet validated.

Too frequently we consider only the benefits brucellosis eradication has had for the livestock producer. Here is a disease which has been a serious public health problem causing unfortunate mental and physical suffering. The progress in eradicating the disease from livestock is reflected in the reduction of human cases reported. Currently most cases can be traced to exposure to infected swine. (Figure 9)

Work projections for 1970 had indicated there would be a total of 3,085 certified counties at the end of the year — 1,534 certified free and 1,541 modified certified. The total certified counties was exceeded by 19 but at the expense of obtaining free counties which fell 36 short of the goal.

Projecting this rate of progress that has been made during recent years indicates that eradication can be achieved by 1978. An all-out effort without diversion of resources could shorten this time. The tools, procedures, trained personnel, legal means, and cooperation are available. Now is the time to apply all of our know-how and finish the program while support from the industry and financial support from the State and Federal legislature is still available. (Figure 10)
Figure 1.

BRUCELLOSIS ERADICATION PROGRAM
CERTIFIED AREAS
JUNE 30, 1970

Figure 2.

*Brucellosis Eradication*

COUNTY CERTIFICATION STATUS

<table>
<thead>
<tr>
<th></th>
<th>June 30, 1969</th>
<th></th>
<th>June 30, 1970</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certified Free</td>
<td>3.4%</td>
<td>Area Work in Progress</td>
<td>0.3%</td>
</tr>
<tr>
<td>Modified Certified</td>
<td>42.0%</td>
<td>Individual Herd Participation</td>
<td>54.3%</td>
</tr>
<tr>
<td>Percent of U.S. Counties</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

U.S. DEPARTMENT OF AGRICULTURE  ANIMAL HEALTH DIVISION  AGRICULTURAL RESEARCH SERVICE
Figure 3.

**BRUCELLOSIS INFECTED HERDS FOUND**

*In Noncertified, Modified Certified and Certified - Free States*

**Number Infected Herds**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Certified-Free</td>
<td>15,049</td>
<td>8,441</td>
<td>9,267</td>
<td>14,391</td>
<td>14,254</td>
<td>10,247</td>
<td>6,027</td>
<td>104</td>
</tr>
<tr>
<td>Modified Certified</td>
<td>111</td>
<td>52</td>
<td>101</td>
<td>157</td>
<td>152</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncertified</td>
<td>9,820</td>
<td>9,743</td>
<td>6,268</td>
<td>8,136</td>
<td>7,671</td>
<td>152</td>
<td>152</td>
<td>104</td>
</tr>
</tbody>
</table>

**Fiscal Year**

- 1964: 14
- 1965: 32
- 1966: 7
- 1967: 9
- 1968: 10
- 1969: 13
- 1970: 19
- 1971: 19

U.S. Department of Agriculture
Animal Health Division
Agricultural Research Service
Figure 4.

DISTRIBUTION OF BRUCELLOSIS REACTOR HERDS

Percent of Total Reactor Herds Found

- 28 STATES, <30 HERDS: 2.0%
- 14 STATES, 30<300 HERDS: 10.8%
- 6 STATES, 300<1,000 HERDS: 17.5%
- 3 STATES, 1,000<3,000 HERDS: 24.6%
- 1 STATE, >3,000 HERDS: 45.1%

FISCAL YEAR 1970

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
Figure 5.

Brucellosis Eradication

**BLOOD TESTING: CATTLE**

**MILLIONS CATTLE TESTED**

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Farm or Ranch</th>
<th>MCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1966-67</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td>1967-68</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>1968-69</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>1969-70</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>1970-71</td>
<td>10.3</td>
<td></td>
</tr>
</tbody>
</table>

**THOUS. REACTORS FOUND**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>121</td>
<td>147</td>
<td>149</td>
<td>130</td>
<td>119</td>
</tr>
</tbody>
</table>

**FISCAL YEAR**

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
Figure 6.

Brucellosis Eradication

Milk Ring Testing:
% Suspicious Tests

27.0%

0.4% (1970)

Fiscal Year

Percent of Total Herd Tests

U.S. Department of Agriculture
Animal Health Division
Agricultural Research Service
Figure 7.

**Brucellosis Eradication**

**CALVES VACCINATED**

MILLION CALVES VACCINATED

![Graph showing the number of calves vaccinated from 1953 to 1971.]

FISCAL YEAR

- **1953** - 0
- **1955** - 1
- **1957** - 2
- **1959** - 3
- **1961** - 4
- **1963** - 5
- **1965** - 6
- **1967** - 7
- **1969** - 6
- **1971** - 5

Figure 8.

**Brucellosis Eradication**

**BLOOD TESTING: SWINE**

**HERDS-LOTS TESTED**

<table>
<thead>
<tr>
<th>Year</th>
<th>Herds-Lots Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1966</td>
<td>59,328</td>
</tr>
<tr>
<td>1967</td>
<td>61,372</td>
</tr>
<tr>
<td>1968</td>
<td>75,760</td>
</tr>
<tr>
<td>1969</td>
<td>60,904</td>
</tr>
<tr>
<td>1970</td>
<td>59,899</td>
</tr>
<tr>
<td>1971</td>
<td></td>
</tr>
</tbody>
</table>

**INFECTED HERDS-LOTS**

<table>
<thead>
<tr>
<th>Year</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1966</td>
<td>3.1%</td>
</tr>
<tr>
<td>1967</td>
<td>2.4%</td>
</tr>
<tr>
<td>1968</td>
<td>1.8%</td>
</tr>
<tr>
<td>1969</td>
<td>1.3%</td>
</tr>
<tr>
<td>1970</td>
<td>1.1%</td>
</tr>
<tr>
<td>1971</td>
<td></td>
</tr>
</tbody>
</table>
Figure 9.

**BRUCELLOSIS**

*Human Cases-Certified Counties*

<table>
<thead>
<tr>
<th>YEAR</th>
<th>1953</th>
<th>'55</th>
<th>'57</th>
<th>'59</th>
<th>'61</th>
<th>'63</th>
<th>'65</th>
<th>'67</th>
<th>'69</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUMBER (THOUS.)</td>
<td>2.5</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

- **Human cases**
- **Certified counties**

U.S. DEPARTMENT OF AGRICULTURE  
ANIMAL HEALTH DIVISION  
AGRICULTURAL RESEARCH SERVICE

Figure 10.

**BRUCELLOSIS ERADICATION GOALS**

*Status of County Certification*

| FISCAL YEAR | 1960-61 | '62-'63 | '64-'65 | '66-'67 | '68-'69 | '70-'71 | '72-'73 | '74-'75 | '76-'77 | '78-
<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>CERTIFIED COUNTRIES</td>
<td>22</td>
<td>62</td>
<td>110</td>
<td>118</td>
<td>124</td>
<td>127</td>
<td>130</td>
<td>135</td>
<td>140</td>
<td>145</td>
</tr>
<tr>
<td>CERTIFIED FREE</td>
<td>104</td>
<td>106</td>
<td>110</td>
<td>112</td>
<td>114</td>
<td>116</td>
<td>118</td>
<td>120</td>
<td>122</td>
<td>124</td>
</tr>
<tr>
<td>MODIFIED CERTIFIED</td>
<td>110</td>
<td>112</td>
<td>114</td>
<td>116</td>
<td>118</td>
<td>120</td>
<td>122</td>
<td>124</td>
<td>126</td>
<td>128</td>
</tr>
<tr>
<td>NOT-CERTIFIED</td>
<td>1,153</td>
<td>1,200</td>
<td>1,250</td>
<td>1,300</td>
<td>1,350</td>
<td>1,400</td>
<td>1,450</td>
<td>1,500</td>
<td>1,550</td>
<td>1,600</td>
</tr>
<tr>
<td>AREA TESTING</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>
</tr>
<tr>
<td>INDIVIDUAL HERD PARTICIPATION</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>
</tr>
</tbody>
</table>

U.S. DEPARTMENT OF AGRICULTURE  
ANIMAL HEALTH DIVISION  
AGRICULTURAL RESEARCH SERVICE

Total Counties in U.S. = 3,153
STUDIES ON CATTLE VACCINATED WITH LIVE STRAIN 19 AND/OR 45/20 ADJUVANT BRUCELLA ABORTUS VACCINES

by

J. B. Hendricks* and W. C. Ray**

INTRODUCTION

Interest was renewed in Brucella abortus Strain 45/20 as a vaccine against bovine brucellosis after satisfactory immunity in guinea pigs was demonstrated using a preparation of killed organisms in an adjuvant. Subsequent studies in cattle indicated that the level of protection provided by two doses of bacterin, administered at intervals of 6 to 12 weeks was comparable to that elicited by vaccination with Strain 19. In a recent review on adjuvant vaccines prepared from killed Strain 45/20 organisms, Morgan and McDiarmid concluded that the effectiveness of such products as judged by serologic, immunologic and local tissue responses was largely dependent upon the nature of the adjuvant. However, Roerink showed that the method of cultivating Strain 45/20 cells can influence the serologic response of an adjuvant vaccine. Other factors, such as the age of the animal at time of vaccination and the size of the challenge dose, may also influence the level of resistance.

In 1969, the Subcommittee on Research summarized the experimental results of 45/20 bacterins for the National Brucellosis Committee and concluded that further knowledge was required. The Subcommittee indicated that this knowledge should include information on the duration of protection afforded by 45/20 adjuvant vaccines, the effect of annual vaccination on immunologic and serologic responses, and the factors, other than adjuvants, which may be responsible for the inconsistent immunologic responses in cattle.

Roerink indicated that the administration of killed 45/20 cells in adjuvant (Duphavac NA†) to cattle previously vaccinated with strain 19 simulated greater serologic responses than it did when administered to cattle not vaccinated previously with Strain 19. Therefore, it seemed important to determine the effect of 45/20 bacterins on the serologic diagnostic procedures used in the Cooperative State-Federal Brucellosis Eradication Program.

This paper presents the results of a study conducted to gain information on the responses in cattle given 45/20 bacterins in adjuvants particularly with regard to:

A. Serologic responses of adult cattle previously vaccinated as calves with Strain 19;

*Brucellosis Epidemiologist, Animal Health Division, Agricultural Research Service, United States Department of Agriculture, Columbia, South Carolina.

**Chief Staff Veterinarian, Brucellosis Epidemiology, Cattle Diseases, Animal Health Division, Agricultural Research Service, United States Department of Agriculture, Hyattsville, Maryland.

†Trade name – N. V. Phillips-Duphar, Weesp, Netherlands
B. Serologic responses of adult cattle having had no previous vaccination against brucellosis;
C. Degree of local tissue reactions following administration of 45/20 bacterins;
D. Duration of local tissue reactions following administration of 45/20 bacterins; and
E. Protection afforded by 45/20 bacterins in adult cattle challenged 14 months after the administration of one annual booster.

SELECTION AND TREATMENT OF GROUPS
In June 1967, 72 three-to-four-year-old cows in their second pregnancy were purchased from herds in North and South Carolina. Records were available to verify that 36 cows had been officially vaccinated as calves with *Brucella abortus* Strain 19. The remaining 36 cows had not been vaccinated against brucellosis. Holstein, Guernsey, Jersey, and Brown Swiss breeds were represented in this study.

The vaccinated and nonvaccinated animals were divided into groups utilizing a randomized block design. Within each set of 36 animals, 12 blocks, each consisting of three animals, were formed. The animals within each block were as homogeneous as possible based on breed, tube agglutination test titer, and size (weight). After the blocks had been established, assignment to the three treatment groups; namely, Duphavac NA, Bortin 45*, and Controls, was done by random selection using a table of random numbers. This design resulted in the following six treatment groups:

**Group 1**
Twelve cows officially vaccinated as calves with Strain 19: Each animal was given a 2 ml. dose of Duphavac NA intramuscularly according to recommended procedures on July 18, 1967. The second dose (2 ml.) was injected intramuscularly on the opposite side of the neck 10 weeks later. Fourteen months after the second dose, a booster (2 ml.) was given intramuscularly on the same side of the neck as the first dose.

**Group 2**
Twelve cows officially vaccinated as calves with Strain 19: Each animal was given a 2 ml. dose of Bortin 45 subcutaneously according to recommended procedures on July 18, 1967. The second dose and a booster of Bortin 45 were given at the same intervals as in Group 1. The first and second doses were given on alternate sides of the neck, but the third dose was injected high on the chest behind the right shoulder.

**Group 3**
Twelve cows officially vaccinated as calves with Strain 19: This group served as Strain 19-vaccinated controls.

**Group 4**
Twelve cows that had not been vaccinated against brucellosis: Each animal was

*Trade name of 45/20 bacterin produced by Glaxo Laboratories, Greenford, Middlesex, England
given Duphavac NA as indicated for animals in Group 1.

Group 5 –
Twelve cows that had not been vaccinated against brucellosis: Each animal was
given Bortin 45 as indicated for animals in Group 2.

Group 6 –
Twelve cows that had not been vaccinated against brucellosis: This group
served as nonvaccinated controls.
The cows mingled freely on pasture after vaccination until 2 weeks before
challenge with virulent Brucella abortus Strain 2308.

I. POST VACCINATION SEROLOGIC RESPONSES

Blood samples were collected from each cow at weekly intervals beginning 2
weeks before the first injection of 45/20 bacterin. Twelve weeks following the
second vaccine injection, the interval was extended to 4 weeks. Blood sampling was
continued at 4-week intervals until the annual booster dose was administered. After
the annual booster, blood samples were again collected at weekly intervals for 12
consecutive weeks, then at 4-week intervals until the cows were challenged with virulent Brucella abortus Strain 2308.

Serum harvested from each blood sample was tested for brucella antibody
using the standard tube agglutination test (STT) at dilutions of 1:25, 1:50, 1:100,
and 1:200; the acidified plate antigen test (APA) at pH 3.75 and 3.0; the brucellosis
card test (card); the rivanol precipitation plate agglutination test (RIV) at dilutions
of 1:25, 1:50, 1:100, and 1:200; and the complement fixation test (CF) at
dilutions of 1:10, 1:20, 1:40 and 1:80 according to previously described
methods\textsuperscript{7,12}. Suspects and reactors to the STT were classified in accordance with
the Brucellosis Eradication Recommended Uniform Methods and Rules\textsuperscript{1}. Animals
vaccinated with 45/20 bacterins, but not with Strain 19 vaccine, were considered as
nonvaccinates for classification purposes because of the reported nonagglutinogenic
properties of this strain. Positive classification for the other serologic tests were:
APA – agglutination at pH 3.75; card – macroscopic clumping after 4 minutes of
continuous rocking; RIV – complete agglutination at the 1:25 dilution; CF – 2+ at
the 1:20 dilution for cattle not vaccinated with Strain 19 and 2+ at the 1:40
dilution for cattle previously vaccinated as calves with Strain 19.

Results

Although there were differences in the number of cows which became positive
to the various serologic tests, both Duphavac NA and Bortin 45 stimulated the
production of agglutinating and complement fixing antibodies (Table 1). Animals
that had been vaccinated as calves with Strain 19 usually developed higher titers
than the cows that were given 45/20 bacterins only.

In general, Duphavac NA stimulated greater serologic responses than Bortin 45,
particularly in cattle vaccinated as calves with Strain 19. This included the
production of significant levels of complement fixing antibody.

The duration of the serologic responses following the administration of
Duphavac NA and Bortin 45 are shown in Table 2. These data indicate that Duphavac NA stimulated production of both agglutinating and complement fixing antibodies over a longer period of time than Bortin 45. However, most of the positive serologic reactions were transient, with many receding or having receded within 3 months after the bacterins were administered. Five months after the bacterins were given, there was no apparent difference in the serologic responses between the groups treated with Duphavac NA and Bortin 45. Comparison of the serologic responses for cattle vaccinated as calves with Strain 19 showed that there were three suspects to the STT and four positive to the CF test with Duphavac NA and no diagnostic titers in the comparable Bortin 45 group 10 weeks after the second dose. At 20 weeks after the second dose of bacterin, there was still one suspect to the STT and two positive to the CF in the Duphavac NA group and no diagnostic titers in the Bortin 45 group. A greater number of cows vaccinated as calves with Strain 19 had reactions to the agglutination, but not to the complement fixation test, 10 weeks after the annual booster than was found 10 weeks after the second dose. This was particularly noticable with Bortin 45 because of the greater differences in duration following the second and annual booster doses.

Comparing the serologic responses for cattle without previous experience with Strain 19 vaccine showed that most of the positive reactions disappeared by the 10th week after the second dose, but suspect titers to the STT persisted longer than 10 weeks after the annual booster. At 20 weeks after the annual booster, there were still two suspects to the STT in each bacterin group.

From the 20th week to the 58th week following the second dose, there was little difference between the two bacterin groups in the persistence of the titers regardless of vaccination history. Only two animals in the Duphavac NA-Strain 19 group continued to have significant serologic reactions. One animal was a suspect to the STT at 6 months and the other was positive to the card test until the 7th month and positive to the APA test until the 10th month. Only two animals in the Bortin 45-Strain 19 group continued to have significant serologic reactions. One animal was a suspect and positive to the APA test until the 11th month but positive to the card test only until the 9th month. The other animal was positive on the APA test until the 7th month. In cattle not previously vaccinated with Strain 19, the only serologic reactions that were significant after the 5th month were in the Bortin 45 group and in the Control group. One cow in the Bortin 45 group was positive to both the APA and card tests 12 months after the second dose. One control animal was suspicious (+50) to the STT for 5 months. Two other animals had titers to the STT of incomplete at 1:50 on one and two test dates respectively during the same period of time.

Discussion

The duration of the serologic response following administration of 45/2C bacterins was one of the primary interests in this study. Based upon the reports of Roerink and others4, 16, persistent reactions were expected to the various supplemental tests in a relatively high percentage of the Strain 19 vaccinated cattle
and low level response in the unvaccinated cattle.

The results of this study differed from those of Roerink and Gue in that most positive titers receded more rapidly in this study. Although some tests were still positive after 12 months in this study, Gue reported that four of twelve heifers given two doses of Duphavac NA retained suspect or reactor STT titers for at least 48 weeks. Roerink reported that 19 percent (15 out of 79) of a group of cattle vaccinated with Strain 19 as calves still were positive 12 to 14 months after injection with Duphavac NA. A slightly higher percentage (22.8 percent or 18 out of 79) were still suspects or reactors on the CF test after the same interval of time. The differences in duration of the serologic response as observed among these investigations may be more apparent than real due to sample variation. However, Cunningham and O’Reilly reported that only two of 54 cattle of various ages developed titers exceeding 30 international units after administration of two doses of 45/20 bacterin.

II. LOCAL TISSUE REACTIONS

Little or no visible local reaction was observed following the intramuscular injection of Duphavac NA. Only three animals vaccinated with this bacterin retained palpable evidence at the site of inoculation 9 months or more after the injection. Two of these animals had small nodules less than one-half inch in diameter, but the reaction of the other animal was considerably larger (16.4 x 9.5 cm.). The number of animals with swellings after injection with bacterins are shown in Table 3.

At the termination of the study, the cattle were slaughtered and the three injection sites on each animal were palpated and incised. There was no evidence of persistent tissue damage or change. These observations were made approximately 36 months after the first dose and 20 months after the annual booster.

Conversely, the administration of Bortin 45 subcutaneously in the neck region or immediately posterior to the shoulder produced local tissue reactions which persisted indefinitely in many animals. The size and persistence of these swellings created an undesirable appearance which would be objectionable to many livestock owners. There was little or no difference in the local tissue reaction in cows which were officially vaccinated and those which were not vaccinated as calves with Strain 19. The reactions were variable, ranging from 1.5 x 1 cm. to 22.0 x 24.0 cm. in size.

At the time of slaughter, the animals which had been vaccinated with Bortin 45 were examined for evidence of tissue response at the three sites of injection. Most cows had granulomatus-type lesions. The size of the lesions varied widely but were large enough to be readily located. The lesions were in the subcutaneous tissue and normally could be trimmed off with the hide without damaging or causing an objectionable blemish to the musculature.

Discussion

In contrast to the observed results of this study when 45/20 bacterin (different serial lot of Duphavac NA) was administered to 67 cattle in a field study, several
cows developed large edematous swellings at the site of the second injection and some became severely lame. Cunningham also observed large edematous swellings with some lameness in his study with 45/20 bacterins. In parallel with Cunningham's observations, a granulomatous lesion was found in the muscle at the site of injection of Duphavac NA when a reactor cow in the field trial was slaughtered. The time lapse between the second injection of bacterin and slaughter was 85 days.

Cunningham suggests that the route of inoculation determines the nature of tissue response following administration of Duphavac NA. MacIntyre and Sizer support the conclusion of the importance of the route of administration and also suggest that the site at which subcutaneous injections of Bortin 45 are given can influence theacceptibility of the tissue response. Cunningham cautioned against allowing leakage of bacterin into subcutaneous tissue if tissue reaction is to be avoided. The explanation for large edematous swellings in the field trial after the second intramuscular injection of Duphavac NA may have been caused by seepage of bacterins into subcutaneous tissue, since no special effort was taken to prevent this possibility. Another factor which might account for this occurrence was that both doses were given on the same side of the neck, contrary to the procedures followed in other experiments.

III. IMMUNOLOGIC RESPONSES

Approximately 14 months following the third injection (or "annual booster"), all of the cattle were randomly assigned to individual 6 feet by 9 feet box stalls in two barns. The barns were of shed-type construction which allowed for maximum ventilation. The solid partitions between stalls were approximately 7 feet high.

On January 35, each animal was exposed as previously described by inoculating approximately 7.5 x 10⁵ viable organisms of Brucella abortus Strain 2308 into the conjunctival sacs of each cow - one-half of the dose into each conjunctival sac.

At the time of exposure, most of the cattle were near the middle of their fourth gestation period. Both the pregnant and non-pregnant cows were included in this final phase of the experiment. During the 2½ years between original purchase and exposure, there were several deaths due to natural causes which resulted in a reduction of the original 12 cows in some groups. The following is the number of pregnant cows over the total number of cows in each of the six groups on the date of exposure:

1. 9/12 4. 8/11
2. 7/11 5. 9/10
3. 8/10 6. 10/10

Within 12 hours after parturition, uterine fluid and quarter milk samples were collected from each animal. Each aborted fetus or full-term calf was necropsied and tissues, as well as stomach contents, were examined bacteriologically. Each sample was inoculated onto plates of two modified selective mediums previously
described\textsuperscript{14}, except that tryptose was substituted for albimi broth.

Cattle which were not proved to be infected by culture of any of the above material were also cultured at slaughter. The following tissues were collected for direct bacteriological examination: lymph nodes-submaxillary, retropharyngeal, iliac, and supramammary; spleen, uterus and sections from each quarter of the udder. Proof of infection was based on the isolation of \textit{Brucella abortus} from one or more of the specimens cultured.

Results and Discussion

In evaluating the results of this investigation, consideration should be given to the fact that all cows were purchased as adults and therefore the administration of Strain 19 vaccine was not under the control nor supervision of this project. The Strain 19 vaccinated group was therefore derived from the general population of animals which were vaccinated under field conditions, but unfortunately a random selection was not possible.

The number of infected animals in each of the six groups and their statistical relationships are presented in Table 4. Using the isolation of \textit{Brucellae} as the criteria for classification as infected or non-infected, there were 10 of the 12 non-vaccinated controls infected, compared to 4 of 10 and 1 of 10 for the groups treated with Duphavac NA and Bortin 45 respectively.

The difference between the nonvaccinated control and either group treated with bacterin alone was statistically significant; however, there was no significant difference between the two bacterin groups.

Groups of animals which received Strain 19 as calves and bacterin as adults were not significantly different from groups receiving bacterin alone.

The results of this investigation confirm those of others: that a serviceable immunity may be obtained by the use of 45/20 bacterin. There are areas in the world where such protection could outweigh any disadvantages associated with persisting serologic responses. In this country, however, the serologic responses demonstrated in this and other studies would be detrimental to the continued progress of the brucellosis eradication program. It is conceivable, however, that the use of this product might be feasible in future program situations as a temporary measure to take advantage of the protection conferred among various age groups of cattle if proper supervision could be maintained.

Summary

Under the conditions of this investigation, both bacterins produced a serviceable immunity comparable to that of Strain 19 vaccine. Bortin 45 produced objectionable tissue reactions at the sites of injection which was not observed following the administration of Duphavac NA. Both products produced persistent serologic reactions when administered to either calf-vaccinated (Strain 19) or nonvaccinated cattle.
<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>TEST RESULTS AFTER SECOND DOSE</th>
<th>TEST RESULTS AFTER ANNUAL BOOSTER DOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TUBE</td>
<td>TUBE</td>
</tr>
<tr>
<td></td>
<td>SUSP.</td>
<td>REACT.</td>
</tr>
<tr>
<td>COWS VACC. AS CALVES WITH STRAIN 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DUPHAVAC NA</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>BORTIN 45</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>COWS NOT VACC. WITH STRAIN 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DUPHAVAC NA</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>BORTIN 45</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

1/Tube agglutination test: Susp., 150 non VACC., 1100 VACC. - Reactor, +100 non VACC. +200 VACC.
2/Brucellosis Card test: + complement fixation test: +20 non VACC., 2+40 VACC.
3/12 cows in denominator of each group.
4/12 cows in denominator of both groups for Bortin 45 bacterin and 11 cows for both groups of Duphavac NA bacterin

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>COWS WITH SIGNIFICANT SEROLOGICAL REACTIONS* 10 AND 20 WEEKS AFTER THE SECOND AND ANNUAL BOOSTER DOSES OF 45/20 BACTERINS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BACTERIN AND TEST USED</td>
<td>VACC AS CALVES WITH STRAIN 19</td>
</tr>
<tr>
<td></td>
<td>SECOND DOSE</td>
</tr>
<tr>
<td></td>
<td>10 WKS</td>
</tr>
<tr>
<td>DUPHAVAC NA</td>
<td></td>
</tr>
<tr>
<td>TUBE - SUSPECT</td>
<td>3</td>
</tr>
<tr>
<td>- REACTOR</td>
<td>0</td>
</tr>
<tr>
<td>APA</td>
<td>3</td>
</tr>
<tr>
<td>CARD</td>
<td>5</td>
</tr>
<tr>
<td>RIV</td>
<td>0</td>
</tr>
<tr>
<td>CF</td>
<td>4</td>
</tr>
<tr>
<td>BORTIN 45</td>
<td></td>
</tr>
<tr>
<td>TUBE - SUSPECT</td>
<td>0</td>
</tr>
<tr>
<td>- REACTOR</td>
<td>0</td>
</tr>
<tr>
<td>APA</td>
<td>3</td>
</tr>
<tr>
<td>CARD</td>
<td>2</td>
</tr>
<tr>
<td>RIV</td>
<td>0</td>
</tr>
<tr>
<td>CF</td>
<td>0</td>
</tr>
<tr>
<td>CONTROLS</td>
<td></td>
</tr>
<tr>
<td>TUBE - SUSPECT</td>
<td>0</td>
</tr>
<tr>
<td>ALL OTHER TESTS</td>
<td>0</td>
</tr>
</tbody>
</table>

*Tube agglutination test Susp., 150 non VACC., 1100 VACC. - Reactor, +100 non VACC. +200 VACC.
Acidified plate antigen test +3 75
Brucellosis Card test + Rivanol plate precipitation test +25.
Complement fixation test 2+20 non VACC., 2+40 VACC.
### Table 3

**Number of Cows with Swellings Observed after Administration of 45/20 Bacterins**

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Observation at Site of ---</th>
<th>1st Injection 1/</th>
<th>2nd Injection 1/</th>
<th>Annual Booster After ---</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>After 1 Year</td>
<td>After 9 Months</td>
<td>1 Week</td>
</tr>
<tr>
<td>D U P H A V A C N A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C A L F V A C C., ST. 19 --</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N O T V A C C., ST. 19 ---</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>B O R T I N 45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C A L F V A C C., ST. 19 --</td>
<td>1</td>
<td>6</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>N O T V A C C., ST. 19 ---</td>
<td>7</td>
<td>8</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>C O N T R O L S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C A L F V A C C., ST. 19 --</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N O T V A C C., ST. 19 ---</td>
<td>0</td>
<td>1/</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1/ Recorded on day annual booster administered.
2/ No sham injections administered to controls - cause of deep swelling unknown.

### Table 4

**Evaluation of 45/20 Bacterins by Comparison of Intergroup Differences after Challenge with Br. abortus Strain 2308**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number Infected</th>
<th>Proportion Infected</th>
<th>Statistical Significance 1/</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control - Not Vacc. St. 19</td>
<td>10/12</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Control - Calf Vacc. St. 19</td>
<td>6/11</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Duphavac NA - Not Vacc. St. 19</td>
<td>4/10</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Duphavac NA - Calf Vacc. St. 19</td>
<td>4/11</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Bortin 45 - Not Vacc. St. 19</td>
<td>1/10</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Bortin 45 - Calf Vacc. St. 19</td>
<td>0/10</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

1/ The values connected by a solid line are not significantly different at 95% probability using tables of exact probabilities for 2 x 2 contingency tests (ARS 20-15 Dated Nov. 1967). The tables are based on Fisher's exact probability test.
REFERENCES


A SURVEY OF THE INCIDENCE OF RAM EPIDIDYMITIS IN IDAHO CAUSED BY BRUCELLA OVIS

R. E. Simmons, D.V.M., G. M. Brown, D.V.M.,
C. R. Ranger and D. E. Pietz, D.V.M.

Abstract
A survey of the commercial flocks of sheep in Idaho, with 1,000 or more ewes, was conducted to determine the clinical, serologic and cultural incidence of epididymitis caused by Brucella ovis. Of the 38 flocks examined and tested, 37 contained one or more rams with clinical and serologic evidence of epididymitis caused by Brucella ovis. Brucella ovis was isolated from the semen of one or more rams from 27 of the 38 flocks.

Epididymitis in rams, caused by Brucella ovis, was first reported in the United States by McGowan and Shultz in 1956. Since then epididymitis caused by B. ovis has been diagnosed mainly in the Western States. The economic importance of this disease has been uncertain. A 10 to 25 percent morbidity in rams in the Western United States has been reported. Crenshaw and McGowan stated that the incidence of the disease in commercial flocks in California was variable, but high enough in most flocks to be of significance.

Because of the increasing interest among Idaho sheepmen in the cause of dry ewes and a possible relationship between the number of dry ewes and the incidence of ram epididymitis, the Idaho Sheep Commission conducted a survey during the summer, fall and winter of 1969-1970 to determine the incidence of epididymitis caused by B. ovis. The survey was conducted in a random sampling of the commercial flocks in Idaho containing 1,000 or more ewes. Herein reported are the results of that survey.

Materials and Methods
Selection of Flocks
A list was compiled of all owners of commercial sheep flocks in Idaho with 1,000 or more ewes. From this list, 38 flocks were selected at random for inclusion in the survey.

To assure an equal representation of flocks from all parts of Idaho, the state was divided into three areas. The number of flocks selected from each area was in proportion to the total number of flocks in the area.
Selection of Rams
Eight rams from each flock were tested and cultured for B. ovis. All rams in

R. E. Simmons is the Veterinarian-in-Charge, Idaho Sheep Commission, Boise, Idaho. G. M. Brown, C. R. Ranger and D. E. Pietz are with the Biologic Reagents Section, Diagnostic Services, Animal Health Division, National Animal Disease Laboratory, Ames, Iowa. The authors acknowledge Messrs. G. Booth and C. K. Graham, Biometrical Services, National Animal Disease Laboratory, Ames, Iowa, for their assistance in designing the survey and analyzing data.
each flock were clinically examined for palpable lesions epididymitis. If gross lesions were detected in more than 8 rams, a random selection of 8 rams was made for serologic testing and semen culture. When lesions were detected in 8 or less rams in a flock, all animals with lesions were tested and rams without lesions were selected at random to make up the deficiency.

Collection of Laboratory Specimens

Semen samples were collected by electroejaculation in sterile Whirl-Pak* bags as described previously.4 Blood samples were collected, the clots removed, and the serum and semen samples were shipped to the laboratory in insulated shipping containers with frozen cans of water as the refrigerant.

Laboratory Procedures

Complement fixation tests were conducted using the procedure described by Biberstein and McGowan5 except that the antigen was prepared by autoclaving a suspension of B. ovis at 121°C for 15 minutes rather than by sonication of the suspension. Semen samples were cultured on selection medium as described.4

Results

The percentage of rams in each flock having detectable lesions of epididymitis ranged from 0 to 45 percent. Clinical lesions were not detected in the rams of only one flock.

A total of 1,890 rams were clinically examined. Lesions of epididymitis were detected in 452 (23.9 percent) of the rams. The clinical, serologic and cultural results are shown in Table 1.

There were 37 flocks that contained rams with clinical evidence of epididymitis. An average of 6.84/8 rams selected for testing and culturing had lesions of the epididymitis. All 37 clinically positive flocks contained one or more rams with titers of > 1:10 to the complement fixation test for B. ovis. An average of 5.4/8 rams tested in each flock had positive serologic titers (> 1:10).

Brucella ovis was isolated from the semen of one or more rams in 27 of the flocks. An average of 2.04/8 rams cultured in each flock were shedding B. ovis in the semen.

A statistical analysis was made of this data, and the estimated percent of commercial flocks in Idaho with 1,000 or more ewes with serological evidence of B. ovis infection in their rams was 97.40 percent with a lower 95 percent confidence limit of 93.75 percent. The estimated percent of flocks with cultural evidence of B. ovis was 71.06 percent with a lower 95 percent confidence limit of 60.35 percent.

Discussion

A very high percentage of the rams in the commercial flocks of sheep in Idaho that were included in this survey had clinical lesions of epididymitis and this condition was caused mainly by B. ovis.

The source of B. ovis infection in the individual commercial flocks in Idaho

*Nasco Veterinary Supply, Fort Atkinson, Wisconsin.
was difficult to ascertain. In certain instances infection may have been introduced through newly acquired rams, and in other instances newly acquired rams may have been exposed to infection after introduction into the native ram flock.

In general, commercial flock owners maintained their rams as separate flocks except during the breeding season when rams were placed with ewes. However, some owners kept their rams together in a community flock during the non-breeding season. This practice may have contributed significantly to the high flock incidence of the disease.

The role purebred flocks played in the overall incidence and spread of the disease in the commercial flocks of the state was problematical. Most of the replacement rams for commercial flocks were purchased from purebred breeders in the state. In the management of their flocks, most purebred breeders kept their ram lambs separate from their mature rams and there was little or no vaccination of rams for \( B. ovis \) in these flocks. Although purebred flocks were excluded from this survey, rams with palpable lesions of the epididymides of two of the larger purebred flocks in Idaho were tested and cultured. All rams in both flocks were serologically and culturally negative for \( B. ovis \).

The majority of rams in the commercial flocks were one of the black-face breeds or black-face crosses. Of the rams examined in this survey, 70 percent were Suffolk, 20 percent were Suffolk-Hampshire cross, and 10 percent were white-faced breeds (Primarily Rambouillet and Columbia). Lesions of epididymitis were not detected in the white faced rams. The lack of clinical evidence of epididymitis in white faced rams, along with the relatively high incidence of epididymitis caused by \( B. ovis \) in black faced rams, may be indicative of a breed difference in susceptibility to this disease.

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>No. examined and tested</th>
<th>No. clinically positive</th>
<th>No. serologically positive</th>
<th>No. culturally positive</th>
<th>No. clinically, serologically &amp; culturally negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flocks</td>
<td>38*</td>
<td>37**</td>
<td>37***</td>
<td>27****</td>
<td>1</td>
</tr>
<tr>
<td>Rams</td>
<td>304</td>
<td>253</td>
<td>206</td>
<td>55</td>
<td>8</td>
</tr>
</tbody>
</table>

* Eight rams examined/flock
** One or more rams with clinical evidence of epididymitis
*** One or more rams with C.F. titer > 1:10
**** *Brucella ovis* isolated from one or more rams
REFERENCES

REPORT OF THE COMMITTEE ON BRUCELLOSIS

H. G. Wixom, Sacramento, California, Chairman
J. R. Bishop, Tipton, Indiana; G. E. Burch, Albany, New York; Vernon D. Chadwick, Jackson, Mississippi; Charles Dancer, Dewey, Oklahoma; Billy L. Deyoe, Ames, Iowa; J. B. Finley, Jr., Encinal, Texas; D. C. Flagg, Bismarck, North Dakota; A. E. Janawicz, Montpelier, Vermont; W. D. Knox, Fort Atkinson, Wisconsin; R. L. Laramore, Gillette, Wyoming; Forrest Lee, Brownlee, Nebraska; C. A. Manthei, Ames, Iowa; R. J. McClennaghan, Ottawa, Ontario, Canada; S. H. McNutt, Madison, Wisconsin; J. O. Pearce, Jr., Okeechobee, Florida; Fred Phillips, Keating, Oregon; H. C. King, Hyattsville, Maryland; Urban Spanier, Belgrade, Minnesota; A. O. Wilson, Hysham, Montana; W. C. Tobin, Denver, Colorado

The brucellosis committee met in open session on Tuesday to give opportunity for free expression by all concerned on the progress, proposed improvements, problems, and objectives of the brucellosis eradication program. This included requests by officials and associations for clarification of recommended procedures and suggestions for implementing certain phases of the program. In addition, a survey of the incidence of ram epididymitis in Idaho caused by Brucella ovis was presented by R. E. Simmons, D.V.M. et al. A report of the survey will be included in the proceedings of the Association. All active members of the committee were present except two and these sent by telephone and telegram words of counsel and advice to the committee.

PROGRESS

Since our last report the States of Delaware, Minnesota, Virginia, and West Virginia have been added to the Certified Free States making a total of twenty, and the Virgin Islands. All other States are Modified Certified except the States of Florida, Louisiana, Oklahoma, South Dakota and Texas. In both Oklahoma and Louisiana only one county must be remodeled to have the entire state recertified. Florida should achieve modified certified status by the end of this year. Texas is making good progress with over 200 counties modified certified. There are only three counties in that state which have not established an organized testing program. Court cases have been the major cause of the delay in this state. This is also true in South Dakota, where only a few cattle remain to be tested. There is evidence that swine brucellosis is gradually decreasing and there is a small increase in the number of validated free herds. The market swine testing program is still being emphasized. The committee recommends investigation of the feasibility of revalidation of swine herds by market swine testing. Identification of animals is still the stumbling block to expanding this program. We were encouraged to be informed that the swine industry of Illinois is ready to carry out a pilot identification program thru L.C.I.
The committee heard a summary of the field studies conducted by USDA on 45/20 vaccine. The committee believes that further studies of the vaccine should be made before making any recommendation for its use.

ANIMAL IDENTIFICATION

September 9, 1970, the United States Department of Agriculture published regulations in the Federal Register to become effective thirty days thereafter, requiring cattle two years of age or older to be properly identified when moved interstate for slaughter. This regulation was recommended on several occasions by the United States Animal Health Association and should assist materially in tracing to the ranch of origin in the market cattle testing program. A similar model law was recommended by the Legislative Committee of the Council of State Governments for enactment by the States. States are urged to give consideration to strengthening their animal identification laws to implement the national program.

MOVEMENT OF OFFICIAL VACCINATES

On July 20, 1970, the United States Department of Agriculture amended its regulations on brucellosis to lower the testing age of officially vaccinated beef cattle to twenty-four months of age, when such cattle are required to be tested at the time of interstate movement. This makes the interstate regulations and the uniform methods and rules consistent as recommended by your committee.

MEXICAN CATTLE

United States Department of Agriculture officials are to be commended for their prompt action in preventing the introductions of brucellosis infected cattle from Mexico by amending on November 20, 1969, the importation regulations.

MARKET CATTLE TESTING PROGRAM

Eradication of brucellosis depends on an adequate Market Cattle testing program. To strengthen this program, the committee recommends that a staff assignment be made in each state to give specific emphasis to the MCT program within a state and to work out the problems which have resulted in an inadequate level of testing and test results returned. In addition, the committee recommends that animal health personnel, where possible and practical, be assigned to slaughtering establishments where large numbers of breeding type beef cattle are slaughtered to assist Consumer and Marketing Services personnel in obtaining MCT samples and related information.

Further, your committee urges that USDA promptly adopt compulsory
regulations requiring blood samples to be taken from all back tagged cattle or otherwise identified breeding type beef cattle consigned to slaughter and the results returned to the state of origin.

The committee recommends the following amendment to the uniform methods and rules to broaden the coverage of cattle included in the MCT program:

Page 3 under Part I paragraph G, amend the definition for Market Testing Animals to read:

"Animals moving in trade through auction markets, stockyards, or to slaughtering establishments; also those animals assembled at farms or ranches that are readied for immediate movement to markets, stockyards or slaughtering establishments or other sales. (Includes market cattle testing (MCT) and market swine testing (MST) programs.)"

It is the intent of the committee to include in the MCT program, breeding cattle over twenty-four months of age which may be assembled at farms or ranches for direct sales of the same type that occurs at the market or stockyards.

ENFORCEMENT

Concern is expressed that an attitude of complacency may develop in regard to brucellosis eradication when a state may achieve Modified Certified Status. Numerous states have been in this status unduly long. In some cases MCT positive herds are not quarantined and tested as soon as possible. In other cases there has been too much delay in testing of herds suspicious to the BRT. State and Federal officials in each state should critically evaluate their programs and make whatever adjustments may be necessary to achieve eradication. If there are problems which are difficult to resolve, these problems should be brought to the attention of this committee for study, as soon as possible. Your committee has received indication from industry members that the objectives of the program should be achieved as quickly as possible.

DEPOPULATION OF INFECTED HERDS

The depopulation of infected herds has already proven to be a valuable procedure to be applied to herds when infection cannot be eliminated within a reasonable time, using established methods. Owners agreeing to depopulate in the brucellosis eradication program find themselves at a serious disadvantage as compared to indemnities applied to the tuberculosis eradication program. The committee recommends that the United States Department of Agriculture amend its regulation pertaining to indemnity to permit payments in bovine brucellosis depopulated herds to be consistent with that applied to bovine tuberculosis depopulated herd.
SPECIAL PROBLEMS

Requests have been made to the committee to recommend changes in the certified herd plan and the use of the card test. We have studied the suggestions and do not recommend that a change be made in the certified herd program. The current standards have been developed to assure that the certified herd status is a reliable one. We do not recommend lowering the standards. The committee reaffirms its recommendation pertaining to the card test that the test be used under the supervision of the state animal health official.

SURVEY

A rather comprehensive questionnaire has been developed by your committee and submitted to the committee on Evaluation and Development of this association. When approved, this questionnaire will be sent to all states for completion. It is the intent that this questionnaire will be helpful in developing uniformity in the national brucellosis eradication program.

OBJECTIVE AND GOAL

The committee reaffirms that the goal of complete eradication by 1975, be our objective. To achieve this objective adequate funding must be provided and all the tools available must be applied with vigor. It is noted that Federal funds for brucellosis eradication have been diverted in the last several years to other animal health programs or possibly for other purposes. The results have been that herds infected were not tested promptly, the MCT program has wavered, the swine validation program has only moved slowly ahead; our nation is behind in achieving Modified Certified Status. Some delays perhaps were unavoidable. However, delays in the program result in spread of the disease and areas which are free are continually being threatened. Undue delay increases the over all cost of the program, and lowers the cost/benefit ratio; but perhaps more important we chance the possibility of all becoming weary of well doing. There is still time to achieve our agreed objective, but there will not be if the program is crippled by insufficient funds and effort. USDA officials are therefore cautioned to review carefully the funds for brucellosis eradication and arrange for sufficient allocation to accomplish our national objective. We are reminded that tremendous effort has been applied at county, state and national levels to assure that sufficient funds would be available to achieve our objective. The great expenditure of funds which have already been applied to this program plus the losses and the efforts by industry, the States and the Federal Government and add to this the concerns of all of us, demand that this eradication effort be of high priority. We are determined to stay on course and will not be satisfied with delays.
VACCINATION OF CATTLE WITH LEPTOSPIRAL BACTERINS
I. SEROLOGIC AND CULTURAL RESULTS OF LEPTOSPIRAL CHALLENGE

A. H. Killinger, Ph.D., L. E. Hanson, D.V.M., Ph.D.,
M. E. Mansfield, D.V.M., and H. A. Reynolds, D.V.M., Ph.D.
Department of Pathology and Hygiene
College of Veterinary Medicine
University of Illinois at Urbana-Champaign

Commercial *Leptospira pomona* bacterins were developed in response to recognition of *L. pomona* as the major cause of losses from leptospirosis of cattle and swine in the United States. The Veterinary Biologics Division, ARS-USDA, currently has issued 24 licenses for the production of *L. pomona* bacterin. Bioassay of this product is prescribed in Veterinary Biologics Division B-20 Standard Requirement for *Leptospira pomona* Bacterin. The potency test is conducted in hamsters.

*L. pomona* bacterins have been widely employed and have been useful in preventing overt clinical leptospirosis in cattle and swine. The degree to which bacterins may prevent the development of subclinical renal shedders following natural exposure to *L. pomona* and its epidemiologic significance in the control of leptospirosis is not well understood.

One objective of this investigation was to attempt to correlate bio-assay of the potency of *Leptospira pomona* bacterins in hamsters with bio-assay of bacterin in cattle. A second objective was to attempt to evaluate the immune status and renal protection of cattle at periods of ten months to a year following vaccination with bacterin.

**MATERIALS AND METHODS**

*Cattle*
Weaned Hereford calves approximately six months of age at the time of vaccination from the University of Illinois Dixon Springs Agricultural Center (DSAC) herd were used. However, eight unvaccinated Angus yearlings were purchased and added to the control group before challenge in Experiment B. The animals were rotated on several pastures at DSAC during the prechallenge period. After challenge the cattle were confined in a metal pole barn with an adjacent concrete floored drylot.

*Leptospiral Bacterins*
Coded commercial *L. pomona* bacterins, previously potency bio-assayed in accordance with Veterinary Biologics Division Standard Requirement B-20, were supplied by the Veterinary Biologics Division of ARS-USDA. Bacterin was injected subcutaneously in two or five ml. doses as recommended by the manufacturer.
Five ml. of an experimental *L. hardjo* bacterin* was injected subcutaneously in Experiment B.

Serum

Fifty ml. of blood was collected from the jugular vein by a 12 gauge California bleeding needle fitted in an adapter to hold a 25 x 150 mm test tube. The tube was closed with a polypropylene cap** and incubated at 37°C overnight for retraction of the blood clot. Clear serum was either poured off or the tube centrifuged in a 50 ml. carrier in an International No. 2 centrifuge in order to obtain maximum yield of serum.

Urine Collection

Cattle were tightly crowded in a line chute. Urine was collected in 25 x 150 mm test tubes during natural voiding, following manual stimulation of heifers or following injection of five ml. of furosemide† intramuscularly. In order to protect personnel, plastic disposable gloves and plastic protective face shields were worn during urine collection.

Bacteriologic Culture

Blood for bacteriologic culture for leptospires was collected in sterile disposable ½ ml. syringes fitted with a 20 gauge one inch needle. Two drops of blood were added to five ml. of semisolid bovine albumin-polysorbate 80 medium (2) and incubated at 30°C.

For culture of urine, 0.1 ml. was added to five ml. of fluid bovine albumin-polysorbate 80 medium and 0.1 ml. from this tube added to a similar tube for further dilution of the original urine and incubated at 30°C.

At slaughter, kidney tissue was aseptically collected from each animal and placed in petri dishes. One gram quantities of kidney tissue were placed in a 2½ ml. disposable syringe and the tissue forced through the syringe into 5 ml. of liquid medium. From this original tube 0.1 ml. was transferred to another tube of liquid and to a tube of semisolid medium for further dilution. Hamsters were injected intraperitoneally with 0.5 ml. of tissue suspension from the first culture tube.

Microscopic Agglutination (MA) Test

MA tests were conducted according to the procedure described by the Committee on Leptospiriosis of the 64th Annual United States Livestock Sanitary Association. All sera causing agglutination or lysis of 50 percent or more organisms in dilutions of 1:100 or greater were considered positive reactors in the MA test.

---

*Affiliated Laboratories, a division of Rohm and Haas Company, White Hall, Illinois

**Kap-uts, Belco Glass, Inc., Vineland, N.J.

†Lasix, National Laboratories, Division of American Hoechst Corp., Kansas City, Mo.
RESULTS OF LEPTOSPIRAL CHALLENGE

Leptospiral Challenge

Cattle were challenged by the intraperitoneal injection of one ml. of \textit{L. pomona} MLS strain, ARS No. 11,000, in liquid bovine albumin-polysorbate 80 medium. Direct counts in a Petroff-Hauser chamber with darkfield illumination indicated 48 billion leptospires were used as challenge in Experiment A and 64 billion in Experiment B.

RESULTS

\textbf{MA Serologic Response to Vaccination}

Forty-three calves were treated in the following manner: 15 were vaccinated with \textit{L. pomona} bacterin Lot 4, 15 with Lot 6 and 13 were unvaccinated controls. Sera were collected for MA testing and hamster passive protection assay before vaccination and at bimonthly intervals from two to 12 months postvaccination. Two of 15 calves vaccinated with Lot 6 had MA titers of 1:100 against \textit{L. pomona} two months postvaccination. All other sera were negative to the MA test during the entire 12 month postchallenge testing. Serum samples were sent to the Veterinary Biologics Division for hamster passive protection assay.\textsuperscript{3}

Another group of 120 calves were treated in the following manner: 15 were vaccinated with \textit{L. pomona} bacterins Lots 3, 5, 7, 9, 10 and 11 respectively, 15 were vaccinated simultaneously with \textit{L. pomona} and \textit{L. hardjo} bacterin and 15 held as unvaccinated controls. Sera were collected before vaccination and at 2, 4, 6 and 12 months postvaccination. One animal vaccinated with Lot 11 had an MA titer of 1:100 against \textit{L. pomona} on serum collected 12 months postvaccination. All other sera were negative during the entire 12 month postchallenge testing period. Serum samples were sent to VBD for hamster passive protection assay.

EVALUATION OF IMMUNE STATUS AND RENAL PROTECTION

Experiment A

MA Serologic Response to \textit{L. pomona} Challenge

Approximately one year after vaccination, 15 cattle vaccinated with Lot 4 bacterin, 15 cattle vaccinated with Lot 6 bacterin and 13 unvaccinated control cattle were challenged with \textit{L. pomona} culture. The animals were bled at the time of challenge and on postchallenge days 7, 14, 21 and 27. Results of the MA tests on the sera are presented in Table 1. Cumulative totals of animals which had titers of 1:100 or greater during the 27 day postchallenge period were 53 percent for cattle vaccinated with Lot 4 bacterin, 60 percent for Lot 6 and 100 percent of the unvaccinated controls.

Leptospiral Cultural Results

Cultural attempts to isolate leptospires were made from the blood on postchallenge days 0, 4, 6, 8 and 10, from the urine on days 0, 7, 14, 21 and 27, and from the kidney on postinoculation day 28. Cumulative results of cultural isolation attempts are presented in Table 2. A transient leptospiremia developed in three of 15 animals vaccinated with Lot 4 bacterin and in two of 13 control
animals. Leptospirosis developed in two of 13 control animals. No leptospires were isolated from the kidneys. Histopathologic examination of hematoxylin and eosin stained sections revealed one or two small foci of interstitial infiltration in the kidney of four of 15 control animals. No changes were detected in other control animals. Thus, the animals in this experiment did not develop a persistent leptospiral renal infection following challenge with *L. pomona* MLS strain.

**Experiment B**

**MA Serologic Response to *L. pomona* Challenge**

Ten months after vaccination, 15 cattle vaccinated with *L. pomona* bacterin and *L. hardjo* bacterin and 15 unvaccinated control cattle were challenged with *L. pomona* culture. The animals were bled at day 0, 7, 14, 21 and 27 postchallenge. The MA results on the sera are presented in Table 3. Cumulative totals of animals which had titers of 1:100 or greater during the 27 day postchallenge period were 60 percent for *L. pomona* vaccinates, 60 percent for *L. pomona* and *L. hardjo* vaccinates and 67 percent for unvaccinated controls.

MA serologic tests against *L. hardjo* on the day of challenge with *L. pomona* indicated that the cattle in Experiment B had had a field exposure to *L. hardjo*, since as indicated in Table 4, 60 percent of the *L. pomona* vaccinates, 73 percent of the *L. pomona* and *L. hardjo* vaccinates and 87 percent of unvaccinated controls had titers to *L. hardjo*. The cumulative totals at the end of the 27 day postchallenge period were 73 percent, 73 percent and 87 percent respectively.

**Leptospiral Cultural Results**

Cultural attempts to isolate leptospires from the blood, urine and kidney were done at equivalent time schedules to Experiment A above. Cumulative results are presented in Table 5. A transient *L. pomona* leptospiremia occurred in two of 15 cattle which had been vaccinated with *L. pomona*, in two of 15 vaccinated simultaneously with *L. pomona* and *L. hardjo* and in one of 15 unvaccinated control animals. None of the animals developed leptospirosis or cultural evidence of renal leptospiral infection with *L. pomona*.

One of the *L. pomona* vaccinates developed a transient *L. hardjo* leptospiremia. All postchallenge urine cultures were negative for *L. hardjo*. However, *L. hardjo* was isolated from 12 of 15 (80 percent), six of 14 (43 percent) and 14 of 15 (93 percent) of kidneys at slaughter of *L. pomona* vaccinates, *L. pomona* and *L. hardjo* vaccinates, and unvaccinated control cattle respectively. (Table 6)

**DISCUSSION**

In the control of veterinary biologics it is desirable to attempt to correlate the routine potency bio-assay of the product in laboratory animals with bio-assay in the animal in which the product is to be used. Since *L. pomona* MLS strain had been used for many years in the bio-assay of the bacterins, it was anticipated that the use of this challenge strain would make possible maximum correlation between
evaluation of bacterins in hamsters and cattle.

Although it regularly produced fatal infections in hamsters, the MLS strain had a low pathogenicity for cattle by the intraperitoneal route. No clinical signs of weight loss, anorexia, or hemoglobinuria developed postchallenge. A transient *L. pomona* leptospiremia was detected on day 4 postchallenge in seven of 60 vaccinates and in three of 28 unvaccinated control cattle. *L. pomona* was isolated from the urine of two of 13 control animals on day 14 postchallenge in Experiment A. All other cultures of urine and kidneys in both Experiments A and B were negative. Thus, *L. pomona* MLS did not establish a persistent renal infection. Therefore, the degree of renal protection provided by vaccination could not be evaluated in this study.

Antibody measured by the MA test was detected at a titer of 1:100 or more in only two of 135 animals at two months following vaccination with *L. pomona* bacterin. Antibody measured by the hamster passive protection test persisted for months in these vaccinated cattle as reported by Huhn, Claus and Macheak. These data suggest that vaccination will not seriously cause conversion of cattle to leptospiral reactor status if criteria for reactors is a titer of 1:100 or greater.

Crawford demonstrated that two types of antibodies, IgM (19S) and IgG (7S) develop in response to leptospiral infection. IgM antibody can be measured by the MA test.

In an independent study with representative sera from cattle in Experiment A, Negi detected hamster passive protective antibody in both the IgG and IgM fractions in sera of cattle twelve months after vaccination. The protective antibody was primarily in the IgG fraction of the serum.

The presence of a natural outbreak of *L. hardjo* infection in the cattle in Experiment B was detected by MA serologic testing of the sera collected at the time of *L. pomona* challenge. The source of the *L. hardjo* field exposure was unknown, however, *L. hardjo* has been endemic in DSAC cattle. After negative urine cultures, the high incidence of *L. hardjo* cultural isolation from the kidneys at slaughter was unexpected. As bacterial contamination occurred in the urine cultures, it probably was responsible for the lack of isolations.

Although the route, number of organisms, time and extent of the natural exposure to *L. hardjo* are unknown, the observation that *L. hardjo* was only isolated from the kidneys of 43 percent of dual *L. hardjo* and *L. pomona* vaccinates but from 80 percent of the *L. pomona* vaccinates and 93 percent of the unvaccinated control cattle is of interest.

Further study is required to determine the ideal route for experimental challenge exposure to *L. pomona* in order to more closely approximate natural field exposure.

The effect of the influence of a second injection of bacterin on the leptospiral immune response of cattle should be investigated.
SUMMARY

The immune status of cattle vaccinated with coded *Leptospira pomona* bacterins was evaluated by intraperitoneal challenge with *L. pomona*, MLS strain, ten to 12 months postvaccination. The MLS strain was selected for challenge in an attempt to correlate the routine bio-assay of *L. pomona* bacterins for potency in hamsters with a vaccination and challenge bio-assay of *L. pomona* bacterins in cattle.

Postvaccination immune status was evaluated by the leptospiral microscopic agglutination (MA) test and the hamster passive protection test of the cattle sera tested at bimonthly intervals. Three of 120 cattle had MA titers of 1:100 when tested two months postvaccination. Subsequent MA titers were negative. Hamster passive protection assays were conducted by the Veterinary Biologics Division.3

Postchallenge MA titers of 1:100 or greater developed in 53 percent of cattle vaccinated with *L. pomona* Lot 4 bacterin, in 60 percent vaccinated with Lot 6 bacterin and in 100 percent of unvaccinated control cattle in Experiment A. This is in agreement with previous studies which indicate some vaccinates do not develop significant MA titers following challenge.

Postchallenge attempts to evaluate the protection to *L. pomona* renal infection were unsuccessful because of *L. pomona* MLS challenge strain was of low pathogenicity for cattle and did not establish a persistent renal infection in the unvaccinated cattle.

MA serologic tests indicated that a natural outbreak of *L. hardjo* infection had occurred in Experiment B cattle before challenge with *L. pomona* MLS strain. Renal infection developed. *L. hardjo* was isolated in culture at slaughter from 80 percent of cattle vaccinated with *L. pomona* bacterin, from 93 percent of unvaccinated control cattle and from 43 percent of cattle vaccinated simultaneously with both *L. pomona* and *L. hardjo* bacterin.

ACKNOWLEDGEMENTS

This study was supported in part by the Veterinary Biologics Division of the Agricultural Research Service, USDA Contract Nos. 12-14-140-1748-94 and 12-14-140-1918-94 and by the University of Illinois Agricultural Experiment Station. We wish to thank Dr. D. E. Baldwin for supplying the experimental *L. hardjo* bacterin and express our gratitude to V. R. Manuel and Rachel Marlowe for valuable technical assistance.
### TABLE 1

**EXPERIMENT A: SEROLOGIC RESULTS**

<table>
<thead>
<tr>
<th>Vaccination L. pomona Bacterin</th>
<th>Postchallenge Day</th>
<th>Cumulative Total*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 4</td>
<td>0% 0% 47% 13% 13%</td>
<td>53%</td>
</tr>
<tr>
<td>Lot 6</td>
<td>0% 0% 53% 20% 13%</td>
<td>60%</td>
</tr>
<tr>
<td>Control</td>
<td>0% 23% 92% 77% 69%</td>
<td>100%</td>
</tr>
</tbody>
</table>

* Individual animals with titers sometime during 27 day postchallenge period
### TABLE 2

**EXPERIMENT A. CULTURAL RESULTS**

**Postchallenge Positive** L. *pomona*

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>Blood Culture</th>
<th>Urine Culture</th>
<th>Kidney Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 4</td>
<td>3/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>Lot 6</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>Control</td>
<td>2/13</td>
<td>2/13</td>
<td>0/13</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Postchallenge Day</td>
<td>Cumulative Total</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>L. pomona</td>
<td>0%</td>
<td>13%</td>
<td>57%</td>
</tr>
<tr>
<td>L. pomona &amp; L. hardjo</td>
<td>0</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>40</td>
<td>29</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Postchallenge Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>L. pomona</td>
<td>60%</td>
<td>40%</td>
<td>38%</td>
</tr>
<tr>
<td>L. pomona &amp; L. hardjo</td>
<td>73</td>
<td>47</td>
<td>33</td>
</tr>
<tr>
<td>Control</td>
<td>87</td>
<td>87</td>
<td>71</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Blood Culture</td>
<td>Urine Culture</td>
<td>Kidney Culture</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------</td>
<td>---------------</td>
<td>----------------</td>
</tr>
<tr>
<td>L. pomona</td>
<td>2/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>L. pomona &amp; L. hardjo</td>
<td>2/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>Control</td>
<td>1/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
</tbody>
</table>
## TABLE 6

**EXPERIMENT B. CULTURAL RESULTS**

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>Blood Culture</th>
<th>Urine Culture</th>
<th>Kidney Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. pomona</td>
<td>1/15</td>
<td>0/15</td>
<td>12/15 (80%)</td>
</tr>
<tr>
<td>L. pomona &amp; L. hardjo</td>
<td>0/15</td>
<td>0/15</td>
<td>6/14 (43%)</td>
</tr>
<tr>
<td>Control</td>
<td>0/15</td>
<td>0/15</td>
<td>14/15 (93%)</td>
</tr>
</tbody>
</table>
REFERENCES


VACCINATION OF CATTLE WITH LEPTOSPIRA POMONA BACTERINS.
II. POTENCY ASSAYS USING THE HAMSTER PASSIVE PROTECTION TEST

by
Ronald G. Huhn, D.V.M., Ph.D.
Kenneth D. Claus, B.S., M.S.
Merlin E. Macheak, D.V.M., M.S.

The idea behind artificial immunization is to enhance the resistance of the immunized subject to a disease and to do so at a total cost that is attractively less than the total cost of the disease if said immunization procedures are not used.

To be attractive to the user, an immunizing product must be safe and must be potent, both in the level of and duration of immunity induced by its use.

The potency assay of *Leptospira pomona* bacterins as defined in Standard Requirement (S.R.) B-20 (September 12, 1961) was at question in this study. Using this test, potency is assayed by inducing active immunity in hamsters and subsequently testing this by inoculating the immunized hamsters with virulent *Leptospira pomona* organisms. The criterion for passing a product as satisfactory in this test is the ability of 80 percent of the vaccinated, challenged hamsters to survive, while at least 80 percent of the unvaccinated challenged hamsters die.

The study related in part here was constructed to determine several things:
1) The relation between potency of a bacterin as expressed in the results of a S.R. B-20 test and protection of immunized cattle from clinical disease or renal infection when inoculated with *Leptospira pomona* organisms. 2) The relation between potency of a bacterin as expressed in the results of a S.R. B-20 test and the level of and duration of protective circulating antibody induced in cattle as a result of immunization with the bacterins. 3) The objectives as stated in 1 and 2 but with the potency assay conducted using a 1/800 cattle dose instead of the 1/200 cattle dose (S.R. B-20 as amended and issued January 1, 1970, requires that the 1/800 cattle dose be used in the assay.) 4) The relation between protective circulating antibody and agglutination antibody. 5) Possible interference phenomena when using more than one Leptospiral serotype in a vaccination procedure.

The present paper will cover those parts of the study related to the results obtained through use of the hamster passive protection test and the serums supplied by the University of Illinois.

MATERIALS AND METHODS

Participants in Study. This study was performed by personnel of the College of Veterinary Medicine, University of Illinois, Urbana, Illinois, (Contractee) under the authority of Sec. 302 (c) (5) of the Federal Property and Administrative Services Act of 1949 and in cooperation with personnel of the VBD Agricultural Research Service, U.S.D.A. (Contractor).

178
Bacterins. Ten bacterins were used in the study. Nine were commercially produced using *L. pomona*; one bacterin was experimentally produced using *L. hardjo* as the antigen. Eight commercial bacterins were potency tested at the VBD Laboratory using the S.R. B-20 procedure; one *L. pomona* bacterin and the *L. hardjo* bacterin were inoculated with a 5.0 ml. dose in addition to the standard dose of *L. pomona* bacterin.

**Cattle (Allocation and Treatment).** The cattle used in the study were mainly Herefords. They originated from the herd maintained by the University of Illinois at the Dixon Springs Research Station in Southern Illinois. The cattle in this herd have been serologically tested annually against *Leptospira* spp. antigens for a number of years. A detailed history is available for each animal on the Station and for its ancestors.

Fifteen head of cattle were allotted to each of eleven vaccine groups and to three unvaccinated control groups. The vaccine and control groups are detailed in Table 2. [Note: With relation to group number in this table and in subsequent references the first two numbers (01-01) refer to sets of samples which were run at one time in the hamster passive protection test; this is distinguished from the last two numbers (01-01) which refer to the treatment given the cattle (either a bacterin or no bacterin)].

The treatment of the cattle at the research test site with regard to collection of serum, urine, blood, and renal tissues as well as the inoculation with live *L. pomona* organisms has been reported by Killinger and Hanson.2

**Microscopic Agglutination Test.** All blood samples collected for serological study were tested for the presence of agglutinating antibodies to *Leptospira autumnalis, Leptospira canicola, Leptospira grippotyphosa, Leptospira icterohaemorrhagiae, Leptospira hardjo, and Leptospira pomona*. A microscopic agglutination test was performed at the University of Illinois, Urbana, Illinois.

**Hamster Passive Protection Test.** A portion of the serum from each blood sample collected for serological study was sent to the VBD Laboratory to be used in a hamster passive protection test. This test was designed to detect antibody in the serum sample which would protect hamsters when they were challenged with hamster virulent *L. pomona* organisms.

Each serum sample was injected intraperitoneally (IP) into five hamsters (0.5 ml. per hamster). Twenty to 24 hours later each hamster was inoculated IP with a standardized challenge of *L. pomona* MLS (approximately 2000 hamster MLD50). The hamsters were then observed for two weeks; at this time surviving hamsters were killed and examined. One kidney was aseptically removed and cultured. Cultures (incubated at 28-30°C) were examined for growth of Leptospires after two and four weeks incubation. The main concerns were the proportion of dead hamsters and proportion of infected hamsters.

Hamsters given inoculum only (positive controls) and hamsters given neither serum nor inoculum (negative controls) were included in each test. Between 1400 and 1500 samples from 180 head of cattle were tested utilizing close to 10,000 hamsters.
**Culture Medium.** Ellinghausen's medium\(^1\) in either the semisolid or liquid form was used in culturing all blood samples and tissues collected in the course of this study.

**Analysis.** All data collected in the trial was coded and prepared for statistical analysis using a computer. Summary tables were also prepared.

**RESULTS AND DISCUSSION**

The results of the microscopic agglutination test were discussed by Drs. Killinger and Hanson.\(^2\) Their results showed that immunization with *L. pomona* bacterin induced no significant or persistent agglutination antibody to *L. pomona*. Such immunization did not induce significant or persistent agglutination antibody to any of the other five antigens used in the test (*L. canicola, L. hardjo, L. autumnalis, L. grippotyphosa, or L. icterohaemorrhagiae*).

The results of the hamster passive protection test (Tables 3-7) indicate that the *L. pomona* bacterins do induce the production of circulating antibody which will protect hamsters when inoculated with *L. pomona*. The question of why the results of the two detection systems differ is raised.

The explanation may take two forms: The hamster passive protection test (as performed) may be much more sensitive than the agglutination test (as performed) in detecting protective antibody. This appears plausible when the results of the hamster passive protection test and the agglutination test are considered for those serums collected from cattle after they were inoculated with *L. pomona*. (Tables 8-12 and previous paper). Comparison here shows a positive correlation of agglutination antibody and protection of hamsters in the hamster passive protection test (e.g., perhaps protective antibody is in concentrations sufficient to be detected by agglutination as well as by the hamster passive protection test.)

The second possibility is that *L. pomona* bacterins induce at least two types of antibody, one which protects and another which reacts with homologous antigen in the agglutination test. The project was not designed to distinguish between these two possibilities.

Correlation of response after challenge of positive control hamsters over the entire series of tests was shown to occur. By inference, the inoculum was considered to be consistent throughout the period that hamster passive protection tests were performed.

An analysis of variance was performed to see if the proportion of dead or infected hamsters varied significantly among vaccine treatments (bacterins 3, 4, 5, 6, 7, 9, 10, and 11) for each sample collected (prevaccination, plus 2, 4, 6 and 12 months postvaccination) Since comparisons were not independent, Dunnett's procedure was used rather than least significant differences. The results of this analysis are present. (Table 13 and Figures 1 and 2)

These results show that serum collected from cattle vaccinated with bacterins which passed the potency assay at 1/800 cattle dose would significantly protect hamsters from renal infection up to one year postvaccination. [Note: Hamster renal
infection was a more sensitive criterion of protection than was hamster death.]
However, serum from cattle vaccinated with bacterins which had passed the
potency assay at 1/200 cattle dose (only) would not significantly protect hamsters
from renal infection at any sampled time postvaccination, not even at two months.
This suggests that of the two dilutions used in testing bacterins (1/200 cattle dose
and 1/800 cattle dose) the bacterins which pass at 1/800 cattle dose tend to
produce a higher level of circulating protective antibody in cattle and that this level
will remain higher longer than it will cattle vaccinated with bacterins capable only
of passing a potency assay at 1/200 cattle dose.

Although this study did not allow a direct comparison to be made between
potency assay results of bacterins and actual protection of cattle from renal
infection, it would appear logical that the bacterins which produced a persistently
high level of circulating protective antibody would protect cattle from renal
infection better than bacterins which induced a low level of such antibody. This
suggested in light of the fact that a circulatory phase (Leptospiremia) is imperative
in the pathogenesis of renal leptospiral infection. Even though this conclusion
appears logical, caution should be used in drawing the inference.

The results of the hamster passive protection test using serums obtained from
cattle just before and periodically after inoculation with hamster-virulent L.
pomona organisms show that the vaccinated animals respond to the infection more
quickly than do the unvaccinated control animals (Figure 3), i.e. the vaccinated
cattle gave an anamnestic type response while the unvaccinated cattle didn’t. This
was expected.

SUMMARY

A project was undertaken by the Veterinary Biologics Division, ARS, USDA,
and the University of Illinois to determine some of the relationships between
Leptospira bacterins, potency assays, and immunity in vaccinated cattle as
measured by resistance of the cattle when inoculated with live Leptospira pomona
organisms and by the results of a hamster passive protection test performed on
serums from the tested cattle.

There appeared to be little or no correlation between agglutination antibody
and circulating protective antibody in the majority of samples tested. This may
have been due to a difference in the sensitivity of the two tests or due to the tests
measuring two different types of antibody.

The duration of circulating protective antibody in test cattle as measured by
the hamster passive protection test was positively related to the results of the S.R.
B-20 potency assays for the bacterins used in the project. Bacterins which passed
the S.R. B-20 at 1/800 cattle dose (80 percent plus survivors) induced higher levels
of circulating protective antibody than did bacterins which failed at this dosage
(less than 80 percent survivors). Again bacterins which passed the S.R. B-20 at
1/800 cattle dose induced protective antibody for a longer period of time than did
bacterins which failed at this dosage.
Freedom from renal infection of hamsters was a much more sensitive criterion of protection than was freedom from death in the hamster passive protection test. Since Leptospira organisms must cross a blood barrier (leptospiremia) to initiate a renal infection it can be concluded the bacterins which induce the higher, more persistent levels of circulating protective antibody (e.g. pass the S.R. at 1/800 cattle dose) should be more effective in preventing renal infection than those bacterins which fail to do this (e.g. pass the S.R. only at 1/200 cattle dose).

Table 1
Results of S.R. B-20 potency tests conducted on vaccines used in study.
Tests were performed in 1968, near the beginning of dating period of vaccines.

<table>
<thead>
<tr>
<th>Vaccine Number (Coded)</th>
<th>Percent Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/200 Cattle Dose</td>
</tr>
<tr>
<td>2a)</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>87</td>
</tr>
<tr>
<td>4</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td>94</td>
</tr>
<tr>
<td>6</td>
<td>96</td>
</tr>
<tr>
<td>7b)</td>
<td>76</td>
</tr>
<tr>
<td>8c)</td>
<td>---</td>
</tr>
<tr>
<td>9</td>
<td>91</td>
</tr>
<tr>
<td>10</td>
<td>57</td>
</tr>
<tr>
<td>11</td>
<td>34</td>
</tr>
</tbody>
</table>

a) *L. hardjo* bacterin – Not potency tested.
b) In 2 of 4 tests conducted on vaccine 7, this product would have passed S.R. B-20 at 1/200 cattle dose but in no case would this product have passed the S.R. B-20 at 1/800 cattle dose.
c) *L. pomona* bacterin used by University of Illinois. Not potency tested at VBD Laboratories.
TABLE 2
Allocation of cattle into groups; treatment of groups.

<table>
<thead>
<tr>
<th>No. of Cattle</th>
<th>Group Number</th>
<th>Vaccine Number</th>
<th>Serology Studies</th>
<th>Infected 1 year Postvaccination with L. pomona</th>
<th>Slaughtered and Cultured</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>01-01</td>
<td>... a)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>15</td>
<td>01-04</td>
<td>04</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>15</td>
<td>01-06</td>
<td>06</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>14</td>
<td>02-03</td>
<td>03</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>15</td>
<td>02-05</td>
<td>05</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>15</td>
<td>02-07</td>
<td>07</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>14</td>
<td>03-09</td>
<td>09</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>14</td>
<td>03-10</td>
<td>10</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>15</td>
<td>03-11</td>
<td>11</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>15</td>
<td>04-02</td>
<td>02b)</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>15</td>
<td>04-14</td>
<td>... a)</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>15</td>
<td>05-02</td>
<td>02b)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>15</td>
<td>05-08</td>
<td>08</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>15</td>
<td>05-13</td>
<td>... a)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

a) Unvaccinated Control Animals  
b) Vaccinated with L. pomona bacterin and with experimental L. hardjo bacterin.
### TABLE 3
Hamster passive protection test. Data is from tests using serum samples collected just prior to immunization.

<table>
<thead>
<tr>
<th>No. of Cattle</th>
<th>Group Number</th>
<th>Vaccine Number</th>
<th>% Hamsters Dead</th>
<th>% Hamsters Infected</th>
<th>Average day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>01-01</td>
<td></td>
<td>61.5</td>
<td>80.0</td>
<td>8.3</td>
</tr>
<tr>
<td>15</td>
<td>01-04</td>
<td>04</td>
<td>62.3</td>
<td>81.0</td>
<td>8.3</td>
</tr>
<tr>
<td>15</td>
<td>01-06</td>
<td>06</td>
<td>68.0</td>
<td>86.6</td>
<td>8.2</td>
</tr>
<tr>
<td>14</td>
<td>01-03</td>
<td>03</td>
<td>64.2</td>
<td>90.0</td>
<td>6.8</td>
</tr>
<tr>
<td>15</td>
<td>02-05</td>
<td>05</td>
<td>73.3</td>
<td>94.6</td>
<td>7.9</td>
</tr>
<tr>
<td>15</td>
<td>02-07</td>
<td>07</td>
<td>77.3</td>
<td>96.0</td>
<td>6.9</td>
</tr>
<tr>
<td>14</td>
<td>03-09</td>
<td>09</td>
<td>68.5</td>
<td>90.0</td>
<td>7.0</td>
</tr>
<tr>
<td>14</td>
<td>03-10</td>
<td>10</td>
<td>81.4</td>
<td>98.5</td>
<td>6.3</td>
</tr>
<tr>
<td>15</td>
<td>03-11</td>
<td>11</td>
<td>63.6</td>
<td>96.0</td>
<td>7.1</td>
</tr>
<tr>
<td>15</td>
<td>04-02</td>
<td>02(^{b)})</td>
<td>69.0</td>
<td>94.0</td>
<td>6.9</td>
</tr>
<tr>
<td>15</td>
<td>04-14</td>
<td>... (^{a)})</td>
<td>80.0</td>
<td>92.0</td>
<td>6.7</td>
</tr>
<tr>
<td>15</td>
<td>05-02</td>
<td>02(^{b)})</td>
<td>... (^{c)})</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>15</td>
<td>05-08</td>
<td>08</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>15</td>
<td>05-13</td>
<td>... (^{a)})</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

a) Unvaccinated Control Animals  
b) Vaccinated with *L. pomona* bacterin and with experimental *L. hardjo* bacterin  
c) ... = not done.
TABLE 4

Hamster passive protection test. Data is from tests using serum samples collected two months postimmunization.

<table>
<thead>
<tr>
<th>No. of Cattle</th>
<th>Group Number</th>
<th>Vaccine Number</th>
<th>% Hamsters Dead</th>
<th>% Hamsters Infected</th>
<th>Average day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>01-01</td>
<td></td>
<td>72.3</td>
<td>91.5</td>
<td>7.3</td>
</tr>
<tr>
<td>15</td>
<td>01-04</td>
<td>04</td>
<td>14.0</td>
<td>51.6</td>
<td>10.0</td>
</tr>
<tr>
<td>15</td>
<td>01-06</td>
<td>06</td>
<td>00.00</td>
<td>22.6</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>02-03</td>
<td>03</td>
<td>22.0</td>
<td>80.0</td>
<td>9.0</td>
</tr>
<tr>
<td>15</td>
<td>02-05</td>
<td>05</td>
<td>34.0</td>
<td>84.0</td>
<td>8.6</td>
</tr>
<tr>
<td>15</td>
<td>02-07</td>
<td>07</td>
<td>26.6</td>
<td>72.0</td>
<td>9.4</td>
</tr>
<tr>
<td>14</td>
<td>03-09</td>
<td>09</td>
<td>12.8</td>
<td>58.5</td>
<td>8.7</td>
</tr>
<tr>
<td>14</td>
<td>03-10</td>
<td>10</td>
<td>47.1</td>
<td>88.5</td>
<td>6.7</td>
</tr>
<tr>
<td>15</td>
<td>03-11</td>
<td>11</td>
<td>38.6</td>
<td>92.0</td>
<td>6.8</td>
</tr>
<tr>
<td>15</td>
<td>04-02</td>
<td>02b)</td>
<td>25.3</td>
<td>80.0</td>
<td>8.8</td>
</tr>
<tr>
<td>15</td>
<td>04-14</td>
<td></td>
<td>72.0</td>
<td>92.0</td>
<td>6.1</td>
</tr>
<tr>
<td>15</td>
<td>05-02</td>
<td>02b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>05-08</td>
<td>08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>05-13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) Unvaccinated Control Animals
b) Vaccinated with *L. pomona* bacterin and with experimental *L. hardjo* bacterin
c) No deaths
d) ... = not done
Hamster passive protection test. Data is from tests using serum samples collected four months postimmunization.

<table>
<thead>
<tr>
<th>No. of Cattle</th>
<th>Group Number</th>
<th>Vaccine Number</th>
<th>% Hamsters Dead</th>
<th>% Hamsters Infected</th>
<th>Average day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>01-01</td>
<td>. . a)</td>
<td>90.7</td>
<td>95.3</td>
<td>5.9</td>
</tr>
<tr>
<td>15</td>
<td>01-04</td>
<td>04</td>
<td>30.6</td>
<td>80.0</td>
<td>7.6</td>
</tr>
<tr>
<td>15</td>
<td>01-06</td>
<td>06</td>
<td>14.6</td>
<td>65.3</td>
<td>8.6</td>
</tr>
<tr>
<td>14</td>
<td>02-03</td>
<td>03</td>
<td>41.4</td>
<td>90.0</td>
<td>7.9</td>
</tr>
<tr>
<td>15</td>
<td>02-05</td>
<td>05</td>
<td>52.0</td>
<td>92.0</td>
<td>7.4</td>
</tr>
<tr>
<td>15</td>
<td>02-07</td>
<td>07</td>
<td>30.6</td>
<td>75.6</td>
<td>8.2</td>
</tr>
<tr>
<td>14</td>
<td>03-09</td>
<td>09</td>
<td>20.0</td>
<td>70.0</td>
<td>10.2</td>
</tr>
<tr>
<td>14</td>
<td>03-10</td>
<td>10</td>
<td>58.5</td>
<td>91.4</td>
<td>7.5</td>
</tr>
<tr>
<td>15</td>
<td>03-11</td>
<td>11</td>
<td>50.6</td>
<td>96.0</td>
<td>6.5</td>
</tr>
<tr>
<td>15</td>
<td>04-02</td>
<td>02b)</td>
<td>25.3</td>
<td>74.7</td>
<td>9.0</td>
</tr>
<tr>
<td>15</td>
<td>04-14</td>
<td>. . a)</td>
<td>88.0</td>
<td>98.7</td>
<td>6.1</td>
</tr>
<tr>
<td>15</td>
<td>05-02</td>
<td>02b)</td>
<td>. . c)</td>
<td>. .</td>
<td>. .</td>
</tr>
<tr>
<td>15</td>
<td>05-08</td>
<td>08</td>
<td>. .</td>
<td>. .</td>
<td>. .</td>
</tr>
<tr>
<td>15</td>
<td>05-13</td>
<td>. . a)</td>
<td>. .</td>
<td>. .</td>
<td>. .</td>
</tr>
</tbody>
</table>

a) Unvaccinated Control Animals  
b) Vaccinated with *L. pomona* bacterin and with experimental *L. hardjo* bacterin.  
c) . . . = not done
**TABLE 6**

Hamster passive protection test. Data is from tests using serum samples collected six months postimmunization.

<table>
<thead>
<tr>
<th>No. of Cattle</th>
<th>Group Number</th>
<th>Vaccine Number</th>
<th>% Hamsters Dead</th>
<th>% Hamsters Infected</th>
<th>Average day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>01-01</td>
<td>...a)</td>
<td>92.3</td>
<td>96.9</td>
<td>6.6</td>
</tr>
<tr>
<td>15</td>
<td>01-04</td>
<td>04</td>
<td>30.6</td>
<td>73.3</td>
<td>8.2</td>
</tr>
<tr>
<td>15</td>
<td>01-06</td>
<td>06</td>
<td>12.3</td>
<td>57.3</td>
<td>9.0</td>
</tr>
<tr>
<td>14</td>
<td>02-03</td>
<td>03</td>
<td>47.1</td>
<td>84.2</td>
<td>8.5</td>
</tr>
<tr>
<td>15</td>
<td>02-05</td>
<td>05</td>
<td>45.3</td>
<td>85.3</td>
<td>6.7</td>
</tr>
<tr>
<td>15</td>
<td>02-07</td>
<td>07</td>
<td>32.0</td>
<td>73.3</td>
<td>8.2</td>
</tr>
<tr>
<td>14</td>
<td>03-09</td>
<td>09</td>
<td>12.8</td>
<td>74.2</td>
<td>10.2</td>
</tr>
<tr>
<td>14</td>
<td>03-10</td>
<td>10</td>
<td>45.7</td>
<td>85.7</td>
<td>7.5</td>
</tr>
<tr>
<td>15</td>
<td>03-11</td>
<td>11</td>
<td>56.0</td>
<td>90.6</td>
<td>6.5</td>
</tr>
<tr>
<td>15</td>
<td>04-02</td>
<td>02b)</td>
<td>45.3</td>
<td>81.7</td>
<td>7.9</td>
</tr>
<tr>
<td>15</td>
<td>04-14</td>
<td>...a)</td>
<td>88.0</td>
<td>97.3</td>
<td>6.0</td>
</tr>
<tr>
<td>15</td>
<td>05-02</td>
<td>02b)</td>
<td>...c)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>15</td>
<td>05-08</td>
<td>08</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>15</td>
<td>05-13</td>
<td>...a)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

a) Unvaccinated Control Animals
b) Vaccinated with *L. pomona* bacterin and with experimental *L. hardjo* bacterin.
c) ... = not done
TABLE 7
Hamster passive protection test.
Data is from tests using serum samples collected approximately 12 months postimmunization.

<table>
<thead>
<tr>
<th>No. of Cattle</th>
<th>Group Number</th>
<th>Vaccine Number</th>
<th>% Hamsters Dead</th>
<th>% Hamsters Infected</th>
<th>Average day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>01-01</td>
<td>...a)</td>
<td>89.2</td>
<td>95.3</td>
<td>6.0</td>
</tr>
<tr>
<td>15</td>
<td>01-04</td>
<td>04</td>
<td>45.6</td>
<td>81.3</td>
<td>7.8</td>
</tr>
<tr>
<td>15</td>
<td>01-06</td>
<td>06</td>
<td>14.6</td>
<td>70.6</td>
<td>8.4</td>
</tr>
<tr>
<td>14</td>
<td>02-03</td>
<td>03</td>
<td>56.4</td>
<td>92.8</td>
<td>7.8</td>
</tr>
<tr>
<td>15</td>
<td>02-05</td>
<td>05</td>
<td>70.6</td>
<td>96.0</td>
<td>6.4</td>
</tr>
<tr>
<td>15</td>
<td>02-07</td>
<td>07</td>
<td>45.3</td>
<td>88.0</td>
<td>7.2</td>
</tr>
<tr>
<td>14</td>
<td>03-09</td>
<td>09</td>
<td>18.4</td>
<td>73.8</td>
<td>8.2</td>
</tr>
<tr>
<td>14</td>
<td>03-10</td>
<td>10</td>
<td>61.0</td>
<td>88.5</td>
<td>6.7</td>
</tr>
<tr>
<td>15</td>
<td>03-11</td>
<td>11</td>
<td>78.6</td>
<td>85.3</td>
<td>6.0</td>
</tr>
<tr>
<td>15</td>
<td>04-02</td>
<td>02b)</td>
<td>96.9</td>
<td>98.5</td>
<td>4.8</td>
</tr>
<tr>
<td>15</td>
<td>04-14</td>
<td>...a)</td>
<td>100.0</td>
<td>100.0</td>
<td>4.6</td>
</tr>
<tr>
<td>15</td>
<td>05-02</td>
<td>02b)</td>
<td>...c)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>15</td>
<td>05-08</td>
<td>08</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>15</td>
<td>05-13</td>
<td>...a)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

a) Unvaccinated Control Animals
b) Vaccinated with *L. pomona* bacterin and with experimental *L. hardjo* bacterin.
c) ... = not done
TABLE 8
Hamster passive protection test. Data is from tests using serum samples collected 12 months postimmunization, just before inoculation of test cattle with live *Leptospira pomona* organisms.

<table>
<thead>
<tr>
<th>No. of Cattle</th>
<th>Group Number</th>
<th>Vaccine Number</th>
<th>% Hamsters Dead</th>
<th>% Hamsters Infected</th>
<th>Average day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>01-01</td>
<td>...a)</td>
<td>87.6</td>
<td>95.3</td>
<td>6.7</td>
</tr>
<tr>
<td>15</td>
<td>01-04</td>
<td>04</td>
<td>48.5</td>
<td>90.0</td>
<td>8.8</td>
</tr>
<tr>
<td>15</td>
<td>01-06</td>
<td>06</td>
<td>28.0</td>
<td>76.0</td>
<td>9.1</td>
</tr>
<tr>
<td>15</td>
<td>05-02</td>
<td>02b)</td>
<td>68.5</td>
<td>97.1</td>
<td>6.7</td>
</tr>
<tr>
<td>15</td>
<td>05-08</td>
<td>08</td>
<td>77.3</td>
<td>96.0</td>
<td>6.0</td>
</tr>
<tr>
<td>15</td>
<td>05-13</td>
<td>...a)</td>
<td>70.0</td>
<td>84.2</td>
<td>6.6</td>
</tr>
</tbody>
</table>

a) Unvaccinated control animals
b) Vaccinated with *L. pomona* bacterin and with experimental *L. hardjo* bacterin.
TABLE 9
Hamster passive protection test. Data is from tests using serum samples collected seven days after inoculation of test cattle with live *Leptospira pomona* organisms.

<table>
<thead>
<tr>
<th>No. of Cattle</th>
<th>Group Number</th>
<th>Vaccine Number</th>
<th>% Hamsters Dead</th>
<th>% Hamsters Infected</th>
<th>Average day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>01-01</td>
<td>. . a)</td>
<td>40.0</td>
<td>90.7</td>
<td>9.7</td>
</tr>
<tr>
<td>15</td>
<td>01-04</td>
<td>04</td>
<td>26.6</td>
<td>41.3</td>
<td>11.3</td>
</tr>
<tr>
<td>15</td>
<td>01-06</td>
<td>06</td>
<td>6.6</td>
<td>28.0</td>
<td>11.9</td>
</tr>
<tr>
<td>15</td>
<td>05-02</td>
<td>02b)</td>
<td>20.0</td>
<td>48.0</td>
<td>9.6</td>
</tr>
<tr>
<td>15</td>
<td>05-08</td>
<td>08</td>
<td>9.3</td>
<td>40.0</td>
<td>9.5</td>
</tr>
<tr>
<td>15</td>
<td>05-13</td>
<td>. . a)</td>
<td>14.7</td>
<td>62.7</td>
<td>9.5</td>
</tr>
</tbody>
</table>

a) Unvaccinated control animals
b) Vaccinated with *L. pomona* bacterin and with experimental *L. hardjo* bacterin.
**TABLE 10**

Hamster passive protection test. Data is from tests using serum samples collected 14 days after inoculation of test cattle with live *Leptospira pomona* organisms.

<table>
<thead>
<tr>
<th>No. of Cattle</th>
<th>Group Number</th>
<th>Vaccine Number</th>
<th>% Hamsters Dead</th>
<th>% Hamsters Infected</th>
<th>Average day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>01-01</td>
<td>...a)</td>
<td>00.0</td>
<td>7.6</td>
<td>---</td>
</tr>
<tr>
<td>15</td>
<td>01-04</td>
<td>04</td>
<td>1.3</td>
<td>10.6</td>
<td>5.0</td>
</tr>
<tr>
<td>15</td>
<td>01-06</td>
<td>06</td>
<td>1.3</td>
<td>17.3</td>
<td>13.0</td>
</tr>
<tr>
<td>15</td>
<td>05-02</td>
<td>02b)</td>
<td>1.3</td>
<td>6.6</td>
<td>7.0</td>
</tr>
<tr>
<td>15</td>
<td>05-08</td>
<td>08</td>
<td>2.9</td>
<td>8.6</td>
<td>9.5</td>
</tr>
<tr>
<td>15</td>
<td>05-13</td>
<td>...a)</td>
<td>2.9</td>
<td>12.9</td>
<td>12.0</td>
</tr>
</tbody>
</table>

a) Unvaccinated control animals

b) Vaccinated with *L. pomona* bacterin and with experimental *L. hardjo* bacterin.

c) ----- = No deaths
TABLE 11
Hamster passive protection test. Data is from tests using serum samples collected 21 days after inoculation of test cattle with live *Leptospira pomona* organisms.

<table>
<thead>
<tr>
<th>No. of Cattle</th>
<th>Group Number</th>
<th>Vaccine Number</th>
<th>% Hamsters Dead</th>
<th>% Hamsters Infected</th>
<th>Average day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>01-01</td>
<td>. . . a)</td>
<td>4.6</td>
<td>27.6</td>
<td>5.7</td>
</tr>
<tr>
<td>15</td>
<td>01-04</td>
<td>04</td>
<td>10.6</td>
<td>49.3</td>
<td>10.3</td>
</tr>
<tr>
<td>15</td>
<td>01-06</td>
<td>06</td>
<td>4.0</td>
<td>32.8</td>
<td>6.0</td>
</tr>
<tr>
<td>15</td>
<td>05-02</td>
<td>02 b)</td>
<td>2.8</td>
<td>15.7</td>
<td>12.0</td>
</tr>
<tr>
<td>15</td>
<td>05-08</td>
<td>08</td>
<td>0.0</td>
<td>22.6</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>05-13</td>
<td>. . . a)</td>
<td>1.3</td>
<td>25.3</td>
<td>12.0</td>
</tr>
</tbody>
</table>

a) Unvaccinated control animals  

b) Vaccinated with *L. pomona* bacterin and with experimental *L. hardjo* bacterin.  

c) —— = No deaths.
**TABLE 12**
Hamster passive protection test. Data is from tests using serum samples collected 28 days after inoculation of test cattle with live *Leptospira pomona* organisms.

<table>
<thead>
<tr>
<th>No. of Cattle</th>
<th>Group Number</th>
<th>Vaccine Number</th>
<th>% Hamsters Dead</th>
<th>% Hamsters Infected</th>
<th>Average day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>01-01</td>
<td>...a)</td>
<td>7.1</td>
<td>23.5</td>
<td>4.0</td>
</tr>
<tr>
<td>15</td>
<td>01-04</td>
<td>04</td>
<td>13.3</td>
<td>39.0</td>
<td>9.0</td>
</tr>
<tr>
<td>15</td>
<td>01-06</td>
<td>06</td>
<td>8.0</td>
<td>24.0</td>
<td>5.8</td>
</tr>
<tr>
<td>15</td>
<td>05-02</td>
<td>02b)</td>
<td>0.0</td>
<td>7.1</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>05-08</td>
<td>08</td>
<td>2.7</td>
<td>9.3</td>
<td>10.5</td>
</tr>
<tr>
<td>15</td>
<td>05-13</td>
<td>...a)</td>
<td>2.7</td>
<td>14.6</td>
<td>12.0</td>
</tr>
</tbody>
</table>

a) Unvaccinated control animals
b) Vaccinated with *L. pomona* bacterin and with experimental *L. hardjo* bacterin.
c) — = No deaths.
TABLE 13
One way analysis of variance to apply Dunnett’s procedure. A comparison of eight *L. pomona* bacterins’ immunogenicity in cattle as measured by the hamster passive protection test. Serum from vaccinated cattle was compared with serum from unvaccinated cattle.

<table>
<thead>
<tr>
<th>Vaccine Number</th>
<th>Results of Potency Test</th>
<th>Pre-immunization</th>
<th>Post immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/200</td>
<td>1/800</td>
<td>2 Months</td>
</tr>
<tr>
<td></td>
<td>Cattle Dose</td>
<td>Cattle Dose</td>
<td>Y₁ c)</td>
</tr>
<tr>
<td>3</td>
<td>87b</td>
<td>57</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>98</td>
<td>91</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>94</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>96</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>76</td>
<td>55</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>91</td>
<td>93</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>57</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>34</td>
<td>20</td>
<td>-</td>
</tr>
</tbody>
</table>

---

a) See Table 1 and S.R. B-20.
b) Number represents percent survivors in B-20 potency test
c) Y₁ = Proportion dead hamsters in test
d) Y₂ = Proportion infected hamsters in test
e) + = Significant at P < .05
f) - = Not significant at P < .05
g) Approximates significance at P < .05
Figure 1. Protective circulating antibody in cattle as measured by the hamster passive protection test. Hamster deaths. Vaccine 3 passed S.R. B-20 potency assay at 1/200 cattle dose only; Vaccine 6 passed the same assay at 1/800 cattle dose.
Figure 2. Protective circulating antibody in cattle as measured by the hamster passive protection test. Hamster renal infections. Vaccine 3 passed S.R. B-20 potency assay at 1/200 cattle dose only; Vaccine 6 passed the same assay at 1/800 cattle dose.

Figure 3. Delayed response to *I. pomona* infection in unvaccinated cattle as measured by protection against renal infection in hamsters (passive protection test).
REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

R. L. Morter, Chairman

The Committee reviewed the previous year's report and the information that has become available during the current year. Leptospirosis is an infectious disease with a host range including most vertebrates. It is global in occurrence with more than 100 pathogenic serotypes having been isolated. Of these, *Leptospira pomona*, *L. canicola*, *L. icterohemorrhagiae*, and *L. grippotyphosa*, have been isolated from cattle and swine in the United States, as well as *L. hardjo* from cattle.

The host range in the United States includes domestic animals and more than 20 common species of wildlife. This results in an enzootic and cyclic disease. The multiplicity of hosts, and the persistence of infections (in many animal populations for years) result in continued exposure of susceptible animals and perpetuation of the disease.

Evidence accumulated during the past several years indicate that the incidence of *L. pomona* has been decreasing in many areas. During the same period the relative incidence of the other four serotypes has increased. The Committee believes that the widespread use of *L. pomona* bacterins has been influential in reducing the incidence of infections due to this serotype.

It is again recommended that the Biologics Division of the Agricultural Research Service, evaluate and license all leptospiral antigens used in the macroscopic agglutination test for diagnosing leptospirosis in animals.

The Division should initiate a program of continued testing and evaluation for all such antigens to assure that specificity and sensitivity are maintained within established limits. It is also recommended that the American Association of Veterinary Laboratory Diagnosticians develop standard procedures for the macroscopic agglutination test including the use of negative and positive control sera. Further assurance of uniformity in the application of serologic procedures could result from the development of training programs in Leptospiral Laboratory. The Committee again recommends that the microscopic agglutination test is the preferred test and should be utilized whenever possible.

The widespread distribution of serotypes other than *L. pomona*, creates a need for additional mono- or multivalent bacterins. Biologic manufacturers are urged to meet this demand by producing monovalent bacterins for *L. grippotyphosa* or *L. hardjo* or a multivalent bacterin combining *L. pomona*, *L. hardjo*, and *L. grippotyphosa*.

The committee urges that Biologics Products Memo No. 44, September 1, 1969, be reviewed and updated to make it consistent with newer knowledge of leptospiral infections and the immune response to leptospiral antigens. Special considerations should be given to the requirements for data on the agglutination
lysis (microscopic agglutination) test and leptospiruria subsequent to challenge. Available data indicates that the 19S antibody (IM) is primarily responsible for agglutination whereas the 7S antibody (IA) is primarily responsible for protection. The 7S antibody can be measured by the passive hamster protection test, which provides an indicator for the immunogenic protective efficacy of a bacterin. Data on leptospiruria following challenge may be desirable, but because of the diversity of hosts and nature of the disease (described above) such data should not be a determining criteria for the licensing of leptospiral bacterins. The committee recognizes that its primary responsibility and concern is protection of the health of livestock and urges expediency by all parties to provide the bacterins urgently needed to meet this objective.

We respectfully submit this report to the Executive Committee for approval and suggest that the work of this committee be continued.
A typical textbook description of foot-and-mouth disease (FMD) in adult cattle will describe an incubation period of 1 to 8 days and clinical signs of salivation, lameness, and vesiculation of the epithelium of the tongue, gums, lips and interdigital spaces of the feet. Fever is described as preceding or accompanying the appearance of the "primary" vesicles. Those familiar with FMD will not refute such a description; however, observations made in our laboratory during recent experiments on FMD transmission from infected to susceptible cattle showed that forms of the disease, heretofore not described, exist which are fundamental to understanding the reservoir state and dissemination of the disease in the field.1,2 This report attempts to describe the different clinical patterns of experimentally-induced FMD that may be encountered in susceptible cattle after exposure to the virus.

It is well known that atypical lesions of FMD may be produced in cattle by infection with viruses modified by frequent passage in tissue culture, different animal species, or eggs. It is also possible to produce modified reactions with virulent field viruses in animals that have been immunized by vaccination or passively-immunized with immune serum. In each of these cases either the virus or the recipient animal has been put in what may be considered an abnormal state. The clinical disease patterns to be discussed here are those that may be seen when the virus is fully virulent and the recipient steer completely susceptible to FMD.

The beginning of any experimental study of the pathogenesis of FMD is the method used to expose the susceptible animal to the virus. Customarily, infection has been induced by inoculating the virus into the epithelium of the tongue. The clinical syndrome seen in cattle after use of this method of infection has been the basis for many of the descriptions of the disease found in the literature. When it became clear to us that the pharynx is the major site of initial virus growth in the bovine, we turned our attention to the type of disease seen after intranasal (IN) exposure to the virus.

To show that the disease pattern is influenced by the inoculation route, two steers, isolated from each other, were each inoculated with 100,000 tissue culture plaque-forming units of type 0, subtype 1 FMD virus. One was infected by inoculating this amount of virus in a volume of 2 ml. at several sites into the epithelium of the tongue with a 27 gauge, 1/4 inch needle. The other was infected by intranasally inoculating the same amount of infectious virus in 5 ml. This was done by slowly instilling 2.5 ml. of inoculum into each nasal cavity via a 10-cm. latex tube attached to a 5 ml. syringe. The temperature of each steer was

From the Plum Island Animal Disease Laboratory, Veterinary Sciences Research Division, Agricultural Research Service, U.S. Department of Agriculture, Greenport, New York 11944.
continuously recorded throughout the 3-day observation periods. Blood samples were taken every 6 hours and oesophageal-pharyngeal (OP) fluid samples, consisting of mucus, desquamated epithelial cells, food particles and saliva, were collected with the cup probang. The titration of infectious virus in serum extracted by centrifugation from the clotted blood samples or in OP fluid samples was done by plaque assay in primary cultures of bovine kidney cells.

A diphasic temperature response occurred (Fig. 1) in the steer inoculated in the tongue with the first peak corresponding to the development of a large vesicle at the inoculation site and the second with the appearance of vesicular lesions of the feet. A single temperature rise was seen in the intranasally-inoculated steer which coincided with the appearance of vesicular lesions on the gums, lips, muzzle and interdigital spaces of the feet. The titer of virus found in the OP fluid and serum samples helps to explain the sequence of infection occurring in the two different inoculation methods. In the steer inoculated in the tongue epithelium, the growth of the virus in the pharyngeal region was preceded by vesiculation of the tongue which resulted in the release of a large amount of infectious virus between the 12th and 20th hour after inoculation. Virus was found in the blood 8 hours after inoculation which was also attributed to the production of virus in the tongue. Thus, in this steer, the disease pattern was established by the release of a large amount of infectious virus from vesiculation of the tongue which then infected other susceptible sites in the body. The disease in the intranasally-inoculated steer followed a different sequence of events. Infection was established in the pharynx within 4 hours after IN inoculation. Growth of virus was limited to this area until the 30th hour when the first virus was apparent in the blood which was then followed 24 hours later by vesicular lesions on the feet and mouth.

The type of lesions seen also differed depending on the method of inoculation. Twenty-four hours after the inoculation of the tongue, the steer was drooling copiously. There was a large vesicular lesion limited to the area of virus inoculation. The vesicular covering was readily peeled back revealing a raw denuded submucosa. Needless to say this animal was not eating. At this same time the intranasally-inoculated steer appeared clinically normal. It responded as most have after IN inoculation of this virus in that lesions were not found on the tongue even though several were present on the adjacent areas of the lips. The major overall difference in the lesion pattern was that the IN inoculation resulted in more involvement of the nasal area characterized by copious serous and later mucopurulent discharge from the nose and far more vesiculation and involvement of the nares and muzzle.

From these results it is easy to see how the concept described in many textbooks of “primary” vesiculation of the tongue with subsequent generalization of infection to the lips, gums, and feet came about when inoculation of the tongue was used to infect the steer. However, this sequence is rarely seen when FMD transmission is studied by putting infected steers with susceptible steers. There remains little doubt as based on observations made during such transmission experiments that FMD in cattle is transmitted by aerosolation of virus from the infected animal to the upper respiratory tract of the recipient resulting in primary
infection of the pharynx.

When we began a series of studies\(^1,2\) on transmission of FMD from infected "donor" steers to a series of susceptible contact steers, the IN route of inoculation was used to infect the donors. As a result of using this procedure, clinical forms of FMD were seen which would not have been seen with the overwhelming infection induced by inoculation of the tongue. Based on these studies, we have divided the different clinical forms of FMD into three basic groups, simply called Form 1, Form 2, and Form 3. The parameters considered in describing each disease pattern were the presence of virus in the pharynx and blood as determined from OP and serum samples, presence of lesions of FMD on the head or feet, and the presence of fever and the transmissibility to susceptible steers. Each of these factors was related to the time after known exposure of the contact steer to a steer infected by IN inoculation or in steers intranasally infected in which the signs were studied directly. The summary charts presented here are the composite of many observations of similarly-treated animals.

Form 1 FMD might be called "classic" disease. It is the type of disease picture described in many textbooks (Fig. 2). The virus established itself in the pharynx shortly after the steer's exposure to the infected donor, grows to peak infectivity by day 3, and day 4 drops to lower infectivity levels primarily as secretory antibody develops. Virus may remain in the pharynx of some carrier animals for a considerable period of time. Virus rarely is found in the blood before 24 hours and remains at a uniform level for about 5½ days. Fever appears about 30 hours after exposure to the virus and is followed within a few hours by vesiculation of the feet, lips, dental pad, muzzle, and, in some cases, the tongue. Maximum lesion development is seen between the 3rd and 4th day. Antibody appears at about 5½ days and its rapid rise coincides with the rapid disappearance of circulating virus. Two peaks of maximum neutralizing activity are seen — one about the 9th day after exposure to the virus, the second at 21 days. These peaks correspond to the production of 19S and 7S antibody, respectively. Antibody remains detectable for at least 4 years after initial infection.\(^5\) Experimentally-infected steers with this form of the disease transmitted FMD to susceptible steers in which the contact period was 24 hours, for 7 days after infection. Transmission only occurred to 2 of 6 steers on day 8 and none from day 9 on. The most rapid transmission occurred on day 3 which coincides with the period of maximum lesion development. It is unlikely that this form of FMD in the field would long go undetected.

Before proceeding with the description of Form 2 and Form 3 FMD, it is necessary to mention some recent findings from our laboratory. It was found, in some cases, that after IN infection of steers with FMD virus there was frequent and unusual recovery of bovine enterovirus from OP fluid and serum samples. This virus is ubiquitous in our cattle as evidenced by significant virus-neutralizing antibody in essentially all steers. It was readily isolated from the feces but rarely was found in OP fluid samples and only from serum samples under the conditions described here. It has not produced any clinical signs of disease when inoculated into the tongue or after IN instillation in adult steers. Based on several rather involved experimental
observations, it was postulated that in some instances after IN inoculation, FMD virus stimulated the growth of resident bovine enterovirus in the pharynx so that the resulting progeny consisted of the ribonucleic acid genome of FMD virus in the viral protein coat of bovine enterovirus. Further it was postulated that virus in this form repeatedly replicated in the inoculated steer and was readily transmitted to susceptible contact steers. This proposed dual virus interaction is useful to explain the pattern of the next two forms of the disease.

Form 2 FMD has only been seen in steers after IN inoculation of a high level of infectious FMD virus after the same steers had been intranasally inoculated with a high level of infectious bovine enterovirus some 2 months before (Fig. 3). Our knowledge about some of the parameters of this form of the disease is incomplete. After inoculation, the presence of recoverable FMD virus in the pharynx has not been established, but it is known that it was never found circulating in the blood. Lesions were limited to the external nares and lips and appeared ulcerous more than vesicular. Foot lesions were never found. Fever did not occur or was transient in some steers. Of interest were the antibody responses equal to or exceeding that seen after frank infection. The transmissibility of the disease in this form is not known.

The most ominous form of FMD in reference to its epizootiology is that seen in Form 3 (Fig. 4). It was first seen in our laboratory in cattle intranasally-infected with 1000 plaque-forming units of fully virulent virus that failed to develop signs of disease in the usually expected time and in steers that had been in contact with these animals. Its major characteristic was an exceptionally long incubation period varying from 40 to greater than 120 days after exposure to an infected but asymptomatic donor steer. Foot-and-mouth disease virus was never found in the OP fluid samples even though it could be isolated from the blood of about half of the susceptible contact steers for about 4 days after contact. A fever of less than 12 hours occurred in 1 of the 8 sequentially exposed contact steers and was the only sign in any animal that might have been attributable to FMD during the incubation period. Of a group of 8 steers with this form of the disease, 6 developed clinical signs of FMD between 42 and 57 days after contact with the donor. One came down with FMD at 119 days, and 1 remained free of signs of the disease until disposed of at 160 days even though FMD virus was isolated from its blood at the end of its 24-hour contact with the apparently normal donor, and 42 days later. When disease signs did not appear in these steers, there was no evidence of modification of the disease nor was antibody ever found until after the frank disease had developed. A disturbing aspect of these experiments was the observation that this form of disease was readily transmitted from a steer intranasally inoculated with FMD virus that never developed outward signs of the disease to at least 8 consecutive daily contact steers in which the first signs of FMD were seen 42 days later. It is not difficult to see how FMD could spread over large areas with the appearance of multiple foci of infection if exposure to such an asymptomatic donor has occurred.

The conditions necessary to cause the activation of the virus to cause frank
FMD in steers with this form of infection are not known at this time. However, it is now clear that a latent form of FMD virus can occur in cattle which is undetectable except under the most exacting laboratory procedures. It is pointed out that while the main feature of this form of disease is the long incubation period, that once the disease appears it has all of the properties of the Form 1 or classic disease pattern.

In summary (Fig. 5), FMD in cattle can vary in its incubation period, state of virus residence in the pharynx and blood, clinical signs and transmissibility to susceptible animals. The clinical patterns given here are not precise and well-defined entities but are representative of the broad response that may occur when fully susceptible cattle come in contact with fully virulent FMD virus.

Figure 1. – Temperature response and titer of type O foot-and-mouth disease virus in the oesophageal-pharyngeal fluid and serum of steers after tongue epithelial and intranasal inoculation of the virus.
Figure 2. — Response pattern of Form 1 foot-and-mouth disease in cattle.

Figure 3. — Response pattern of Form 2 foot-and-mouth disease in cattle.
Figure 4. – Response pattern of Form 3 foot-and-mouth disease in cattle.
Figure 5. — Response patterns of Form 1, Form 2, and Form 3 foot-and-mouth disease in cattle.
REFERENCES


BOVINE HERPES MAMMILLITIS-LIKE DISEASE DIAGNOSED IN THE UNITED STATES


Plum Island Animal Disease Laboratory
Veterinary Sciences Research Division
Agricultural Research Service
United States Department of Agriculture
Greenport, Long Island, New York

In early August 1970 a local veterinary practitioner was contacted to treat a lame cow on a farm near Mahnomen, Minnesota. The owner discovered the cow had edema of both forelegs and slightly raised skin nodules of pencil-eraser size on all areas of the body.

A return visit was made a couple days later with the State district veterinarian, called because the skin lesions were suspicious of mange. Skin scrapings were submitted to the University of Minnesota's diagnostic laboratory, but mites were not identified. Histopathological examination of a deep scraping did reveal inclusion bodies suggestive of lumpy skin disease.

The Foreign Animal Disease Diagnostician from the Animal Health Division was immediately requested to assist the local practitioner and State Veterinarian in a complete investigation of the herd. They reported it was a closed herd of 17 adult Holstein cows and four yearling heifers. The characteristic skin nodules observed or palpated on six cows, were, on some, so numerous as to appear coalesced. Two cows had pox-like lesions on the teats and two had deep ulcerations of the flexing portion of the knee. The initially affected cow had been treated with lime-sulfur and now appeared definitely improved. The owner reported all the adult herd had experienced some form of the disease this summer.

Blood and serum from the adult cows and nodule specimens (both formalin-fixed and frozen) from two affected animals were couriered to the Plum Island Animal Disease Laboratory (PIADL).

Histopathologic examination of the formalin-fixed nodules revealed no inclusion bodies. Mycelial fragments, indicative of cutaneous streptothricosis, a mycotic dermatitis caused by Dermatophilus congolensis, was observed in one specimen.

The frozen nodules, each ground to a 10% suspension in tryptose phosphate broth containing 1000 LU penicillin and 1000 micrograms of streptomycin per ml., were centrifuged 15 minutes at 1000 rpm. The supernatant fluid was used as the

---

1 University of Minnesota Diagnostic Laboratory, St. Paul, Minnesota.
2 Animal Health Division, ARS, USDA, St. Paul, Minnesota.
3 State District Veterinarian, Ada, Minnesota.
inoculum. Macerated blood clots were present and used undilute.

Two 18-month-old Herford steers were each inoculated with a nodule suspension and a blood sample from one cow by three routes: 3 ml. intravenously (I.V.), 2 ml. intradermally (I.D.) in multiple sites, and 2 ml. subcutaneously (S.C.). Both steer remained clinically normal throughout the observation period.

Each blood and nodule specimen (0.5 ml. each) was inoculated onto two monolayers of primary bovine kidney (BK) tissue cultures in prescription bottles. Following 40-minutes adsorption at 37°C, 10 ml. of Hank’s saline containing 0.5% lactalbumin hydrolysate (HLH) with 200 I.U. of penicillin and 200 micrograms of streptomycin per ml. were added, and incubation continued.

The cultures, observed daily, showed no cytopathogenic effect (CPE) through 7 days post inoculation (DPI), at which time each monolayer was disrupted by freezing and thawing twice and 1 ml. of each subinoculated onto new BK cultures. After 48 hours incubation small foci of CPE were observed in the monolayers subinoculated from blood and tissue of one cow (No. 4506). When these were again passaged in BK monolayers, large areas of CPE typical of herpes virus was observed after 2 days incubation. Syncytia formation and the appearance of multinucleated giant cells preceded complete disruption and detachment of the cell sheet 1-3 days later. At the sixth passage in BK cell cultures the virus titer was \(5 \times 10^5\) per ml., expressed as the greatest dilution of virus producing CPE in at least 1 of 2 inoculated cultures.

Infected BK monolayers were examined in the electron microscope. Sections showed typical developmental stages of herpes virus. Particles were found in the nucleus, budding from the nucleus and in the cytoplasm. Other sections showed enveloped particles in the cytoplasm and extra-cellular spaces. These were very similar to the virus associated with herpes disease in man and ducks.

Two additional steers were moved into the room with the other two, 10 days following the initial inoculations. One was left as an uninoculated contact control, while the other, (No. 539) was inoculated by the same three routes, I.V., I.D., and S.C., with fluid taken from BK cultures at 3 DPI, containing the third passage of the Minnesota isolate. Each steer was examined and its rectal temperature was recorded once each day. Steer No. 539 developed a rise in temperature to 103.6°F at 2 DPI and again at 4 DPI. Small skin eruptions were palpated along both sides of the neck on the 6th day and a few hours later had extended to both front and rear legs, along the back and over the rib cage. The eruptions were very sensitive to pressure and were more easily palpated than seen. Hair could easily be removed from the nodules’ centers.

The uninoculated contact steer did not develop skin lesions, or clinical illness.

A 10% suspension of a nodule, excised at 10 DPI from steer No. 539 produced CPE on BK cultures. A lactating Guernsey cow was inoculated I.D. with fluid obtained from this BK culture at 3 DPI in the teats, mammary-gland area and neck. She developed very large nodules (1 in. x 1 in.) at the site of inoculation on the neck and several smaller nodules (pencil-eraser size) on the uninoculated sites of the neck, abdomen and hind legs. Lesions on two inoculated teats and the skin of the
Udder were small and firm, covered with non viable epithelium, and did not spread to uninoculated areas.

To test the effect of the Minnesota isolate in small laboratory animals, fluid from 6th-passage BK cultures was used. One to three day old suckling mice were each inoculated with 0.05 ml., either S.C. or intraperitoneally (I.P.). Reddened areas of the skin were localized around the site of the S.C. injection. Vesicles appeared on the noses, and reddened bands developed on the skin of the feet, tail and body of those inoculated I.P. Mortality was 100% in both groups within 7-10 days.

Lesions were not produced in guinea pigs inoculated S.C. However, erythema with later exfoliation developed in others in the foot-pad inoculation sites.

Bovine herpes mammillitis (BHM) virus was recently isolated in England4,8,10,14 and Scotland3,6, and the Allerton herpes virus has been associated with a mild form of lumpy skin disease in Africa.1,5 To test for antigenic similarities with these viruses, neutralization tests were performed.

The Allerton virus, received from Kabete, South Africa, as passage No. 8 in lamb testes tissue culture, was passaged at the PIADL in BK cultures. The BHM virus was received from Dr. Gibbs, Bristol University, England as the 8th passage in calf kidney cells, and was also passaged in BK cultures. The Minnesota virus isolate, in its 6th passage BK culture, had initially been recovered from blood of the cow No. 4506.

Serums from cattle from the Minnesota herd (three different bleeding dates), from PIADL steer No. 539 and from rabbits hyperimmune to BHM (Sent by Dr. Gibbs) were tested against all three viruses. All sera were heat-inactivated to 56°C for 30 minutes.

Tests were performed in BK tissue cultures in prescription bottles, using two cultures per dilution. Serial ten-fold dilutions of virus were made. 1/5 dilution of serum was mixed with an equal volume of each dilution of virus and incubated 30 minutes in a 37°C water bath. Each monolayer received 0.2 ml. of virus-serum mixture which was adsorbed for 40 minutes at 37°C before 10 ml. of HLH without serum was added. Cultures incubated at 37°C were observed daily for CPE for 7 days.

Serums from the Minnesota herd causing neutralization were those of the two cows from which skin lesions were initially excised (Nos. 4506 & 4501). Serums from these two cows neutralized both the BHM and Minnesota viruses, as well as the Allerton virus (Table 1). BHM rabbit hyperimmune serum and 16 DPI serum from PIADL steer No. 539 (Minnesota virus inoculated) neutralized all three viruses. Serological relationships between the Allerton herpes virus and BHM have previously been reported.6

This disease syndrome has been previously described in the United States,12 but this is the first isolation of the etiologic agent. Lesions in other animals, however, could easily have been mistaken as insect bites, mange, ringworm or even overlooked because of the inapparent illness associated with the infection. The Minnesota herpes virus, when initially inoculated systemically, produced the skin
lesions characteristic of BHM. Since mycelium, suggestive of *D. congolensis*, was observed upon histological examination of a formalin-fixed nodule from Minnesota, its role in this particular disease syndrome must be further investigated.

ACKNOWLEDGEMENT

The authors acknowledge the services of Dr. Ronald S. Goos, veterinary practitioner, Mahnomen, Minnesota, and for his alertness in contacting State Veterinarian concerning this disease and also Dr. A. C. Pier for his examination for *D. congolensis*.

### TABLE 1

**VIRUS NEUTRALIZATION TESTS OF SERA**

<table>
<thead>
<tr>
<th>Sera Identification</th>
<th>Bleeding Date or DPI</th>
<th>Viruses</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minn</td>
<td>BHM</td>
<td>Allerton</td>
</tr>
<tr>
<td>No. 4501¹</td>
<td>8-21-70</td>
<td>0⁴</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9-3-70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9-18-70</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10-7-70</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>No. 4506¹</td>
<td>8-21-70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9-3-70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9-18-70</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10-7-70</td>
<td>5</td>
<td>5.5</td>
</tr>
<tr>
<td>No. 539²</td>
<td>0 DPI</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. 539²</td>
<td>16 DPI</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>BHM rabbit hyperimmune serum</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

¹Minnesota cattle from which skin lesions were excised  
²PIADL steer inoculated with BK culture  
³Sent by Dr. Gibbs  
⁴V.N. index — recorded as log₁₀ of virus neutralized
REFERENCES

PATHOGENESIS OF AFRICAN SWINE FEVER IN PIGS

A. H. Dardiri and W. R. Hess

INTRODUCTION

The close resemblance in signs and lesions of African swine fever (ASF) and hog cholera (HC) in susceptible pigs\textsuperscript{1,2,3} requires differential diagnosis of the 2 diseases by laboratory means. The ASF virus is detected by the hemadsorption (HA)\textsuperscript{4} and fluorescent antibody (FA) tests\textsuperscript{5,6}. Complement fixation (CF)\textsuperscript{7} and agar gel diffusion precipitation (AGDP)\textsuperscript{8} tests are also useful in detecting antibodies in pigs subsequent to their exposure to the disease.

The purpose of this work explored the pathogenesis of ASF in the acute and chronic disease. The above mentioned tests were used to determine the time of appearance and persistence of the virus and antibodies. Information developed will be beneficial to those who are concerned with the evolution of the disease in the field as well as the diagnosticians responsible for selecting suitable specimens and processing them in the laboratory.

MATERIALS AND METHODS

\textbf{Virus.} Virulent ASF virus isolated from pigs in Dakar, Africa, and further passaged 9 times in pigs was used as a 10\% suspension (w/v) of spleen tissue in blood from a pig which died as a result of ASF infection. Previous infectivity tests using a similar inoculum indicated a survival rate of 20-40\% was likely.

\textbf{Animals and exposure.} Twenty 50-lb. Yorkshire-Tamworth pigs were used. Blood samples were obtained from all the pigs, and then 18 were inoculated intramuscularly with 1 ml. of suspension containing $10^{3}$ pig 50\% lethal doses (LD\textsubscript{50}) of ASF virus.

For determination of the pathogenesis of ASF, the following information was compiled: thermal response, clinical signs and macroscopic lesions, development of leukopenia, staining of leukocyte smears and cultures with fluorescein-conjugated ASF antibodies, viremia, and formation of CF and AGDP antibodies. Every other day, subsequent to exposure of the pigs, 2 blood samples were taken from the anterior vena cava of each pig. One of the blood samples was drawn in heparin to contain 0.1 mg/ml and the other sample was allowed to coagulate, before the serum was collected.

\textbf{Leukocyte cultures and smears.} From 10 ml. of heparinized blood, the leukocyte layer was separated by centrifugation, collected, and mixed with 2 ml. of normal swine serum, 2 ml. of heparinized plasma from the same pig; and 2 ml. of Eagle's essential medium. Leukocyte cultures were made by dispensing 1 ml. in each of four Leighton tubes containing cover glass slides. Five blood smears were also made on the glass slides; they were prepared by spreading evenly to a thin film one drop of the cellular suspension on each slide.

From the Plum Island Animal Disease Laboratory, Veterinary Sciences Research Division, Agricultural Research Service, U.S. Department of Agriculture, Greenport, Long Island, N.Y.
Impression smears. At necropsy, small pieces of tissues were collected aseptically and pressed against a glass slide in several locations of the glass surface.

**Virus assay.** Heparinized blood samples were diluted decimally and tested in leukocyte cultures from noninfected pigs. The cultures were examined daily for 5 days. Tissues collected aseptically at necropsy were weighed, ground in a mortar and prepared as a 10% suspension (w/v) in Eagle’s essential medium. The suspensions were centrifuged, and the supernatant fluid was diluted decimally and inoculated into porcine leukocyte cultures which had been prepared previously. Cultures were observed daily for 8 days for HA. Cultures considered to have a negative or suspicious reaction were subpassaged twice in freshly prepared pig leukocyte cultures. Fifty percent HA endpoints were calculated by the method of Reed and Meunch.9

**Fluorescent antibody tests.** Preparation of the fluorescein-labeled antibody and the technique for the FA test was that described by Heuschele et al.5

**Serology.** The serums were tested for CF and AGDP antibodies during 124 DPI. The CF test followed the method described by Cowan7. Antigen used for these tests was prepared according to the method reported by Hess et al.10

**Detection of virus in surviving pigs.** At 45, 90 and 135 DPI, 30 ml. of blood was collected in heparin from each of the 4 surviving pigs. Each of these samples was inoculated IM at multiple sites, in one susceptible pig. Each inoculated pig was housed separately. Clinical disease signs and their thermal responses were observed and recorded daily during an additional 60 days. Serums from the surviving pigs were examined by the AGDP test.

**RESULTS**

**Clinical features.** Of the 18 pigs given the virus IM, one died at 8 DPI and another at 20 DPI. Two pigs survived, but the rest died between the 9th and 12th DPI. In the animals that died 8-12 DPI, disease signs started with a fever of 103°-104° on the 4th DPI. This was followed by a marked temperature rise to 105°-107°. During the next 4 days, the temperature reached 106°-108° and then declined for 1-2 days to 107°-102.6°, after which the animals died. Two to four days before death, the recumbent pigs were lying huddled together and were reluctant to move. Constipation was followed by diarrhea tinged with blood. The pigs appeared dull and weak, occasionally coughing or making snorting sounds. Before death, the animals were comatose, lying on one side. Their eyes were crusty, they ground their teeth, and they grunted when disturbed. The pig that died 20 DPI had a persistent fever of 105°-106° beginning 4 DPI with two peaks of 107.4° at 7 DPI and 104.8° at 19 DPI. During this period, the pig lost appetite and weight. Diarrhea was relatively mild.

The temperature pattern of the pigs that died was characterized by 3-4 days of normal temperature, a period of hyperthermia, and gradual rise, than a sharp dip only one day before death. The pigs surviving inoculation had a temperature of 104°-106° 10-20 DPI. The next 13 days, the temperature range remained between 101.2° and 103.6°. During the following 22 days, the range was 104°-106.2°, after
which it declined to 101°-102°.

The febrile response in the 2 contact control pigs, 5-12 DPI, was 104°-107.2°. Between the 13th and 19th DPI, the temperature descended to 104°-102.8°, and on the 20th DPI, the temperature was 104.2°; the range for the next 6 days was 105.2°-107.0° after which it gradually declined to normal.

**Detection of ASF virus by HA reaction.** Hemadsorption reaction was detected in leukocyte cultures prepared from the infected blood two days earlier than by inoculation of the same blood on cultures prepared from blood of healthy pigs. The HA reaction on the leukocytes, from the infected blood, appeared 2 days after incubation of the cultures. Viremia was detected by this means throughout the course of the disease until the pigs died. In the surviving pigs, the leukocyte cultures from the infected blood had HA reaction, which was detected at a longer period than when the same blood was inoculated on normal leukocyte cultures. In the 4 surviving pigs, the HA reaction was demonstrated in their leukocyte cultures at 40, 46, 61 and 56 DPI. However, when the same blood was inoculated into normal leukocyte cultures, the HA reaction was produced at 21, 22, 16 and 15 DPI respectively. The nature of the hemadsorption reaction in the 2 types of cultures was identical with the appearance of inclusion bodies.

**Leukopenia.** The daily total leukocyte counts indicated a marked and steady decrease from the first day of temperature rise until death. This observation is in agreement with DeTray and Scott (1957)\(^1\). A similar pattern in leukopenia was observed in pigs that survived, however, all counts decreased gradually for about 2 weeks, and then increased gradually to normal in 3-4 weeks.

**Virus concentration in blood of infected pigs.** Present in Table 1 is the concentration of ASF virus in the blood of 20 pigs involved in this work. Viremia was present in 14 of 16 pigs by 4 DPI and was detected by inoculation of normal leukocyte cultures. In the majority of pigs, the virus concentration was usually highest at 6 and 8 DPI during the hyperemic period. Immediately before death, the level of virus decreased slightly. The level of virus titer in the 2 pigs which survived IM inoculation were similar to those of the pigs that died. Viremia was detected after 6 and 10 DPI respectively and remained through 20 DPI. The virus titer increased gradually, reaching a peak which was maintained for 3-4 days and then declined, until HA could not be detected. Pigs infected by contact had viremia at 10 and 18 days, respectively, with a relatively low titer which was detectable for at least 12 days.

**Distribution of ASF virus in pig tissues.** Virus was found in ample quantity in the 10 tissues harvested from 16 pigs (Table 2). The gastric lymph nodes, spleen, blood, and liver rendered relatively high virus titer and these tissues could readily serve for virus isolation in disease diagnosis.

**Virus lesions in tissue.** The frequency distribution of macroscopic lesions of pigs that died 8, 9, 10, 11, 12, and 20 DPI are summarized in Table 3. Predominant lesions most consistently exhibited conjunctivitis, peripheral hemorrhage in lymph nodes, tonsillitis, petechiation in the epiglottis and pericardium, broncho-pneumonia, pneumatic ecchymosis, gastritis, petechiation of the gastric and
intestinal mucosa, splenic enlargement, renal petechiation and ecchymosis, and congestion of the gall bladder.

**Complement-fixing antibodies.** Complement-fixing antibodies were found in 5 of 16 serums taken one day before the pig died. Pigs having CF antibodies died 8, 10 and 12 DPI. The serums reacted at a dilution of 1:20. The CF antibody level in serums from four surviving pigs is presented in Table 4. Antibodies began to appear on the 14th DPI, the level increasing and remaining in 3 of the pigs through 124 DPI. The CF antibody response in the 4th pig was less than that of his 3 pen-mates, and persisted for only 90 DPI.

**Agar gel diffusion precipitin antibodies.** Precipitating antibodies were not detected in serums of the pigs which died. Data relative to the surviving 4 pigs is presented in Table 4. Two pigs that recovered from IM infection had AGDP antibodies which were first detected 18 DPI, and persisted until the 116th DPI (Table 4). Serum titers reached 1:16 to 1:32 and then declined. The AGDP antibody level in the 2 contact pigs were lower than those in the infected pigs, but it was found within a similar period.

**Immunofluorescence.** Specific fluorescence was visible in the leukocyte smears and cultures as bright fluorescent cytoplasmic inclusion bodies or diffuse, somewhat granular, fluorescence in the entire cytoplasm. Leukocyte smears from pigs that died gave nonspecific fluorescence in specimens obtained 2, 4, and 6 DPI, but specific immunofluorescence was seen in smears made at 8, 10, 12, and 14 DPI. It was also seen 16, and 20 DPI, but with fewer foci. Fluorescence was found at 10-20 DPI in the leukocyte smears from 2 pigs that were given IM inoculation of ASF. Viral antigen was observed in leukocyte cultures with marked intensity, starting 2 DPI and remaining until lysis of the cells. Viral antigen was detected only in leukocyte smears from the pigs which survived IM inoculation, 14-18 DPI; however, it was demonstrated continuously in leukocyte cultures from 10-34 DPI. There were fewer foci and it was found irregularly between 48 and 80 DPI. Specific immunofluorescence was observed in leukocytes cultured from the 2 contact pigs 16 and 20 days after exposure by contact, remained detectable for 20 and 26 days. Impression smears from the spleen, lung, liver, kidneys, gastro-hepatic lymph nodes, brains and bone marrow had viral antigen; the highest incidence of fluorescence was in the spleen, lymph nodes and lung tissue.

**Detection of virus in surviving pigs.** Inoculation into susceptible pigs of 45 DPI blood specimens from pigs surviving experimental ASF infection caused death after 9-14 DPI. Four pigs given 90-DPI blood died after 23, 26, 20, or 37 days. Death was preceded by a febrile response of 104°-107° for 12, 13, 11, and 9 days, respectively. Though samples collected 135 DPI from 3 pigs killed susceptible pigs (Table 5), blood from a fourth pig did not cause death. The disease course in these pigs subinoculated was 23, 27, and 31 days. Death was preceded by high body temperatures, for 10-14 days. Clinical signs and lesions of ASF were observed in the recipient pigs, and their tissues and blood produced hemadsorption on pig leukocyte cultures.
Information was developed relevant to the sequence of infection and serologic response to ASF virus in experimentally infected pigs. Twenty pigs were exposed to the virus: 18 were inoculated with virulent ASF virus and 2 served as contact controls. Two inoculated pigs, and both contact pigs, survived infection following manifestation of the disease features. Fifteen pigs died between 8 and 12 DPI, and one lived for 20 days. The thermal response in 12 pigs was a gradual or sudden rise for 3-4 days, to a peak of 107° to 108°; the temperature then decreased 1-2 degrees and remained constant for several days, at 106°-105° until the pigs died. In 3 pigs, temperature declined sharply the day before death. The thermal pattern of 4 surviving pigs was characterized by a diphasic curve. The temperature climbed to a peak, 105°-107°, declined gradually to 102°-104°, ascended again to 105°-106°, and then later gradually decreased to 102.6°. During the temperature rise, ASF virus was isolated from the pigs' blood. This observation stimulates interest to investigate more fully the mechanism of virus replication and release in the blood of chronically infected pigs as compared to that in the acute disease form. Virus concentrations in 10 organs collected from each pig indicated a high virus titer in the organs with an abundance of reticuloendothelial cells such as lymph nodes, spleen, and blood. The fact that ASF virus was detected sooner and for a longer period in blood of pigs with acute and subacute disease by using leukocyte cultures from the infected blood, offers an additional method for rapid detection of ASF virus by the HA reaction. This method would be advantageous in an outbreak when it might be difficult to find donor pigs not exposed to the disease. A time saving appears evident because the HA reaction in leukocyte cultures of infected blood may appear within 24-48 hrs., the time usually allowed for growing normal leukocyte cultures. Detection of low levels of virus seems more likely in leukocyte cultures from infected blood as the entire culture is, in effect, the inoculum.

Immunofluorescent examination of leukocyte cultures from the IM-inoculated pigs revealed viral antigen in the leukocyte cultures sooner than it was in the smears prepared from the same leukocytes. Viral antigen was detected from 2 DPI until death. Specific fluorescence was found in leukocyte cultures prepared from the 2 surviving contact pigs beginning 12 DPI. Furthermore, specific immunofluorescence was identified clearly in the leukocyte cultures from all the surviving pigs at late as 34 DPI and, intermittently, up to 80 DPI. The delay in the appearance of the fluorescent antigen in the leukocyte smears may be due to the need for a higher concentration of antigenic mass for a strong reaction and subsequent clear visibility. The appearance of intense and specific fluorescence in the leukocyte cultures was probably because of the additional 48-hour multiplication period in the culture, resulting in a larger amount of antigenic material for the fluorescent reaction.

In serums from 3 of the 16 pigs which died, 8, 10, and 12 DPI, respectively, CF antibodies were found at a serum dilution of 1:20. The significance of this level as specific response to ASF virus exposure is not know. In the pigs which survived infection. CF antibodies appeared at 19 DPI and persisted through 88-124 DPI.
Antibodies were detected by AGDP test between 18 and 116 DPI only, in the 4 pigs which recovered. However, AGDP antibody level was lower than CF antibody titers.

Blood from the 4 surviving pigs contained virus at 45 and 90 DPI. Blood collected 135 DPI from 3 pigs produced the disease in susceptible pigs. The blood from one surviving pig did not cause mortality or any serologic response upon inoculation into another pig. Pigs which apparently recover from ASF usually are virus carriers. Further work to define the carrier state of an ASF infection is warranted.

**SUMMARY**

The progressive response of pigs to experimental infection with African swine fever (ASF) virus, Dakar isolate, was pursued in pigs exposed by intramuscular inoculation or by contact. The following information was developed:

1. Most pigs died one or two days after temperature peak. A biphasic thermal response occurred in pigs surviving the acute disease.
2. Hemadsorption and fluorescent antibody reactions were detected earlier and over longer periods in leukocyte cultures prepared from blood of infected pigs than by the "conventional methods" of inoculation of leukocyte cultures from noninfected pigs.
3. Daily total blood count revealed leukopenia.
4. Gastrohepatic lymph nodes, spleen, blood, and liver had high virus titer and appeared to be the tissues of choice for virus isolation.
5. Immunofluorescence was detected in impression smears from the spleen, lungs, kidneys, gastrohepatic lymph nodes, with the spleen and lymph nodes having the highest incidence.
6. Complement-fixation and agar gel diffusion precipitin antibodies were detected in the tissues for about 110 days in sera of pigs which survived the disease.
7. In the absence of detectable viremia, blood collected from 3 of 4 infected pigs 135 DPI was infectious to susceptible pigs.

**ACKNOWLEDGEMENT**

The authors are grateful to Mrs. Joan L. Grohoski, and Messrs. David Perkins and Peter Mikicuik for their technical assistance.
<table>
<thead>
<tr>
<th>Pig No.</th>
<th>1</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>22</th>
<th>24</th>
<th>26</th>
<th>28</th>
<th>30</th>
<th>32-124</th>
</tr>
</thead>
<tbody>
<tr>
<td>1873</td>
<td>3*</td>
<td>5</td>
<td>4</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>
</tr>
<tr>
<td>1874</td>
<td>3</td>
<td>5</td>
<td></td>
<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>1875</td>
<td>4</td>
<td></td>
<td></td>
<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>1876</td>
<td>1</td>
<td>5</td>
<td></td>
<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>1877</td>
<td>2</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>
</tr>
<tr>
<td>1879</td>
<td>4</td>
<td>2</td>
<td>4</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>
</tr>
<tr>
<td>1880</td>
<td>4</td>
<td>5</td>
<td></td>
<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>1883</td>
<td>4</td>
<td>5</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>
</tr>
<tr>
<td>1885</td>
<td>4</td>
<td>5</td>
<td></td>
<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>1889</td>
<td>3</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>
</tr>
<tr>
<td>1890</td>
<td>4</td>
<td>4</td>
<td></td>
<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>1923</td>
<td>2</td>
<td>4</td>
<td>5</td>
<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>1928</td>
<td>4</td>
<td>4</td>
<td>5</td>
<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>1887</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1882**</td>
<td>N</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>1925**</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>1931</td>
<td></td>
<td></td>
<td></td>
<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>1932</td>
<td></td>
<td></td>
<td></td>
<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>Contact</td>
<td></td>
<td></td>
<td></td>
<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>
</tr>
<tr>
<td>2049**</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>U</td>
<td>1</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>2050**</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>U</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

* Virus concentrations - Log 10 HAD50/ml
U Undiluted
** Survivors
N Nondetectable
Table 2. The distribution of ASF virus in the tissue of pigs which died from experimental infection.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Gastro</th>
<th>hepatic</th>
<th>L. node</th>
<th>Pharynx</th>
<th>Tonsils</th>
<th>Spleen</th>
<th>Bone</th>
<th>Marrow</th>
<th>Blood</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Lung</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1873</td>
<td>5.4*</td>
<td>5.6</td>
<td>5.8</td>
<td>6.2</td>
<td>6.0</td>
<td>7.0</td>
<td>4.0</td>
<td>5.6</td>
<td>6.2</td>
<td>6.0</td>
<td>5.8</td>
<td>5.0</td>
<td>4.6</td>
</tr>
<tr>
<td>1875</td>
<td>6.2</td>
<td>5.0</td>
<td>7.0</td>
<td>5.8</td>
<td>6.2</td>
<td>6.0</td>
<td>7.0</td>
<td>6.4</td>
<td>7.0</td>
<td>6.8</td>
<td>4.0</td>
<td>7.0</td>
<td>3.0</td>
</tr>
<tr>
<td>1877</td>
<td>7.0</td>
<td>6.6</td>
<td>7.6</td>
<td>4.0</td>
<td>6.4</td>
<td>7.0</td>
<td>6.2</td>
<td>4.6</td>
<td>4.6</td>
<td>6.2</td>
<td>4.6</td>
<td>7.0</td>
<td>6.0</td>
</tr>
<tr>
<td>1887</td>
<td>6.0</td>
<td>4.0</td>
<td>6.6</td>
<td>6.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>4.4</td>
<td>6.8</td>
<td>6.4</td>
<td>6.8</td>
<td>5.2</td>
<td>5.2</td>
</tr>
<tr>
<td>1889</td>
<td>5.0</td>
<td>4.2</td>
<td>7.2</td>
<td>5.4</td>
<td>5.8</td>
<td>5.4</td>
<td>5.0</td>
<td>7.0</td>
<td>6.0</td>
<td>5.2</td>
<td>6.2</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>1923</td>
<td>7.8</td>
<td>6.0</td>
<td>6.6</td>
<td>6.6</td>
<td>5.6</td>
<td>4.4</td>
<td>5.6</td>
<td>6.0</td>
<td>5.2</td>
<td>5.2</td>
<td>5.2</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>1932</td>
<td>7.6</td>
<td>6.8</td>
<td>5.2</td>
<td>7.0</td>
<td>6.0</td>
<td>7.0</td>
<td>6.4</td>
<td>6.0</td>
<td>5.2</td>
<td>6.2</td>
<td>6.2</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>1874</td>
<td>5.6</td>
<td>5.0</td>
<td>6.2</td>
<td>4.8</td>
<td>6.4</td>
<td>6.0</td>
<td>5.2</td>
<td>6.2</td>
<td>5.2</td>
<td>5.2</td>
<td>5.2</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>1876</td>
<td>7.0</td>
<td>5.8</td>
<td>6.4</td>
<td>4.0</td>
<td>5.8</td>
<td>6.6</td>
<td>4.2</td>
<td>5.0</td>
<td>5.4</td>
<td>5.2</td>
<td>5.2</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>1879</td>
<td>6.8</td>
<td>6.0</td>
<td>7.0</td>
<td>7.0</td>
<td>5.6</td>
<td>6.8</td>
<td>4.4</td>
<td>5.2</td>
<td>4.4</td>
<td>5.2</td>
<td>5.2</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>1880</td>
<td>5.8</td>
<td>6.2</td>
<td>4.8</td>
<td>7.2</td>
<td>6.2</td>
<td>6.2</td>
<td>5.6</td>
<td>6.2</td>
<td>6.2</td>
<td>5.2</td>
<td>6.2</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>1883</td>
<td>7.2</td>
<td>4.6</td>
<td>7.0</td>
<td>6.4</td>
<td>5.8</td>
<td>7.0</td>
<td>4.5</td>
<td>5.2</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>3.8</td>
<td>4.0</td>
</tr>
<tr>
<td>1890</td>
<td>6.4</td>
<td>2.6</td>
<td>6.8</td>
<td>6.8</td>
<td>6.0</td>
<td>5.4</td>
<td>7.0</td>
<td>4.8</td>
<td>4.8</td>
<td>4.8</td>
<td>4.8</td>
<td>3.8</td>
<td>5.2</td>
</tr>
<tr>
<td>1828</td>
<td>6.0</td>
<td>6.0</td>
<td>5.8</td>
<td>5.6</td>
<td>6.0</td>
<td>5.0</td>
<td>5.2</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>1931</td>
<td>7.0</td>
<td>3.8</td>
<td>6.4</td>
<td>4.8</td>
<td>5.8</td>
<td>4.2</td>
<td>5.0</td>
<td>4.6</td>
<td>4.6</td>
<td>4.6</td>
<td>4.6</td>
<td>4.0</td>
<td>5.2</td>
</tr>
<tr>
<td>1885</td>
<td>6.8</td>
<td>5.0</td>
<td>6.8</td>
<td>6.0</td>
<td>5.6</td>
<td>5.0</td>
<td>5.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>5.2</td>
<td>5.2</td>
</tr>
</tbody>
</table>

* Log HAD50/g
AFRICAN SWINE FEVER IN PIGS

Table 3. Incidence of lesions in pigs which died following experimental infection with African swine fever.

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Number affected/Number infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctival congestion</td>
<td>12/12</td>
</tr>
<tr>
<td>Erythema</td>
<td>6/12</td>
</tr>
<tr>
<td>Subcutaneous hemorrhage</td>
<td>1/12</td>
</tr>
<tr>
<td>Lymphatic peripheral hemorrhage</td>
<td>10/12</td>
</tr>
<tr>
<td>Larynx edema</td>
<td>1/12</td>
</tr>
<tr>
<td>Tonsil congestion</td>
<td>6/12</td>
</tr>
<tr>
<td>Hemothorax</td>
<td>1/12</td>
</tr>
<tr>
<td>Epiglottis petechiation</td>
<td>9/12</td>
</tr>
<tr>
<td>Hydropericardium</td>
<td>2/12</td>
</tr>
<tr>
<td>Epicardial petechiation</td>
<td>10/12</td>
</tr>
<tr>
<td>Bronchopneumonia</td>
<td>9/12</td>
</tr>
<tr>
<td>Pneumonic ecchymosis</td>
<td>9/12</td>
</tr>
<tr>
<td>Gastric hemorrhage</td>
<td>8/12</td>
</tr>
<tr>
<td>Gastric edema</td>
<td>4/12</td>
</tr>
<tr>
<td>Gastric serosa petechiation</td>
<td>6/12</td>
</tr>
<tr>
<td>Gastric mucosa petechiation</td>
<td>11/12</td>
</tr>
<tr>
<td>Enteritis small intestine</td>
<td>7/12</td>
</tr>
<tr>
<td>Severe intestinal serosal and mucosa petechiation</td>
<td>8/12</td>
</tr>
<tr>
<td>Button ulcers</td>
<td>1/12</td>
</tr>
<tr>
<td>Colonic petechiation</td>
<td>2/12</td>
</tr>
<tr>
<td>Splenic enlargement</td>
<td>12/12</td>
</tr>
<tr>
<td>Renal cortex petechiation</td>
<td>12/12</td>
</tr>
<tr>
<td>Cholecystic petechiation</td>
<td>7/12</td>
</tr>
<tr>
<td>Cystic petechiation</td>
<td>3/12</td>
</tr>
<tr>
<td>Cerebral congestion</td>
<td>3/12</td>
</tr>
<tr>
<td>Petechiation of omentum and mesentery</td>
<td>1/12</td>
</tr>
</tbody>
</table>
Table 4. Complement-fixation and agar gel diffusion precipitin antibody titers in serum of pigs surviving experimental infection with ASF virus.

<table>
<thead>
<tr>
<th>DPI</th>
<th>#1882</th>
<th>#1925</th>
<th>#2049</th>
<th>#2050</th>
<th>#1882</th>
<th>#1925</th>
<th>#2049</th>
<th>#2050</th>
</tr>
</thead>
<tbody>
<tr>
<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>
</tr>
<tr>
<td>4</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>
</tr>
<tr>
<td>8</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>
</tr>
<tr>
<td>14</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>
</tr>
<tr>
<td>18</td>
<td>#40</td>
<td>80</td>
<td>10</td>
<td>20</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>40</td>
<td>80</td>
<td>20</td>
<td>20</td>
<td>8</td>
<td>16</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>28</td>
<td>80</td>
<td>160</td>
<td>40</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>32</td>
<td>80</td>
<td>40</td>
<td>10</td>
<td>40</td>
<td>16</td>
<td>16</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>38</td>
<td>40</td>
<td>40</td>
<td>10</td>
<td>40</td>
<td>16</td>
<td>16</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>42</td>
<td>40</td>
<td>80</td>
<td>10</td>
<td>40</td>
<td>16</td>
<td>16</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>46</td>
<td>80</td>
<td>80</td>
<td>10</td>
<td>10</td>
<td>16</td>
<td>16</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>52</td>
<td>80</td>
<td>80</td>
<td>10</td>
<td>10</td>
<td>32</td>
<td>4</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>62</td>
<td>160</td>
<td>40</td>
<td>10</td>
<td>40</td>
<td>16</td>
<td>16</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>66</td>
<td>80</td>
<td>80</td>
<td>20</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>72</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>80</td>
<td>640</td>
<td>40</td>
<td>80</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>88</td>
<td>640</td>
<td>40</td>
<td>320</td>
<td>20</td>
<td>8</td>
<td>8</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>90</td>
<td>640</td>
<td>80</td>
<td>640</td>
<td>10</td>
<td>8</td>
<td>4</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>110</td>
<td>640</td>
<td>80</td>
<td>320</td>
<td>0</td>
<td>8</td>
<td>4</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>116</td>
<td>640</td>
<td>40</td>
<td>320</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>124</td>
<td>640</td>
<td>40</td>
<td>320</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>U</td>
<td>U</td>
</tr>
</tbody>
</table>

Reciprocal serum dilution
U Undiluted serum
DPI Days post-inoculation
Table 5. Disease Course and Mortality in Pigs Inoculated with Blood Collected 45, 90, and 135 DPI from Pigs Surviving African Swine Fever.

<table>
<thead>
<tr>
<th>Donor pig No.</th>
<th>45</th>
<th>90</th>
<th>135</th>
</tr>
</thead>
<tbody>
<tr>
<td>1882</td>
<td>9*</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>1925</td>
<td>10</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>2049**</td>
<td>11</td>
<td>20</td>
<td>31</td>
</tr>
<tr>
<td>2050**</td>
<td>14</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

* Days/mortality in recipient pig

** Contact exposure
REFERENCES

JOINT CAMPAIGN AGAINST
RINDERPEST IN AFRICA

D. E. DeTray, D.V.M.1

The most extensive international animal disease control program ever undertaken anywhere is currently underway in Africa. Yet, except for the recognition given the Rinderpest Campaign by the United States Animal Health Association, few U.S. veterinarians are even aware of the Campaign. When one is so immersed in a subject it is disheartening to realize that most people in the U.S. have never heard of rinderpest. A couple of years ago, just before elections, while on home leave I was asked what I did. I replied that I was working on the Rinderpest Campaign. My questioner asked “Who is Rinderpest and what is he campaigning for?”

The Joint Campaign Against Rinderpest was initiated at a conference held at Kano, Nigeria in May 1961 under the aegis of the Inter-African Bureau for Animal Health (IBAH). IBAH is one of several bureaus functioning under the Scientific Technical and Research Commission of the Organization of African Unity (STRC/OAU). (In September 1970 the name IBAH was changed to the Inter-African Bureau for Animal Resources – IBAR).

PURPOSE OF THE CAMPAIGN

The purpose is to control and eventually eradicate rinderpest from the African continent. This is to be accomplished by vaccinating all cattle in the rinderpest belt of Africa each year for three consecutive years and by taking other control measures necessary to eliminate the disease. (Although a single inoculation of viable vaccine into susceptible cattle will produce a lifetime immunity it has been determined that three consecutive annual mass vaccinations are required to raise the immunity of the national herds to a satisfactory level. This is because it is impossible to get complete vaccination coverage in a single year due to logistic and communication problems. Also, colostral immunity may persist in calves to the age of ten months and block the immune response to the vaccine. Therefore calves must be revaccinated after they attain one year of age to assure their immunity).

RINDERPEST VACCINE

Although rinderpest has been the major concern of animal health authorities since its introduction to sub-Saharan Africa in 1889, it is now feasible to think of control and eventual eradication of the disease from Africa. Fortunately there is only one immunologic type of the virus and cattle that survive the disease are not “carriers”. The tissue culture attenuated rinderpest vaccine developed by Dr. W. P. Plowright at the East African Veterinary Research Organization in Kenya is one of

1Regional Livestock Advisor, Bureau for Africa, U.S. Agency for International Development.
the safest and most effective immunizing agents ever devised for man or animal. The vaccine produces a lifetime immunity with no adverse post vaccinal reactions. In the early stages of the Campaign live caprinized vaccines were used. These vaccines were effective but caused serious post vaccinal reactions and an average mortality of 2 percent. When the tissue culture vaccine (TCV) was first introduced the nomadic cattlemen had no faith in it because it produced no reactions. After the first year with no rinderpest in TCV vaccinated cattle they were convinced of its effectiveness. The word soon spread by "bush telegraph" and, for the most part, the cooperation of the cattle owners has been excellent.

Tissue culture rinderpest vaccine for the Campaign has been produced at the following laboratories:

- The Hann Laboratory, Dakar, Senegal
- The Vom Laboratory, Nigeria
- The Farcha Laboratory, Port Lamy, Chad
- The Bamako Laboratory, Bamako, Mali
- The Imperial Vet. Institute, Debre Zeit, Ethiopia

COORDINATION AND ADMINISTRATION

From its inception in 1962 the Campaign has been administered by an International Coordinator's Unit which is directly responsible to the Executive Secretary of the STRC/OAU. The International Coordinator and his Deputy are under contract to STRC/OAU and are selected on the basis of being "qualified veterinarians familiar with animal disease control in Africa and who are competent planners, negotiators and administrators". To date these positions have been held by nationals from France, Great Britain, Holland and Sudan with a combined experience in African animal disease control of about seventy years. The Coordinator's Unit works closely with the Veterinary Departments of the countries participating in the Campaign. Each country selects a senior veterinarian as its National Organizer.

The Campaign has survived a major civil war, two lesser civil wars, at least ten coup d'états and various other changes of governments. This is a great tribute to the cohesive powers of STRC/OAU and to the effectiveness of the International Coordinator's Unit, the various veterinary departments and the National Organizers.

The International Coordinator's Unit was located at Kano, Nigeria for Phase I of the Campaign, at Ougadougou, Upper Volta for Phase II, and Dakar, Senegal for Phase III. Currently the Unit is located in Nairobi, Kenya for Phase IV and later will be moved to Addis Ababa, Ethiopia for Phase V.

ORGANIZATIONS OF THE CAMPAIGN

In the rinderpest belt of Africa there are 22 countries with approximately 80 million cattle covering an area twice the size of the continental U.S. Funds were not
available to launch a simultaneous campaign over this vast area, therefore the Campaign was carried out in Phases. Phase I (1962-65) was initiated in the Lake Chad Basin and covered parts of Nigeria, Niger, Chad and the Cameroun. Phase II (1964-67) involved Nigeria, Niger, Mali, Upper Volta, Togo, Dahomey, Ghana and the Ivory Coast. Phase III (1966-69) involved Mauritania, Senegal, Mali, Gambia, Sierra Leone, Guinea, Ivory Coast, Liberia, and a small section of Chad not covered in Phase I. Phases I, II, and III covered the rinderpest belt of west and central Africa where the cattle population is estimated at 32,750,000. During these three Phases over 81 million vaccinations were carried out and it was estimated that 83 percent of the cattle were vaccinated at least once.

Phase IV (1968-71) initiated the Campaign in Eastern Africa and involves Kenya, Uganda, Tanzania, Sudan and Somalia. As of June 30, 1970 over 10 million vaccinations had been carried out in these countries. Phase V (1970-73) covers the Southern half of Ethiopia. With an estimated 25 million cattle Ethiopia has the largest cattle population in Africa. It is said that 80 percent of the people live 24 hours by mule from the nearest road. The logistical problems are similar to those of the foot-and-mouth campaign in the isolated areas of Mexico in 1947. Nevertheless, during a trial period from July 1, 1969 to June 30, 1970 over 2 and a half million vaccinations were carried out in southern Ethiopia. We are optimistic about the future of the campaign. Phase VI (1972-75) will cover the northern half of Ethiopia and will mark the completion of the Africa-wide rinderpest campaign.

FINANCING

The total cost of the Campaign is estimated at about thirty-two million dollars. Of this the participating countries are contributing one half. One country, Kenya, is financing the campaign entirely from her own resources. The remaining funds provided to STRC/OAU by external donors including the U.S. Agency for International Development (USAID), the European Economic Community (EEC), West Germany, the United Kingdom, France, Canada and the United Nations Development Program (UNDP). The USAID contribution for the life of the project will be about five and a half million dollars. USAID has maintained a veterinarian in Africa in a liaison position with the Campaign since its inception. The writer is the third U.S. veterinarian to fill this position.

The cost for a single vaccination is about 23 U.S. cents. This includes vaccine, personnel, equipment and transportation.

DISCUSSION

The Joint STRC/OAU Campaign Against Rinderpest is the first effort to control and eradicate a livestock disease on an Africa-wide basis. The Campaign is also a prototype for multilateral donor support to self help projects on a regional basis in developing countries.

Follow-up measures by the individual countries are essential to the success of
the Campaign. Each country has agreed to vaccinate increases to herds twice (as calves and yearlings) and any cattle that may have been missed during the three year campaign. Most of Phase I, II, and III countries are doing a good job of follow-up under the surveillance of IBAR. A few countries have not done so well largely because of civil disturbances, lack of personnel and funds. A few small outbreaks of rinderpest have occurred in West Africa since the completion of the Campaign. These outbreaks were quickly controlled by vaccination and quarantine. Overall in West Africa the fall in the number of rinderpest outbreaks has been spectacular from the beginning of the massive vaccination campaign. For example, in Phase I countries there had been an average of one thousand outbreaks a year for the four years prior to the Campaign. During the first year of the Campaign there were 175 outbreaks; during the second year 28, and in the third year only 10 outbreaks were recorded.

Will rinderpest be eradicated in Africa? The knowhow is available. It depends on how it is applied. The logistic and communications problems are formidable. Trained personnel are in short supply and funds for veterinary services are limited. A major problem in many countries is the lack of laboratory facilities for rinderpest diagnosis and for differential diagnosis of mucosal disease and other rinderpest-like diseases.

What about rinderpest in game animals, sheep and goats? There is strong evidence that once the disease is eradicated in cattle it will not persist in these animals.

The Rinderpest Campaign has demonstrated the feasibility and value of cooperative effort on an Africa-wide basis in livestock disease control. Many of us connected with the Campaign sincerely believe that it is the first step toward a healthy and profitable cattle industry in Africa where high quality protein is so badly needed.

**SUMMARY**

The Africa-wide Joint Campaign Against Rinderpest was initiated in 1962 and will be completed in 1975. The Campaign is conducted under the aegis of the Scientific Technical and Research Commission of the Organization of African Unity. Twenty-two countries are involved with 80 million cattle covering an area twice the size of the continental United States. The objective is to control and eventually eradicate rinderpest in Africa by mass vaccination.

**ACKNOWLEDGEMENTS**

Mr. Amos Odelola, Executive Secretary, STRC/OAU, who has been so successful in obtaining cooperation from both the participating countries and the donors.
Dr. Bill Beaton, formerly Director of IBAH. The Campaign was his brain child.
Dr. Henri Lepissier, International Coordinator Phases I, II and III. He got the Campaign off the ground and supplied much of the information in this report.
Dr. Iain M. Macfarland, Deputy International Coordinator Phases I, II and III; International Coordinator Phases IV and V. He planned the Eastern Campaign and keeps the ball rolling. I borrowed heavily from his reports.

Dr. S. J. Henstra, Deputy International Coordinator Phase III. Previously worked on the Campaign in Nigeria and Senegal.

Dr. A. M. Dahab, Deputy International Coordinator Phases IV and V. Formerly Director of Veterinary Services in Sudan.

Drs. John Bill Williamson and Luther Al Fahland, my predecessors in USAID as representatives of USAID with the Campaign.

And — to all the National Organizers, field veterinarians, vaccinators and others who have worked long and hard toward the success of the Campaign. It has been a very great honor for me to be associated with the Campaign and these fine people.
GROWTH OF FOOT-AND-MOUTH DISEASE VIRUS
IN THE BOVINE PHARYNX

by
J. W. McVicar, D.V.M., J. H. Graves, D.V.M.,
and P. Sutmoller, Dr. Vet. Med.

INTRODUCTION

In 1957 Korn, reported on recovery of foot-and-mouth disease (FMD) virus from tissues of inoculated cattle before the appearance of visible lesions. Many of these cattle had been infected by wiping or spraying the virus on the muzzle. He concluded that primary multiplication of FMD virus after this sort of inoculation occurred in the upper respiratory tract probably in the nasal mucosa. He further concluded that virus then entered the blood stream, apparently by way of the lymphatics, to infect susceptible epithelial sites and that what has been referred to as a primary vesicle did not occur following "natural infection".

Hyslop in 1968 recovered FMD virus from saliva and surrounding air before the appearance of lesions in cattle following virus inoculation into the epithelium of the tongue (IDL). Recently there have been reports that following contact of susceptible cattle with infected ones, virus was recovered from oesophageal-pharyngeal (OP) fluid before visible epithelial lesions appeared. Mohanty and Cottra in 1978 have reported the infectivity titers of virus in OP fluid of cattle during the prodromal stages of infection. They inoculated steers by the IDL or pharyngeal route. These and other studies have suggested that indeed an important site of FMD virus multiplication in cattle is in the pharyngeal region.

This report describes studies on the growth of FMD viruses in the pharyngeal region of cattle after intranasal exposure.

MATERIALS AND METHODS

Cattle. Grade Hereford steers approximately 18 months old were used throughout these experiments. All animals were housed in isolation units as described elsewhere.

Virus. Virus strains used were: Type A, subtype 10, strain CANEFA-1 (A10); Type O, subtype 1, strain CANEFA-2 (O1); and Type C, subtype 3, strain CANEFA-3 (C3). All originated from samples of tongue epithelium from diseased cattle in Argentina. In these experiments, the 7th passage in primary bovine kidney cell (BK) cultures was used.

Cattle Inoculation. Undiluted tissue culture harvest was clarified by centrifugation and a 100-fold dilution made in tissue culture medium. One steer of a pair was inoculated with the undiluted harvest and the other with the 100-fold dilution.
of the same virus. The steer's head was held slightly elevated and 2.5 ml. of
inoculum was slowly instilled into the ventral portion of one nasal passage through
a 10 cm. length of rubber tubing. This procedure was repeated on the opposite side
for a total inoculum of 5 ml. per steer.

**Sampling Procedure.** After inoculation, OP fluid samples were obtained with a
cup probang every 20 minutes for the first 2 hours and then every 2 hours
through 24 hours. Blood samples were taken every 6 hours for the first 24 hours
and then both OP fluid and blood samples were taken daily until clinical signs
developed.

**Virus Assay.** Titrations of virus in OP fluid and blood were made by plaque
assay in BK cell cultures.

**RESULTS**

The curves plotted in Fig. 1 represent the growth of FMD virus types A, O, and
C in the pharyngeal region of steers as detected in OP fluid samples. Although there
are some differences, the shapes are generally quite similar. Some steers had
substantial amounts of virus in the early samples no doubt representing inoculum
virus. After inoculation the infectivity titers dropped rapidly, and in some
instances, went below detectable levels before an abrupt rise indicated release of
new virus into the pharyngeal region. This rise occurred somewhat earlier in the
steers inoculated with the higher amounts of virus. After 10 to 12 hours, the
infectivity titers leveled off and then remained relatively constant. With each virus,
the steer that received the higher dose reached and maintained a higher level of
infectivity in the OP fluid. Although not shown in this figure, this plateau
continued until a second rise preceded the appearance of epithelial lesions. Virus
was detected in the blood in the 24-hour sample of only those steers which received
the higher dose of the O and C viruses.

The virus titers in the OP fluid samples from the steers inoculated with the A10
virus were late in rising and had lower plateaus than those titers found for the O
and C viruses. It is interesting that this particular strain of virus is known to contain
a high percentage of noninfectious particles; and although equally virulent as the
O and C viruses, is a slow growing virus in tissue culture and animals.

All steers inoculated with the higher dose of each virus had clinical signs 2 days
after inoculation whereas steers receiving the lower virus doses did not show clinical
signs until day 3.

**DISCUSSION**

The abrupt and continued rise of virus titers in OP fluid samples from steers,
after the intranasal instillation of FMD virus, is evidence that this virus multiplies
in the pharyngeal region. The absence of viremia until many hours after high
infectivity titers were reached in the pharynx also points to early multiplication in
this area. These observations together with those of other workers

---

1. Sampling Procedure. After inoculation, OP fluid samples were obtained with a
cup probang every 20 minutes for the first 2 hours and then every 2 hours
through 24 hours. Blood samples were taken every 6 hours for the first 24 hours
and then both OP fluid and blood samples were taken daily until clinical signs
developed.

2. Virus Assay. Titrations of virus in OP fluid and blood were made by plaque
assay in BK cell cultures.

3. RESULTS

The curves plotted in Fig. 1 represent the growth of FMD virus types A, O, and
C in the pharyngeal region of steers as detected in OP fluid samples. Although there
are some differences, the shapes are generally quite similar. Some steers had
substantial amounts of virus in the early samples no doubt representing inoculum
virus. After inoculation the infectivity titers dropped rapidly, and in some
instances, went below detectable levels before an abrupt rise indicated release of
new virus into the pharyngeal region. This rise occurred somewhat earlier in the
steers inoculated with the higher amounts of virus. After 10 to 12 hours, the
infectivity titers leveled off and then remained relatively constant. With each virus,
the steer that received the higher dose reached and maintained a higher level of
infectivity in the OP fluid. Although not shown in this figure, this plateau
continued until a second rise preceded the appearance of epithelial lesions. Virus
was detected in the blood in the 24-hour sample of only those steers which received
the higher dose of the O and C viruses.

The virus titers in the OP fluid samples from the steers inoculated with the A10
virus were late in rising and had lower plateaus than those titers found for the O
and C viruses. It is interesting that this particular strain of virus is known to contain
a high percentage of noninfectious particles; and although equally virulent as the
O and C viruses, is a slow growing virus in tissue culture and animals.

All steers inoculated with the higher dose of each virus had clinical signs 2 days
after inoculation whereas steers receiving the lower virus doses did not show clinical
signs until day 3.

**DISCUSSION**

The abrupt and continued rise of virus titers in OP fluid samples from steers,
after the intranasal instillation of FMD virus, is evidence that this virus multiplies
in the pharyngeal region. The absence of viremia until many hours after high
infectivity titers were reached in the pharynx also points to early multiplication in
this area. These observations together with those of other workers

---
support the hypothesis$^{2,14}$ that the pharyngeal region is the primary site of FMD virus multiplication in cattle infected by the upper respiratory route.

All of the rising infectivity titers leveled off 10 to 12 hours after inoculation regardless of the inoculum dose or the virus type used. The infectivity level of this plateau was related to the dose of virus given, in that the steers receiving the higher doses had the higher plateau levels.

On the basis of these observations, we propose that:

1. After upper respiratory exposure to virus, the number of cells in the pharyngeal region, which are initially infected is related to the infectivity level of the virus exposure.
2. Early in infection, as a result of local defense mechanisms, a number of primary foci are established from which only limited spread of virus takes place.
3. With the number of infected cells thereby limited, a dynamic equilibrium is reached between virus and host which results in a uniform level of virus release.

A steer from another experiment$^6$ lent support to this hypothesis especially in illustrating the long-term maintainance of uniformly low levels of FMD virus in the OP fluid before the appearance of clinical signs. Twenty-four hours after virus exposure a low infectivity titer (10 PFU/ml) was found in a sample of this steer's OP fluid. This low level of virus was maintained for 3 days. On day 4, the titer had increased; and this rise continued for 2 more days until clinical signs were observed 7 days after exposure. Viremia was not detected in this steer until 4 days after virus exposure or 3 days after virus was isolated from the pharynx. Thus, a rather efficient mechanism was operating to maintain this uniformly low level of virus release over several days.

SUMMARY

Growth curves of foot-and-mouth disease (FMD) virus in the bovine pharyngeal region indicate that the virus multiplies at this site after upper respiratory infection. A hypothesis is advanced to explain the relatively uniform levels of virus infectivity in the bovine pharyngeal region.

ACKNOWLEDGMENTS

The assistance of Mr. W. Parrish was especially helpful; and we would like to acknowledge the technical assistance of Mrs. E. Appelt and Messrs. E. Loper and W. Harris, and the clerical assistance of Mrs. J. R. Faller.
Figure 1 – Virus infectivity titers of oesophageal-pharyngeal (OP) fluid from steers after the intranasal inoculation of foot-and-mouth disease (FMD) virus. PFU = plaque-forming units.
REFERENCES


INFLUENCE OF ENTEROVIRUS ON FOOT-AND-MOUTH DISEASE VIRUS INFECTION: A HYPOTHESIS

by

In the laboratory, foot-and-mouth disease (FMD) has usually been studied by the inoculation of animals at what were believed to be the predilection sites, such as the epithelium of the tongue of cattle, and the coronary band of the feet of sheep and pigs. When this was done, a "primary vesicle" developed at the injection site followed by fever, viremia and lesions at other susceptible sites. However, as a rule, there is no "primary vesicle" when cattle are exposed to virus by contact with an infected animal. We should like to present the following hypothesis for the pathogenesis of FMD as more representative of what happens in nature. Infection of the upper respiratory tract and local multiplication in the pharynx is followed by drainage of virus to the circulatory system, infection of epithelial sites, and finally the formation of vesicles.

In an earlier report we reported on initial infection and local multiplication of FMD virus (FMDV) in the pharynx. Another report covered aspects of the disease signs and transmissibility. It is known that circulating antibody can prevent the spread of the virus via the circulating system but does not affect the infection and the local multiplication in the pharyngeal area.

Results of studies on the growth of FMDV in the pharynx of cattle indicated that there was an inhibition of the growth of FMDV. Such inhibition occurs very early during the infectious process and it has been proposed that a number of primary foci are established by the inoculum from which only limited spread takes place. In this early state, it is likely that some localized mechanism such as interferon is operating, since antibody can be ruled out as a major inhibitor. However, it is difficult to explain how low levels of FMDV alone could be responsible for inducing the protection of the majority of cells of the nasopharyngeal mucosa; particularly in cattle that maintain low levels of virus infectivity in the pharyngeal area several days after exposure.

Based on observations in cattle and tissue culture, we have postulated that resident bovine enterovirus (BEV) in the naso-pharynx of cattle is a major factor in the course of an FMDV infection. Enteroviruses are ubiquitous in our experimental cattle as indicated by high serum antibody levels in essentially all animals, and the relative ease with which they can be isolated from fecal samples. Enteroviruses resemble FMDV in several aspects and both are picorna viruses. They are not serologically related, however, and BEV also differs from FMDV, in that it is acid stable and that it has a lower buoyant density. Bovine enterovirus does not appear...

From the Plum Island Animal Disease Laboratory, Veterinary Sciences Research Division, Agricultural Research Service, United States Department of Agriculture, Greenport, L.I., New York, 11944.
pathogenic for our adult cattle although we have observed that regrouping of cattle results in an active exchange of these viruses as evidenced by significant rises in antibodies.

Bovine enterovirus apparently is able to replicate and remain in the bovine host without damaging too many cells and causing clinical signs of disease. Local antibody against BEV is probably a major factor in maintaining such an equilibrium. It is proposed that when FMDV is introduced into the pharynx of BEV infected cattle, this equilibrium is upset and that this disturbance may ultimately inhibit the spread of FMDV to other pharyngeal cells. This concept may help to explain some unusual clinical manifestations of foot-and-mouth disease.

When a certain percentage of the naso-pharyngeal cells contain BEV, and are exposed to FMDV there is a likelihood that both picornaviruses can replicate in the same cell. As a result, we propose that the following progeny may be produced: The two “parental” viruses, BEV and FMDV, and “mixed” viruses with RNA of one virus and the protein coat of the other (Fig. 1). Such “mixed” particles also are called genomic-masked particles. One type of particle which could upset the balance between the steer and the resident enterovirus is the one consisting of the BEV RNA in a FMDV protein coat. This particle could be an efficient infectious particle, since it has the coat properties of the invasive FMDV and therefore does not react with antibody against BEV. If this particle introduced BEV RNA into a susceptible host cell, the progeny produced would be BEV, because virus replication is determined by the nucleic acid genome. Current work in our laboratory has shown that, in simultaneously infected tissue cultures, BEV grows as fast or faster than FMDV. In light of this efficiency and more rapid spread of this genomic-masked particle and thus the consequent rapid multiplication of BEV, one could explain how the introduction of FMDV could lead to an increased production of BEV. In fact, in support of this explanation, BEV antibody titers are found to increase shortly after experimental exposure of steers to FMDV.

Moreover, it is further proposed that the increased BEV activity is able to trigger the response of an early, nonspecific, local defense mechanism which thereby inhibits the spread of both BEV and FMDV. Subsequently, the production of additional BEV antibody establishes a new balance between the BEV and the host animal. Furthermore, because of increased BEV activity, it would be more difficult for FMDV formed during infection to become adsorbed to cells not yet infected by BEV. This in turn means that increased numbers of “mixed” particles might be produced.

Now we might consider the other extreme of the mixed population, that of a particle consisting of FMDV RNA in a BEV coat. Though such particles are at a disadvantage because of the increasing amounts of BEV antibody, they are not recognized by the host as FMDV in spite of their FMDV RNA. In fact, they are “wolves in sheeps' clothing” since sooner or later when a change occurs in the relationship between BEV and the host animal the reappearance of FMDV in its own coat may result. Several experimental observations are consistent with this hypothesis: The genomic-masked particle consisting of FMDV RNA with BEV
protein coat has been produced in tissue culture.\(^5\) This type of particle was acid-resistant as is BEV and neutralized by BEV specific antiserum but not by FMDV specific antiserum, yet produced FMDV by replication in tissue culture. It also had the density of BEV and could be separated from the higher density "parental" FMDV particle by ultracentrifugation techniques.

We have seen FMDV infections which strongly suggests that a comparable situation does occur in cattle. These observations were made in experiments whereby susceptible cattle were placed in contact with steers inoculated intranasally with relatively low levels of FMDV.\(^6\) From pharyngeal samples and serums of a high proportion of these cattle, BEV could be isolated shortly after exposure. Also FMDV was recovered from the serum of several of the cattle but clinical signs of FMD did not appear within the normally expected incubation period. Such infected steers also failed to produce circulating antibody against FMDV. If a particle consisting of FMDV RNA within a BEV coat were the type virus circulating in these animals, then the above observations can be explained. Eventually, as yet unknown factors enabled FMD to develop in these steers after unusually long incubation periods.

Normally, the isolation of FMDV from viremic blood is a rather straight-forward procedure. In the previous experiments, however, there was considerable difficulty in isolation of FMDV until it was found that FMDV recovery was improved by rinsing the tissue cultures before inoculation and by replacing the tissue culture medium with fresh medium. We now believe that these procedures removed the antibody against BEV that was introduced in the cultures either by the test serum or by the "normal" bovine serum used to grow the cultures. The enhanced recovery after removal of bovine serum suggest that the particle that circulated in the blood consisted of FMDV RNA but with BEV coat properties.

Another series of observations indicating interaction between BEV and FMDV occurred when steers were exposed by intranasal inoculation of BEV followed by intranasal inoculation of high levels of infectious FMDV about 2 months later. The resulting clinical FMD was markedly different from the signs seen in control steers that were not pre-exposed to BEV. In the BEV exposed steers, the FMD lesions were less extensive, healed rapidly, and remained localized on the mouth or nostrils with no spread to the feet. We believe, based upon our clinical observations that the earlier exposure to BEV in these steers resulted in a higher percentage of BEV infected pharyngeal cells so that subsequently a higher exposure to FMDV could be counteracted.

It is possible that these experiments may represent the type of interaction which occurs in carriers of FMDV, as reported in South American and Arica. These animals do not have FMDV antibodies in spite of FMDV growth in the pharynx.\(^7,8\) Here again, the spread of FMDV must be kept in check by some locally operating defence mechanism. It is difficult to visualize the low level of FMDV by itself, mediates this mechanism.

It is also tempting to speculate that in the immune carrier the equilibrium between the bovine host and FMDV at least partly depends on interaction with
other viruses.

Summarizing, we postulate that the course of an FMDV infection depends on the degree of infection of the pharyngeal cells with resident BEV relative to the level of FMDV exposure. If the level of exposure to FMDV is massive, then it can over-ride any inhibitive mechanism. However, if the level of FMDV exposure is relatively low, it could result in a localized or abortive infection only.
Figure 1 — Genomic-masked viral progeny from cells infected with foot-and-mouth disease virus (FMDV) and bovine enterovirus (BEV).
REFERENCES

REPORT OF THE COMMITTEE ON
FOREIGN ANIMAL DISEASES

N. M. Konnerup, Silver Spring, Maryland, Chairman
N. L. Meyer, Hyattsville, Maryland, Co-Chairman

Sub-Committee on Vesicular Diseases:

VENEZUELAN EQUINE ENCEPHALOMYELITIS
Venezuelan equine encephalomyelitis (VEE), on the move in epizootic fashion since 1968, continues active in the Caribbean area. The disease is currently active in Venezuela, Colombia, Costa Rica, and Mexico. The 1969 Central American epizootic and the current epizootics in Costa Rica and Mexico have been caused by the highly virulent subtype IB previously found only in Ecuador and Peru. The continued ominous spread of this highly virulent strain of VEE toward the U.S. poses a most serious threat to the Gulf coast area of the United States.

The U.S. Army Medical Research and Development Command has taken the initiative in helping to protect the equine population and arrest the disease by releasing nearly two million doses of an experimentally produced vaccine and providing the services of specialists to supervise its use. Unfortunately, the supply of vaccine is now nearly exhausted and insufficient progress has been made in obtaining United States Department of Agriculture approval for the commercial production of this vaccine within the United States.

It should be noted that attempts are being made to arrange for vaccine production by the Government of Mexico but this will require considerable time to develop and it is not clear whether this vaccine will be only for use within Mexico or whether it will be made available to all countries of Central and South America. Even in an emergency it is most unlikely that a VEE vaccine produced outside the United States would be used within the United States.
REPORT OF THE COMMITTEE

*The committee endorses the VEE contingency plan and the resolution submitted by the committee on Infectious Diseases of Horses and further recommends that the USDA authorize the commercial production of an initial quantity of ten million doses for export sales to countries where the disease is enzootic until an adequate capability for production of this vaccine is developed in Latin America.

RINDERPEST

Recent outbreaks of rinderpest have been reported in Vietnam, Kuwait, Afghanistan, Iran, and Turkey. The disease which erupted in Afghanistan has invaded Iran and Turkey. Iran has provided emergency supplies of vaccine. International assistance including aid from the Soviet Union has helped to control the Middle East spread of the disease. Containment of rinderpest in the Middle East and other Asian areas will require a massive immunization campaign of equal magnitude to that being carried out on the African Continent.

The joint campaign against rinderpest in Africa with current emphasis on Ethiopia and Somalia continues making progress. While new areas proceed at a remarkable pace, the maintenance of vaccination in the areas previously covered appears to lag. It will indeed be tragic if this failure to adhere to a system of permanent immunization leads to perpetuation of the virus and ultimate resurgence of the disease.

CONTAGIOUS BOVINE PLEUROPNEUMONIA

Contagious bovine pleurpneumonia continues to spread in many parts of Africa. This disease is probably as serious and certainly more insidious than rinderpest. Despite intensive vaccination it continues to be a major problem. It is quite clear that additional methods such as restricted movement of animals from foci of infection and elimination of reactor animals are essential for effective control. Further research on more effective diagnostic techniques and the development of a better immunizing agent are also needed.

INFECTIOUS BOVINE MAMMILLITIS

A closed herd of 17 Holstein cattle from northern Minnesota have all been affected with elevated, pencil-eraser-size nodules in the epidermis. These nodules have been seen on all areas of the body. Two affected animals had teat lesions. A cytopathogenic agent was isolated on second passage in primary bovine kidney tissue culture from an excised nodule and whole blood specimens obtained from one animal.

Virus neutralization studies as well as electronmicrographs suggest the isolate to be a herpes virus. It is similar to both bovine mammillitis virus, recently reported in England, and to Allerton virus which has been associated with a mild skin disease.

*The committee is not in unanimous agreement concerning the recommendation on production of vaccine and only a few members have seen and studied the contingency plan submitted by the Committee on Infectious Diseases of Horses.
in Africa. A current investigation is in progress to determine the extent and evaluate the significance of this disease in the United States.

**RIFT VALLEY FEVER**

Rift Valley Fever was recently confirmed in cattle and sheep in Malawi, and in cattle, sheep and goats in limited areas of Rhodesia. Presence of the disease in cattle and sheep in Rhodesia had been suspected before, but not confirmed.

**FOWL PLAGUE**

An occurrence of classical Fowl Plague was recorded in Hong Kong in 1969.

**HEMOPROTOZOAL DISEASES**

Progress is being made on the development of immunizing techniques to control hemoprotozoal diseases. The U.S. Agency for International Development is supporting research on the control of piroplasmosis, anaplasmosis, and trypanosomiasis in South America. The United Nations Development Program through FAO is carrying out research on immunization against trypanosomiasis and Theileria parva (East Coast Fever) in Africa.

**AFRICAN SWINE FEVER**

African swine fever is endemic in Spain, Portugal and Africa. Sporadic outbreaks of classical, acute African swine fever occur in northern Spain. In southern Spain, the disease has taken on a more chronic pattern with a longer incubation period, lesser symptoms and increasing difficulty in diagnosis. Spain has an active African swine fever eradication program, including diagnosis, slaughter and indemnity payments.

**VESICULAR DISEASE SUBCOMMITTEE**

1970 Report

*Foot-and-Mouth* — Foot-and-mouth disease was sporadic throughout the continent of Europe in the past year with the exception of the Iberian Peninsula, Anatolia, Turkey and two provinces of Greece.

Great Britain, Ireland, and the Scandinavian countries have remained free of the disease except for an outbreak in Denmark. The disease appeared in December 1969, in cattle on 8 farms in the northern part of Jutland. Another outbreak occurred in February about 20 km. from the first. Although the source of infection was never determined, it may have been associated with the vaccination of cattle for export. The Netherlands has been free of the disease for the third consecutive year and Switzerland experienced only one outbreak in 1969, and none thus far in 1970.

Portugal improved markedly from over 900 outbreaks in 1968 to no recorded outbreaks since August 1969. Spain, the only country in Western Europe with a high incidence in 1969, reported 294 outbreaks during the first 6 months of 1970.
France, Germany, and Italy have reduced the level of disease through the use of a systematic vaccination program, although all are still experiencing scattered outbreaks. France, in 1969, reported disease on 36 farms, vaccinated 18,000,000 cattle and about 1,100,000 sheep and goats. In 1970, France has reported only two outbreaks, both in the first 3 months of the year. The Federal Republic of Germany (West Germany) requires that all cattle over 6 weeks of age receive trivalent vaccine. Swine are included in ring vaccination measures around outbreaks. Most outbreaks occurred in young cattle and those vaccinated less than two times. In Italy, trivalent vaccine was administered to 7,500,000 cattle and 3,600,000 sheep and goats during 1969. The 130 outbreaks in 1969 and 124 in 1970 were primarily in young cattle unvaccinated and those recently imported. The majority of the 1970 outbreaks occurred during the first 3 months.

In the Danube region, which is important in animal trade and for exports to the West, conditions were greatly improved. Yugoslavia, Hungary, and Rumania, experienced an epizootic of type C virus in 1968 but have remained free since January 1969.

The European part of Turkey had no foot-and-mouth disease for the second consecutive year. However, in Anatolia, type A22 has caused sporadic outbreaks and type O numerous outbreaks.

Greece experienced 11 outbreaks in 1969 and 10 in early 1970, of types A, O, and C. However, the prime concern centered around a type A virus outbreak first appearing in a piggery in Crete in October 1969 with 9 premises involved. In November, 33 premises were found infected in the Department of Sirres. This strain of type A virus was markedly different from the strains previously isolated in Europe. Although imported meat used at a nearby military airfield was suspected, the origin of the virus “A Greece 1969” was never determined.

The major change in the foot-and-mouth disease situation in the Near East was the appearance of type C virus in Lebanon, which was briefly mentioned in the 1969 report of the FAO committee. This virus was probably introduced in the spring of 1969 by sheep imported from Europe. Its presence was first demonstrated in Lebanon in April where serious losses were suffered among sheep and goats. The virus spread to Syria and two months later to Jordan, where it remained confined to isolated foci in border areas. The appearance of type C virus created difficulties in providing adequate amounts of vaccine. The FAO Emergency Fund assisted in providing 100,000 monovalent and bivalent cattle doses. The vaccine was used to bring the disease under control in heavily infected Lebanon, to encourage and facilitate eradication of the infection in the sporadically affected countries, Syria and Jordan, and to prevent the infection from reaching Iraq, Israel, and Turkey. Although the disease was prevented from spreading, outbreaks continued in Lebanon and Syria during late 1969 and early 1970.

Foot-and-mouth disease is present throughout the Continent of Africa. The virus types reported are: SAT 1, SAT 2, A, O, and C.

Foot-and-mouth disease continues to be enzootic in South America. Types A, O, and C are reported in Argentina, Brazil, Uruguay, Chili, Bolivia, and Peru.
Colombia, Paraguay, Venezuela, and Ecuador have reported both A and O in 1970. Approximately 7,000 sheep and 80 cows were slaughtered in Chili’s southernmost Tierra del Fuego Province, which was thought to be free. Infected meat from infected areas of Chili was thought to be the cause. On July 22, 1970, Argentina reported an outbreak in the locality of El Maiten in the Province of Chubut which had been declared free from foot-and-mouth disease on June 11, 1969. This area was again declared free on August 31, 1970. A total of 982 animals were slaughtered.

The Central American Republics and Panama have been historically free of foot-and-mouth disease — isolated from the infection in South America by the Darien Rain Forest on the Colombian-Panama border. A highway is being cut through this barrier and only 140 miles of jungle remains to be breached.

As transportation is improved, so are the chances for spread of infection. The Central American Governments recognize this danger and are interested in developing organizations similar to the one now in Mexico to prevent and eradicate foot-and-mouth disease and rinderpest. Two Animal Health Division veterinarians are now in Central America working on this project. The Animal Health Division desires to send one additional veterinarian and an administrative officer to this area in fiscal year 1971.

The goal of the Animal Health Division is to formulate an agreement with Panama and as many other Central American countries as possible, to establish cooperative activities similar to those which exist with Mexico under the Mexico-American Commission for the Prevention of Foot-and-Mouth Disease.

The worldwide distribution of foot-and-mouth disease outbreaks and the types of virus isolated are shown in the following chart:
## FOOT-AND-MOUTH DISEASE REPORTS 1968 to 1970

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>European:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albania</td>
<td>1959</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>1966</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>1966</td>
<td>1</td>
<td>O,A,C</td>
<td>3</td>
<td>O,A,C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulgaria</td>
<td>1966</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Czechoslovakia</td>
<td></td>
<td>31</td>
<td>O,A,C</td>
<td>7</td>
<td>O,A,C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyprus</td>
<td>1964</td>
<td>5</td>
<td>O,A,C</td>
<td>8</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>1959</td>
<td>55</td>
<td>O,C</td>
<td>36</td>
<td>O,C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>France</td>
<td></td>
<td>55</td>
<td>O,C</td>
<td>36</td>
<td>O,C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany (East)</td>
<td></td>
<td>3</td>
<td>O,C</td>
<td>4</td>
<td>O,C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany (West)</td>
<td></td>
<td>66</td>
<td>O</td>
<td>12</td>
<td>O,C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Great Britain</td>
<td></td>
<td>187</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hungary</td>
<td></td>
<td>59</td>
<td>C</td>
<td>6</td>
<td>O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>1941</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td></td>
<td>20</td>
<td>O,A,C</td>
<td>131</td>
<td>O,A,C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>1967</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>1952</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poland</td>
<td></td>
<td>6</td>
<td>O</td>
<td>1</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td></td>
<td>923</td>
<td>O</td>
<td>161</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Romania</td>
<td></td>
<td>11</td>
<td>C</td>
<td>2</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Russia</td>
<td></td>
<td>1,392 cas.</td>
<td></td>
<td>473 cas.</td>
<td>O,A</td>
<td>205 cas.</td>
<td>O,A</td>
</tr>
<tr>
<td>Spain</td>
<td></td>
<td>561</td>
<td></td>
<td>409</td>
<td>O,A,C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>1966</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Switzerland</td>
<td></td>
<td>23</td>
<td>C</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yugoslavia</td>
<td></td>
<td>43</td>
<td>O,C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Country</td>
<td>Last Outbreak</td>
<td>1968</td>
<td>1969</td>
<td>1970</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>
</tr>
<tr>
<td></td>
<td></td>
<td>No. Outbreaks</td>
<td>Virus Types</td>
<td>No. Outbreaks</td>
<td>Virus Types</td>
<td>No. Outbreaks</td>
<td>Virus Types</td>
</tr>
<tr>
<td>Asian:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cambodia</td>
<td></td>
<td>26</td>
<td>O</td>
<td>5</td>
<td>O,A,C</td>
<td>1,892</td>
<td>O</td>
</tr>
<tr>
<td>Ceylon</td>
<td></td>
<td>164 cas.</td>
<td></td>
<td>80 cas.</td>
<td>O</td>
<td>10</td>
<td>O</td>
</tr>
<tr>
<td>Greece</td>
<td></td>
<td>111</td>
<td>O,A,C</td>
<td></td>
<td>O</td>
<td>41</td>
<td>O</td>
</tr>
<tr>
<td>Hong Kong</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td></td>
<td>9,146</td>
<td>O</td>
<td>1,842</td>
<td>A</td>
<td>942</td>
<td>A,C</td>
</tr>
<tr>
<td>Iran</td>
<td></td>
<td>234</td>
<td></td>
<td>179</td>
<td>A</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Iraq</td>
<td></td>
<td>2,433</td>
<td>O</td>
<td>8,036</td>
<td>O</td>
<td>114</td>
<td>O,A</td>
</tr>
</tbody>
</table>

FOOT-AND-MOUTH DISEASE REPORTS 1968 to 1970 (continued)
FOOT-AND-MOUTH DISEASE REPORTS 1968 to 1970

<table>
<thead>
<tr>
<th>Country</th>
<th>1968</th>
<th>Virus Types</th>
<th>1969</th>
<th>Virus Types</th>
<th>1970 (Incomplete Reports)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Outbreaks</td>
<td></td>
<td>No. Outbreaks</td>
<td></td>
<td>No. Outbreaks</td>
</tr>
<tr>
<td>Israel</td>
<td>9</td>
<td>O,A</td>
<td>24</td>
<td>O</td>
<td>16</td>
</tr>
<tr>
<td>Jordan</td>
<td>12</td>
<td>O,A</td>
<td>8</td>
<td>O,C</td>
<td>4</td>
</tr>
<tr>
<td>Lebanon</td>
<td>152</td>
<td>O</td>
<td>269</td>
<td>C</td>
<td>214</td>
</tr>
<tr>
<td>Pakistan (West)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syria</td>
<td>164</td>
<td>O</td>
<td>189</td>
<td>O,C</td>
<td>262</td>
</tr>
<tr>
<td>Thailand</td>
<td>159</td>
<td>40</td>
<td></td>
<td></td>
<td>27</td>
</tr>
<tr>
<td>Turkey</td>
<td>302</td>
<td>1,447</td>
<td>O,A</td>
<td></td>
<td>253</td>
</tr>
<tr>
<td>Vietnam</td>
<td>3</td>
<td>O</td>
<td>52</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Kuwait</td>
<td></td>
<td></td>
<td></td>
<td>O,A, SAT 1</td>
<td></td>
</tr>
</tbody>
</table>

Africa:

<table>
<thead>
<tr>
<th>Country</th>
<th>1968</th>
<th>Virus Types</th>
<th>1969</th>
<th>Virus Types</th>
<th>1970 (Incomplete Reports)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algeria</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angola</td>
<td>28</td>
<td>A,SAT 2</td>
<td>20</td>
<td>A,SAT 2</td>
<td></td>
</tr>
<tr>
<td>Botswana</td>
<td>1</td>
<td>SAT 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burundi</td>
<td>4</td>
<td></td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cameroon</td>
<td>56</td>
<td></td>
<td>7</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Dahomey</td>
<td>23</td>
<td></td>
<td>2</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Egypt</td>
<td>3</td>
<td></td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ehtiopia</td>
<td>10</td>
<td></td>
<td>5</td>
<td>O,A</td>
<td></td>
</tr>
<tr>
<td>Ghana</td>
<td>56</td>
<td>SAT 1 &amp; 2</td>
<td>11</td>
<td>SAT 1</td>
<td>2</td>
</tr>
<tr>
<td>Ivory Coast</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kenya</td>
<td>43</td>
<td>O,A</td>
<td>79</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Libya</td>
<td>91</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mocambique</td>
<td>2</td>
<td></td>
<td>10</td>
<td>SAT 2</td>
<td>8</td>
</tr>
</tbody>
</table>
# Foot-and-Mouth Disease Reports 1968 to 1970

<table>
<thead>
<tr>
<th>Country</th>
<th>1968</th>
<th>Virus Types</th>
<th>1969</th>
<th>Virus Types</th>
<th>1970 (Incomplete Reports)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Outbreaks</td>
<td>No. Outbreaks</td>
<td>No. Outbreaks</td>
<td>No. Outbreaks</td>
<td>No. Outbreaks</td>
</tr>
<tr>
<td>Niger</td>
<td>12</td>
<td></td>
<td>10</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Nigeria (Northern)</td>
<td>58</td>
<td>A, SAT 1</td>
<td>3</td>
<td></td>
<td>SAT 1</td>
</tr>
<tr>
<td>Rhodesia</td>
<td>SAT 1&amp;2</td>
<td>SAT 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>3</td>
<td>SAT 1&amp;2</td>
<td>2</td>
<td>SAT 2</td>
<td>SAT 2</td>
</tr>
<tr>
<td>South West Africa</td>
<td>2</td>
<td>SAT 2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MALi</td>
<td>1</td>
<td></td>
<td>2</td>
<td></td>
<td>SAT 2</td>
</tr>
<tr>
<td>Samolia</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>SAT 2</td>
</tr>
<tr>
<td>Sudan</td>
<td>9</td>
<td>A, SAT 1</td>
<td>1</td>
<td></td>
<td>SAT 2</td>
</tr>
<tr>
<td>Tanzania</td>
<td>181</td>
<td>SAT 2</td>
<td>47</td>
<td>SAT 2</td>
<td>8</td>
</tr>
<tr>
<td>Tchad</td>
<td>18</td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Togo</td>
<td>4</td>
<td></td>
<td>43</td>
<td>C</td>
<td>O</td>
</tr>
<tr>
<td>Tunisia</td>
<td>62</td>
<td></td>
<td>43</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>Uganda</td>
<td>13</td>
<td>O,A,SAT 2</td>
<td>24</td>
<td>O,A, SAT 2</td>
<td>14</td>
</tr>
<tr>
<td>Egypt (UAR)</td>
<td>3 cas.</td>
<td></td>
<td>14</td>
<td>O,C,SAT 1&amp;2</td>
<td></td>
</tr>
<tr>
<td>Swaziland</td>
<td>4</td>
<td></td>
<td>4</td>
<td>SAT 2</td>
<td></td>
</tr>
<tr>
<td>Zambia</td>
<td>3</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>South American:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argentina</td>
<td>1,430</td>
<td>O,A,C</td>
<td>2,015</td>
<td>O,A,C</td>
<td>399</td>
</tr>
<tr>
<td>Brazil</td>
<td>241</td>
<td>O,A,C</td>
<td>436</td>
<td>O,A,C</td>
<td>511</td>
</tr>
<tr>
<td>Colombia</td>
<td>177</td>
<td>O,A</td>
<td>254</td>
<td>O,A</td>
<td>60</td>
</tr>
<tr>
<td>Paraguay</td>
<td>54</td>
<td>O,A,C</td>
<td>174</td>
<td>O,A,C</td>
<td>3</td>
</tr>
<tr>
<td>Uruguay</td>
<td>67</td>
<td>O,A,C</td>
<td>45</td>
<td>O,A,C</td>
<td>45</td>
</tr>
</tbody>
</table>

- **Report of the Committee**
FOOT-AND-MOUTH DISEASE REPORTS 1968 to 1970

<table>
<thead>
<tr>
<th>Country</th>
<th>1968</th>
<th>Virus Types</th>
<th>1969</th>
<th>Virus Types</th>
<th>1970</th>
<th>Virus Types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Outbreaks</td>
<td>1968</td>
<td>O,A</td>
<td>81</td>
<td>O,A</td>
</tr>
<tr>
<td></td>
<td>Outbreaks</td>
<td></td>
<td>1969</td>
<td>O,A,C</td>
<td></td>
<td>O,A,C</td>
</tr>
<tr>
<td>Venezuela</td>
<td>30</td>
<td>cas.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chili</td>
<td>O,A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ecuador</td>
<td>A,O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolivia</td>
<td>O,A,C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peru</td>
<td>O,A,C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VESICULAR STOMATITIS

United States

Investigations. — From October 1, 1969 to September 30, 1970, tissue and/or serum specimens from 36 herds were submitted to Diagnostic Services ANH Division, NADL, Ames, Iowa, for vesicular disease examination.

New Jersey (NJ) vesicular stomatitis (VS) virus infection was serologically confirmed in five herds (Table 1); four from the Carolinas (1970), and one from Louisiana (1969). These were the only cases diagnosed serologically on the east coast since 1964 except a single case in Marion County, S.C. in August 1966, and three survey serums with neutralizing antibodies collected from young livestock on Ossabow Island, Chatham County, Georgia in July 1969. New Jersey virus was isolated from a cow in one of the herds in South Carolina. This was the first isolation of New Jersey VS virus on the east coast since 1964.

Bovine Serum Survey. — Bovine serums from six states and Puerto Rico were tested by the serum neutralization (SN) test for antibodies against VS virus. Following is a summary of results obtained from 1454 serums collected between fall 1969 and August 1970.

<table>
<thead>
<tr>
<th>State</th>
<th>No. of Serums</th>
<th>No. of Positive Serums</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>447</td>
<td>0</td>
</tr>
<tr>
<td>Colorado</td>
<td>212</td>
<td>3</td>
</tr>
<tr>
<td>Georgia</td>
<td>215</td>
<td>0</td>
</tr>
<tr>
<td>Indiana</td>
<td>180</td>
<td>0</td>
</tr>
<tr>
<td>Louisiana</td>
<td>200</td>
<td>5</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1454</td>
<td></td>
</tr>
</tbody>
</table>
**TABLE 1**

VESICULAR STOMATITIS POSITIVE HERDS* OCTOBER 1, 1969 THROUGH SEPTEMBER 30, 1970

<table>
<thead>
<tr>
<th>STATE</th>
<th>COUNTY</th>
<th>INFECTED</th>
<th>INVESTIGATION</th>
<th>EPITHELIUM</th>
<th>PROBANG</th>
<th>SERUM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CF</td>
<td>VI</td>
<td>VI</td>
</tr>
<tr>
<td>Louisiana</td>
<td>Union</td>
<td>3/19**</td>
<td>10-6-69</td>
<td>—</td>
<td>—</td>
<td>Neg.</td>
</tr>
<tr>
<td>N. Carolina</td>
<td>Columbus</td>
<td>1/2</td>
<td>5-21-70</td>
<td>Neg.</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>S. Carolina</td>
<td>Marion</td>
<td>1/32</td>
<td>7-2-70</td>
<td>—</td>
<td>—</td>
<td>Neg.</td>
</tr>
<tr>
<td>S. Carolina</td>
<td>Marion</td>
<td>1/54</td>
<td>7-7-70</td>
<td>NJ</td>
<td>NJ</td>
<td>NJ</td>
</tr>
<tr>
<td>S. Carolina</td>
<td>Berkeley</td>
<td>2/19</td>
<td>8-18-70</td>
<td>—</td>
<td>—</td>
<td>Neg.</td>
</tr>
</tbody>
</table>

* Positive samples were all bovine although porcine and equine specimens were also tested from 13 states.

** Numbers infected/number cattle in the herd.

> Complete neutralization of the dilution indicated. Neutralization titers are figured by the Spearman-Karber method and are listed as the logarithm of the reciprocal of the highest serum dilution which neutralized 100-1000 TCID$_{50}$ of virus.

N Negative at .9 the 1:8 serum dilution

--- No sample received.
As noted in the table, Louisiana and Colorado were the only states in which there was definite evidence of VS indicated by the neutralization tests. Three animals in one Caldwell Parish herd, one from Rapides and one from Ascension Parishes had neutralizing titers.

New Jersey VS was last reported in Colorado during 1966 and both Mesa and Montezuma Counties were involved. Two of the three positive animals could have been infected at that time. The two year old hereford in Mesa County must have become infected subsequent to 1966.

Deer Serum Survey. — One hundred thirty-three deer serums collected in the Southeastern Cooperative Wildlife Disease Survey were negative to both types of VS. These serums were collected from summer 1969 through April 1970 in Alabama, Georgia, Maryland, S. Carolina, Virginia, and W. Virginia.

Trainer and Hanson* reported in 1969 on serums obtained during a seven year period from white-tailed deer shot on the Welder Wildlife Foundation Refuge near Corpus Christi, Texas. A positive reaction neutralized 100 tissue culture doses of either New Jersey or Indiana serotypes of VSV.

<table>
<thead>
<tr>
<th>Year</th>
<th>VSV reactors</th>
<th>N.J.</th>
<th>Ind.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1963</td>
<td>3/121**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1964</td>
<td>23/157</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1965</td>
<td>2/96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1966</td>
<td>13/127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1967</td>
<td>12/100</td>
<td>30/100</td>
<td></td>
</tr>
<tr>
<td>1968</td>
<td>0/48</td>
<td>0/48</td>
<td></td>
</tr>
<tr>
<td>1969</td>
<td>6/41</td>
<td>22/41</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>27/690</td>
<td>84/690</td>
<td></td>
</tr>
</tbody>
</table>

The occurrence of VS antibody in deer is consistent with other reports in the literature. An epizootic of VS-Indiana occurred in cattle in Texas in 1964 and the virus was active in cattle at this time.

Mexico

During calendar year 1969, U.S. and Mexico veterinarians from the Mexican-American Commission for the Prevention of Foot-and-Mouth Disease performed 85 suspected vesicular disease investigations. Fifty-four of these were non-vesicular conditions. There were 14 cases of New Jersey VS, 12 cases of Indiana VS and 5 cases of double fixation. In calendar year 1969, while most of the cases occurred in southern half of Mexico, there were 2 cases of New Jersey stomatitis in west central Sonora.

* Ref. The American Journal of Epidemiology.

** No. of positive/no. tested.
As of September 29, in calendar year 1970, there have been 32 investigations of which 23 were non-vesicular and 9 were positive for New Jersey VS. Seven of these occurred in the southern two-thirds of the State of Vera Cruz and 2 were in the State of Chiapas.
RESEARCH ON VESICULAR DISEASES

Vesicular Exanthema of Swine. — This is the first time in many years that specific reference to research on vesicular exanthema of swine has not been made. Its extinct state has relegated it to reference in textbooks of virology or veterinary medicine.

Vesicular Stomatitis — There are many references to vesicular stomatitis virus (VSV) in the current literature; however, the bulk of these refer to its use as a reagent for interferon assays in tissue cultures. Its sensitivity to interferon has made it a popular virus in that regard.

Cartwright et al. have dissected the VSV particle by chemical and enzymatic treatments. Destruction of the outer envelope and fringe structures removed the immunizing capacity of the particles but left an infectious skeleton-like structure. They characterized the ribonucleoprotein in centrifuge gradients and by chemical assay.

Calisher and Muness found that double diffusion in agar gel could be used to identify VSV. The reaction was specific when tested with a group of 75 arboviruses.

Tesh et al. surveyed for the prevalence of VSV neutralizing antibody in various Panamanian human and animal populations. They found VSV-Indiana antibodies were mainly in arboreal and semiarboreal wild animal species. New Jersey VSV antibody levels in feral animals were highest in Chiropteran, Carnivora, and certain rodents. Domestic animal and human antibody rates were high to both viruses. They imply that the two viruses may have different cycles in nature. Their data suggest that VSV-Indiana is arthropod-transmitted while the mode of VSV-New Jersey transmission is unknown. They also found that young animals developed signs of encephalitis after inoculation while adults had inapparent infection but high antibody response. Most mammals were susceptible.

Johnson et al. have presented the interesting hypothesis that VSV-Indiana is basically a plant virus. Their proposal states that when in an infected plant the virus has two protein coats, one of which is removed when the virus is picked up by insects such as Phlebotomus feeding on infected plant juices. The virus in the insect is then ingested or bites the grazing animal which in turn becomes infected but with single-coated virus. They propose that the virus in the infected animal is at a dead-end and is no longer naturally transmitted.

Foot-and-Mouth. — Part 2 of the extensive inquiry into the severe foot-and-mouth disease (FMD) epidemic of 1967/68 known as the “Northumberland Report” has been published. It deals primarily with review of current control policy in Great Britain and recommends improvements. The literature contains new discussions on the feasibility and likelihood of FMD virus (FMDV) spread by wind during the 1967/68 FMD outbreak in Great Britain. They center on theoretical considerations and real evidence that such spread took place remains to be produced.

Significant nonspecific neutralization of A, O and C FMDV by normal bovine serum was reported by Patty. Passage of the O virus in cultures containing serum with high nonspecific activity reduced the sensitivity of the harvested virus to the inhibitors. Dialysis of normal bovine serum against distilled water followed by
hearing to 64°C for 30 minutes reduced the nonspecific activity of the serum but did not alter virus-specific antibody titers.

After adsorption of FMDV with homogenized calf kidney cells, Campbell found that a small amount of FMDV remained that was resistant to further adsorption and was less pathogenic for mice.

American white-collared peccaries were found susceptible to FMDV by contact with infected animals or injection of the virus. The disease was not as severe as that seen in pigs.

Hedger found that a high percentage of cattle vaccinated against FMD became virus carriers when exposed by natural field challenge even though clinical FMD was not seen. The proportion infected was a function of the severity of the exposure. The duration of the carrier state was not influenced by the prior vaccination. Occasional marked differences from the original infective virus in carrier virus from cattle were found. It was found that in two steers infected with type O FMDV, who were carriers of type A virus, a new virus was recovered with the antigenic characteristics of both viruses.

Mohanty and Cottral reported that immunofluorescence could be found as early as 3 hours after inoculation of tissue cultures with esophageal-pharyngeal (EP) fluid samples containing FMDV. Virus in EP samples from carrier cattle required 18 hours before it was detectable in the cell cultures. Kaaden et al. isolated FMDV from oesophageal and pharyngeal scrapings up to 9 months after infection. The virus isolated from these scrapings produced more interferon and was more susceptible to inhibition by interferon. It also had a longer incubation period in mice and produced small plaques.

Crandell and Gomez found that FMDV attenuated by chick-embryo passage produced smaller plaques than the reactor viruses produced by vaccination or field strain. Types A and C field viruses produced non-clean plaques distinguishable from the vaccine or reactor viruses. All viruses produced larger plaques with dextran sulfate in the agar but smaller with DEAE-dextran.

Fish et al. in the study of more than 300 vaccinated cattle in 16 herds in the Netherlands, found that antibody responses were greatest against type A virus and less against O and C in that order. Antibody against all three viruses persisted throughout the 30-month observation period and increased significantly after additional annual vaccinations.

Foot-and-mouth disease virus was inactivated by first order reaction with 2-ethylethyleneimine (EEI) as reported by Bauer. Its serological antigenicity and immunizing capacity were retained.

Maes and Fernandes found that oil emulsions used for experimental FMD vaccines absorb up to 400 times more virus than aluminium hydroxide. The oil did not destroy the virions. Diethylaminoethyl-Dextran (DEAE-D) proved to be a good adjuvant for the immunization of guinea pigs with inactivated FMDV as reported by Wittmann. It was not as good as Freund's adjuvant but was better than adjuvant 65 or alginate.

McKercher and Gailinunas found satisfactory immunity in pigs vaccinated
with oil-adjuvanted vaccine. A study of the appearance of the inoculation sites was made over a 365-day period.

A passive hemagglutination test was reported by Tokuda and Warrington.\textsuperscript{22} Virus at concentrations of 10 to 40 \(\mu\)g/ml was coupled to sheep red blood cells with glutaraldehyde. They reported the test to be two to four times more sensitive than complement fixation.

The antibody-combining sites on the surface of FMDV were studied by Brown and Smale.\textsuperscript{23} By treatment with trypsin and adsorption of serum with virus fractions, they concluded there are three specific sites. Cowan\textsuperscript{24} found that 7S and 19S antibody classes differed in the specificity of reaction indicating fundamental differences in reaction site on the virus of the two antibody classes.

Ascione and Vande Woude\textsuperscript{25} and Vande Woude \textit{et al.}\textsuperscript{26} studied the mechanism of host cell activity during FMDV infection. They found a marked inhibition of ribosomal RNA methylation when cells were infected with FMDV. Polatnick and Arlinghaus\textsuperscript{27} studied the influence of various temperatures on protein synthesis and RNA polymerase activity when cells were infected by FMDV.

Pringle \textit{et al.}\textsuperscript{28} working with temperature-sensitive mutants of FMDV found that the 17 mutants studied could be arranged in five groups depending on the genetic recombinations observed. Attenuation of FMDV by treatment with the mutagen, hydroxylamine, was shown by Maes.\textsuperscript{29} Plaque purification of the surviving mutated virus was required but immunogenicity was lost upon continued passage in all culture. He also found that 39\% of the surviving plaque-forming units remaining after treatment were mutants if the total infectivity dropped 90\%.\textsuperscript{30}

Maes and Fernandes\textsuperscript{31} studied the influence of coating the strands of nucleic acid with DEAE-D on interferon induction in mice and protection against FMDV infection.

Gailiunas, Cottral and Scott\textsuperscript{32} demonstrated that FMDV remains infectious at 4\(^{\circ}\)C, for 33 to 398 days on meat-packaging materials contaminated with infected blood, serum, lymphoid tissue and fat.
REFERENCES

EVALUATION OF THE IMMUNODIFFUSION TEST FOR THE DIAGNOSIS OF EQUINE INFECTION ANEMIA


INTRODUCTION

Equine infectious anemia (EIA) was first described in 1843 and recognized as a virus disease in 1904. It is an acute or chronic disease of Equidae, characterized by intermittent fever, depression, weakness, loss of weight, edema and anemia. The disease is difficult to diagnose because of the lack of a reliable diagnostic test other than horse inoculation.

Almost every conceivable method of diagnosis has been tried. A precipitin test was developed at Texas A&M University and found to be reliable at certain stages of the disease. Saxer has described a gel diffusion test using pancreas as the antigen. Complement fixation (CF) has been reported using a variety of antigens including spleen. Kono and Kobayashi have reported a CF test using infected horse leukocyte cultures as the antigen. Other serological tests have been reviewed by Dreguss and Lombard, Ishii and Johnson. None of the previously described tests could consistently detect the chronically infected horse.

An immunodiffusion test has been described by Coggins and Norcross. This test was reported to detect antibody starting as early as 18 days postinoculation (DPI) and continuing for the life of the animal.

The immunodiffusion test is being evaluated for accuracy and reliability. This phase of the evaluation is based primarily on testing of samples from known susceptible and experimentally infected animals.

MATERIALS AND METHODS

Antigen

A pony (No. 220) was used as an antigen source. It was infected with 110 ml. of serum that had been drawn 16 days postinfection (DPI) from a horse with acute EIA. Pony No. 220 also developed acute EIA and was moribund at 9 DPI. The spleen was harvested, divided into 1-gram quantities, and stored at -70° C. Before use the spleen was finely minced with sharp pointed scissors. Spleens were also prepared from a pony proved susceptible by the cross transfusion technique and from 14 ponies infected with EIA for periods of 14 to 218 days.

Serum

Serum or plasma samples were tested from 57 noninfected horses and 72 horses experimentally infected with EIA. All horses were first proved susceptible by a previously described technique. The Wyoming strain of EIA virus was used to infect 71 horses; the other horse was infected with a field strain from Kentucky.
total of 1,280 samples were available from preinoculation and during all stages of the disease. Several chronic carriers were sampled for periods up to 3 years. Twenty-one of the horses were sampled every 1-5 days so that the time of antibody appearance could be determined. All serum samples were coded so that the infectivity status was unknown until the results were determined.

Colostrum samples were available from two infected horses and one non-infected horse.

In addition, 385 field samples were tested. One hundred and ninety-nine of these samples were obtained at the Central Nebraska Packing Co., North Platte, Nebraska, from Western type horses from Montana, Wyoming and Colorado. The remainder of the field samples were from almost every type and breed of horse or pony.

**Agar Gel Plates**

The immunodiffusion test procedure was very similar to that previously described by Coggins\(^1,2\), with the following modifications. The agar was 0.69% Noble special agar\(^*\) dissolved in Tris buffer. The buffer was prepared by mixing 7.02 gm. Trizma HC1\(^**\) and 0.67 gm. Trizma base\(^**\) in 1000 ml. distilled water. The buffer was 0.05 molar, pH 7.2.

The agar was prepared by mixing the following components:

- Noble special agar 0.69 gm.
- Tris buffer 100.0 ml.
- Sodium chloride 8.5 gm.

The solution was autoclaved at 15 pounds of pressure for 5 min. The agar was removed from the autoclave and immediately added to the plates using a pipette. Plastic tissue culture plates\(\dagger\) 60 mm. in diameter were used and 6 ml. of agar (3 mm. deep) was added to each plate.

A Perspex template with 7-well pattern was placed over the uncovered Petri dish and a No. 4 (8 mm.) cork borer was introduced through the holes to cut the agar. The distance from the center well to the outside wells and between each outside well was 3 mm.

Antigen was placed in the center well. A known positive serum was placed on each side of the serum to be tested. A total of 3 samples were tested on each plate. Plates were incubated at room temperature in an air tight chamber and observed daily for 4-7 days.

**RESULTS AND DISCUSSION**

Antibody was detected in serum samples from all infected horses. Once a horse became positive on the immunodiffusion test, all subsequent samples were positive.

---

* Difco Laboratories, Detroit, Mich.
** Sigma Chemical Co., 3500 DeKalb St., St. Louis, Mo.
\(\dagger\) Falcon Plastics, 5500 W. 83rd St., Los Angeles, Calif.
No samples tested from susceptible horses gave a specific precipitin reaction (Table 1).

The time of first appearance of antibody varied with incubation period (Table 2). Incubation period was the time from inoculation until the temperature rose to over 101°F for 24 hours. Antibody was first detected between 14 and 40 DPI. Antibody was detected in all samples drawn within 14 days after the first temperature rise. In the cases of long incubation periods the serum gave a precipitin reaction before the first temperature rise. It appears that a sample taken 14 days or more after clinical signs are seen will be positive for antibodies against EIA virus.

The 199 samples collected at Central Nebraska Packing Co. were all negative. All the positive field samples were from 3 herds (Table 1). One of the field samples, which was the only positive sample from that herd, was verified positive by horse inoculation. The remainder of the samples are being checked by horse inoculation.

The 2 colostrum samples from infected horses produced lines of precipitation while the sample from the susceptible horse did not. The positive samples were checked by horse inoculation and found to be infective.

Antigen and 2 reference serums were supplied by Coggins (Cornell University, Ithaca, New York) for comparison. Potency was compared by endpoint dilution to our antigen and reference serums. The potency was very similar and results were identical with those observed by Dr. Coggins.

The spleen from a noninfected pony was checked against 350 samples, both negative and positive. No specific lines of precipitate were observed with any sample. The spleens from horses infected more than 14 days also produced no lines when tested against known positive and negative serums. As reported previously, it appears the production of an acceptable antigen requires the rapid onset of acute clinical signs and harvesting the spleen 8-14 DPI.

The precipitin reaction is enhanced by the use of a positive serum on each side of a sample being tested. Figure 1 illustrates that the sample in well No. 5 (the same serum as in well No. 2) would not have been identified as positive if it had been next to 2 negative samples. The control positive lines also check the antigen and aid in the identification of nonspecific lines which are seen occasionally. Figure 2 illustrates how the test is normally set up and shows a negative, a weak and strong positive samples.

Several of the serum samples tested by the immunodiffusion test have been found to contain antibodies to other agents. Antibodies to the following equine viruses were demonstrated. Herpes I and II, influenza (A1 equine and A2 equine)* Eastern and Western encephalomyelitis.* Also, six samples that were CF positive for piroplasmosis** were tested as were samples from clinical signs of strangles.† The other agents had no observable effect on the immunodiffusion test.

Heat inactivation of the serum at 56°C for various periods up to 1 hour had

---

* Courtesy of Dr. L. Sinclair, Veterinary Biologics Division, NADL, Ames, Iowa.
** Courtesy of Dr. E. A. Carbrey, Animal Health Division, NADL, Ames, Iowa.
† Courtesy of Dr. L. Sinclair, Veterinary Biologics Division, NADL, Ames, Iowa.
no effect on our ability to detect EIA antibody with this test. The addition of 0.1%, 0.5% and 1.0% phenol to the serum did not affect the test results.

There are certain limitations to this test. It can detect only antibody and not virus. As the EIA-infected horse is considered to be a carrier for life, a horse positive for antibody should be a virus carrier. There have been exceptions to this carrier status reported\textsuperscript{14,15} Our observations also indicate that if repeated horse inoculation tests are made on the same chronic carrier, virus will not always be demonstrated. It appears though that very few if any animals ever completely recover.

The spleen does contain a variety of antigens which could lead to spurious precipitin lines. A few of these lines have been observed and do add confusion to the test even though they have been easily identified. This is a potential problem and a standardized antigen that will produce repeatable results must be used.

The results of this evaluation of the immunodiffusion test have been excellent. The test has shown 100% reliability on all samples tested. Also, Dr. Coggins' results have been duplicated in this laboratory. This initial evaluation has revealed no serious problems in the use of this test to diagnose EIA.

ACKNOWLEDGMENTS

The authors express their appreciation to Dr. L. J. Kemeny for his advice and assistance, Mr. Dennis Somers for technical assistance and Mr. Ralph Glazier for the illustrations.

SUMMARY

Evaluation of the immunodiffusion test indicates that it is an accurate method for the diagnosis of equine infectious anemia (EIA). Multiple serum or plasma samples from 57 known susceptible and 72 horses experimentally infected with EIA have been tested. The results were 812 negative and 468 positive samples. All infected horses became positive and remained positive for as long as they were alive. Antibody to EIA was not detected in samples from any susceptible horse, or in experimentally infected horses until 14 to 40 days after infection.

Antibodies to other agents, heat inactivation of the serum, and addition of phenol had no recognizable effect on the test.
TABLE 1
Immunodiffusion Test Results

<table>
<thead>
<tr>
<th>Source of Specimen</th>
<th>EIA Infectivity Status</th>
<th>No. of Horses tested</th>
<th>Positive Samples</th>
<th>Negative Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental horses</td>
<td>Proven susceptible</td>
<td>129</td>
<td>0</td>
<td>812</td>
</tr>
<tr>
<td>Experimental horses</td>
<td>Experimentally infected</td>
<td>72</td>
<td>468</td>
<td>0</td>
</tr>
<tr>
<td>Field samples</td>
<td>Unknown</td>
<td>385</td>
<td>20</td>
<td>365</td>
</tr>
</tbody>
</table>

TABLE 2
Comparison of incubation period to first detection of antibody by the immunodiffusion test.

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Antibody first detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI**</td>
<td>Individual horses</td>
</tr>
<tr>
<td>8</td>
<td>15, 16</td>
</tr>
<tr>
<td>9</td>
<td>16, 16</td>
</tr>
<tr>
<td>10</td>
<td>14, 16, 16, 24</td>
</tr>
<tr>
<td>11</td>
<td>14, 16</td>
</tr>
<tr>
<td>12</td>
<td>18, 18, 18</td>
</tr>
<tr>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>15</td>
<td>17, 21</td>
</tr>
<tr>
<td>16</td>
<td>20, 22</td>
</tr>
<tr>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>61</td>
<td>40</td>
</tr>
</tbody>
</table>

*To temperature over 101°F for 24 hr.
**DPI – Days postinoculation
Figure 1 – Immunodiffusion test in which antigen is in center well, control positive serum in wells 1 and 3, the same weak positive serum in wells 2 and 3, and negative serum in wells 4 and 6.
Figure 2 – Immunodiffusion test which has antigen in center well; control positive serum in wells 1, 3 and 5; negative serum in well 2; weak positive serum in well 4 and strong positive serum in well 6.
EVALUATION OF THE IMMUNODIFFUSION TEST

REFERENCES

V.E.E., A DISEASE ON THE MOVE

Richard O. Spertzel*, V.M.D., Ph.D., and Robert W. McKinney, Ph.D.

Venezuelan equine encephalomyelitis (VEE) is a zoonotic arbovirus disease affecting both the horse and man. In the horse, the infection may be expressed as (a) subclinical, with no overt symptoms; (b) mild, consisting primarily of anorexia, high fever and depression; (c) severe, but nonfatal, consisting of anorexia, high fever, stupor, weakness, staggering, blindness, and occasionally with permanent sequellae; or (d) fatal, with the same sequence of symptoms, but terminating in death. Signs of illness may appear from 3 to 10 days after infection and persist from a few hours to several days. In man, however, the disease occurs more commonly as a mild-to-severe respiratory illness, associated with a severe frontal headache and high fever; overt encephalitis is rare and occurs primarily in children. The severity of the disease, in terms of morbidity and mortality, varies with virus subtype and background immunity of the host population. The etiologic agent of VEE is a member of the A Group of arboviruses. Minor antigenic variations exist among different VEE virus isolates, and 8 subtypes have been identified in vitro. However, infection with one of the subtypes results in immunity to the others.

The virus is endemic throughout major portions of Central and South America, and parts of North America, and has been isolated in the state of Florida, Fig. 1. Outbreaks, or “waves”, of the disease occur sporadically immediately adjacent to these endemic areas; these have been of low magnitude and were self-limiting.

An epizootic of equine encephalomyelitis was diagnosed in Colombia and Venezuela, in 1935 and 1936, respectively. The etiology of this disease was shown to be a previously unidentified virus and was named Venezuelan equine encephalomyelitis virus. The disease spread eastward across Venezuela and erupted on the island of Trinidad in 1943. Subsequently, additional outbreaks appeared in Peru, Ecuador, and Colombia. The most recent outbreaks have originated in Venezuela (1962 and 1968), Colombia (1967-68), Ecuador (1969), Guatemala (1969), Mexico (1970), and Costa Rica (1970). The outbreak, which started on the Venezuela-Colombia border in 1968, is gradually moving eastward across Venezuela.

In 1969 a major epizootic erupted on the Pacific Coastal Plain of Guatemala, adjacent to the El Salvador border. From this area, the disease rapidly swept westward and northward in Guatemala, and eastward through El Salvador, Honduras and Nicaragua. Morbidity and mortality were high. The severity of the outbreak correlated well with the laboratory finding that the epizootic strain was similar to the highly virulent subtype 1B previously found only in Ecuador and the adjacent part of Colombia.

*From the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701

268
An attenuated live viral vaccine was used to control the 1969 Central American epizootic. Vaccination of the horse population at the periphery of the epizootic was accomplished to create an immune barrier in an attempt to limit the spread of VEE infection. Vaccination of horses also was practiced within the epizootic area to control the disease. Seven to 10 days after vaccination, even on ranches where active cases were occurring, all equine cases of VEE ceased. Complete protection occurred in local areas, e.g., valleys or large ranches within the eventual epizootic area, in which vaccination was completed prior to spread of the disease into the area. In Guatemala, an immune barrier of vaccinated horses, about 50 kilometers in width, was established on the Pacific Coastal Plain. This barrier prevented the spread of VEE westward in Guatemala, Fig. 3. In addition, in the upper and lower Motagua Valley, vaccination was successfully employed to halt the disease. By the use of a massive VEE vaccination program, the disease was confined to the Pacific Coastal Plain of Honduras and was contained in the southwestern corner of Nicaragua.

Although the 1969 epizootic in Guatemala and Nicaragua was contained, the disease erupted in 1970 in Guanacaste Province of Costa Rica. It is postulated that the infection originated in Nicaragua. Such transmission would imply that VEE infection breached the barrier of immunized horses established the previous summer, since over 90% of the equines in the province of Rivas in Nicaragua were vaccinated and there were no cases of VEE reported there in 1969, or in 1970. In early June 1970, VEE erupted in the Grijalva River Valley of Mexico, adjacent to Guatemala, and from there it spread throughout the upper Grijalva Valley, into the Pacific Coastal Plain and the isthmus of Tehuantepec. In both Costa Rica and Mexico, spread of the epizootic again appears to be halted by massive immunization campaigns using the attenuated vaccine.

It must be emphasized that the vaccination programs employed a live attenuated virus vaccine. A word of caution is necessary on the use of inactivated vaccines prepared from virulent VEE virus. In the spring of 1970, an inactivated VEE vaccine, prepared commercially in Central America in accordance with standards established for Eastern and Western equine encephalomyelitis vaccines in the United States, was used in Nicaragua. Concurrent with the use of this vaccine, encephalitis appeared in horses, and deaths in equines were traced to the vaccine. This event corresponds to that which occurred in the 1950's when laboratory personnel, immunized with inactivated VEE vaccine prepared in essentially the same manner, developed disease due to VEE virus. Although safety tests in both situations had indicated the absence of residual live virus, use of the vaccine resulted in clinical disease. The inability to produce an inactivated vaccine that was safe and effective prompted the development of the attenuated vaccine used in Central America.

This attenuated virus vaccine, developed for use in laboratory personnel, has

---

*This vaccine was developed at the U.S. Army Medical Research Institute of Infectious Diseases at Fort Detrick, Frederick, Maryland and was furnished from research supplies. The Institute also provided technical assistance to the several countries.
been extensively used in Mexico and Central America during epizootics. Immunization campaigns in these countries have been highly effective in preventing equine deaths, and potentially served to reduce the human illness which follows the equine disease. Although the epizootic was arrested in 1969, the disease erupted beyond the vaccine immune barrier in 1970.

Thus, the disease continues to move, Fig. 4. It is the opinion of the authors that it is not a question of if, but when an epizootic of VEE will erupt in the United States. As indicated earlier, virus of Venezuelan type has been isolated in Florida, and 3 human cases have been reported there.\textsuperscript{13,14} With these findings, plus the continued spread of the highly virulent subtype 1B, the potential for its introduction into the United States must be recognized. The present state of the knowledge concerning the ecology of this virus does not permit saying when and where this will occur.
ENDEMIC DISTRIBUTION
OF VEE SUBTYPES

FIGURE 1
EPIZOOTICS
OF VEE SUBTYPES

FIGURE 2
CENTRAL AMERICAN EPIZOOTICS
AND IMMUNE BARRIERS, 1969-70

FIGURE 3
PROVEN OUTBREAKS
VEE SUBTYPE IB

JAN 1969
JUNE 1969
JUNE-NOV 1969
SEPT 1970

FIGURE 4
REFERENCES


REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

C. L. Campbell, Tallahassee, Florida, Chairman


The horse disease upon which you have just heard a rather thorough treatise, that of Venezuelan Equine Encephalomyelitis, has assumed greater significance of late to animal health officials of this nation. In 1969 your Committee on Infectious Diseases of Horses reported that there had been extensive outbreaks of this disease in South and Central America. However, it appeared at that time that the incidence may have peaked and was on the decline. Such is not the case. VEE has moved some 250 miles up into Mexico where more than 6,000 horses are reported to have succumbed from the malady in recent weeks.

It is the opinion of your Committee that VEE constitutes a major threat to the United States equine industry at this moment, and that vigorous plans for preventing its introduction into this country should be initiated at once. To this end, the following Resolution is presented as a part of this report and for proper reference to the Committee on Nominations and Resolutions for appropriate action. I should like to point out that more than 1½ million doses of VEE vaccine have been used up to this point with apparent safety and efficacy in connection with field outbreaks.

RESOLUTION

Your Committee also considered and wishes to propose the following VEE Contingency Plan:

Introduction

This Plan recognizes that: (1) VEE is a zoonotic arthropod-borne disease, (2) It is apparent that the health of people will have first priority over that of other hosts, and obviously U.S. Public Health Service must play a major role in any program, and (3) Equidae and rodents seem to be major amplifying hosts for the virus of VEE.

The Organization

Full responsibility for the prevention, control and eradication of VEE in equidae must rest with the U.S. Department of Agriculture. In fulfilling this
responsibility USDA should utilize the Emergency Animal Disease Eradication Organization (using the USDA Red Book as a guide) where practicable.

Because of the related human health problem a close planning and working relationship must be maintained with the United States Public Health Service. This relationship should be established now.

USDA should at once establish a Select Advisory Committee making available all the expertise on VEE existing in USDA, USPHS, U.S. Army Medical R & D Command, and any other possible sources. Said Committee should also include the President of the U.S. Animal Health Association, representing the states.

USDA should, with the advice of above select committee, appoint a National Campaign Chief who will be given full responsibility and support if VEE is diagnosed in the United States. The National Campaign Chief should be an individual mutually acceptable to USDA and USPHS, but independent from either agency. The chief state animal health official in each state should serve as the campaign chief in his state.

I. The National Campaign Chief and his staff, in addition to those stated in the "Emergency Animal Disease Eradication Organization Plan" should have the following responsibilities:

1. Issuing all news releases relating to VEE outbreaks. It is recognized that because of the human health involvement distorted and "scare" headlines and stories are apt to appear in the press and that broadcast of erroneous information may cause mass hysteria resulting in the American populace in general completely avoiding all horse sports, activities and areas where horses are located. This obviously would deal a catastrophic economic blow to the $7 billion equine industry. Consequently, an aggressive, factual information and news release program closely coordinated with USPHS and other health officials must be provided.

2. Planning the strategy that should be used in the campaign against VEE in the United States, and neighboring countries where applicable.

3. Establishing and issuing policy relating to the campaign in coordination with the chief animal health official in the states concerned.

II. The USDA and State Animal Health Regulatory Officials should be responsible for the following:

1. Establishing a diagnosis through collection of samples from equidae and information relating to outbreaks.

2. Controlling the international, interstate, and intrastate movement of animals through quarantines and similar actions.

3. Establishing a VEE vaccination program for equidae where indicated.

4. Carry out their responsibilities utilizing the Emergency Animal Disease Eradication Organization wherever possible.

III. The U.S. Public Health Service should be responsible for the following:

1. Providing laboratory services relating to virus isolation from humans, equidae, and insects and serology relating to VEE problems.

2. Protecting people and animals against biting insects through application of
appropriate insecticides and larvacides.

3. Trapping insects and small vertebrates to determine the vectoring and reservoir potential in a given geographical area.

**The Threat**

Venezuelan Equine Encephalitis has caused serious epizootics in the equine populations of several South American Countries. In 1969 serious outbreaks occurred in Central America. In 1970 additional outbreaks have occurred in Central America and epizootic disease has been identified for the first time in Mexico. The first outbreaks in Mexico were near the Mexico-Guatemalan border. The disease now appears to have moved north and west, an estimated 250 miles. Horses and other equidae in the United States are believed to be nearly 100 percent susceptible to VEE.

**Control Actions**

The slaughter of equidae as the method of control is judged not appropriate for use against VEE, except in specific instances where indicated. The causative agent of VEE, a virus, is arthropod-borne and may be hosted by other species. Therefore, the slaughter of affected horses in an area would not eliminate the disease. For instance, susceptible horses introduced into an affected area may contract VEE from non-equine animals or insects.

I. There are four control tools available. They are:

1. Control of vectors to reduce spread of disease and further exposure to man and animals.
2. Control of movement of equidae.
3. Control husbandry practices and the transport of dogs and other mammals, including exotic animals, that may constitute reservoirs.
4. Vaccination (assuming the vaccine is further tested and meets the minimum Veterinary Biologics Division requirements for such vaccines).

II. If VEE is diagnosed in equidae in the United States, the following actions should be initiated:

1. Control regional and local movement of equidae to control spread from epizootic area and to control spread within epizootic area.
2. Immediately institute appropriate area-wide vector control.
3. Urge equine owners to practice rigid vector control among their animals. Vector control should include appropriate and careful use of pesticides and where possible housing of equine in insect-proof facilities.
4. Vaccinate equidae to form an adequate (epidemiologically defined) vaccine immune barrier around the periphery of the epizootic in designated high risk areas. This vaccination to be an ANH and State agency supervised and programmed owner-practicing veterinarian cooperative venture. For the first six months USDA should provide the vaccine, and private practitioners should do the vaccination. If sufficient practitioners are not available, vaccine should be administered by State and/or USDA veterinarians and possibly DOD
5. Control husbandry and movement of dogs and other mammals, including exotic animals, that may constitute reservoirs.  

III. If VEE in equidae is diagnosed anywhere North of Mexico City, but not in the United States:  
1. Use the same vector control procedures outlined above.  
2. Compulsory vaccination of equidae in nearby high-risk areas. (Zone of vaccination to be based on factors such as presence of and potential movements of vectors and non-equine hosts.)  
3. Discontinue importation of horses and other equidae from the affected country.  
4. Alert U.S. equine owners, including explanation of vector control procedures.  
5. If other arrangements for vaccine production have not been completed, USDA should issue temporary license for production of five million doses of VEE vaccine.  

It is consistent with the forementioned plan that the United States give high priority to research designed to evaluate the efficacy and safety of a vaccine to be used against VEE in the United States.

EQUINE PIROPLASMOSIS

Your Committee received and considered revised data compiled on equine piroplasmosis since the last meeting of this Association and wishes to make the following observations and recommendations.

1. The 1969 report suggested a revision to the Prospectus on chemotherapeutic measures for horses infected with Babesia equi or Babesia equi and caballi, using diampron at the rate of 4 mg. per pound body weight intramuscularly for four consecutive days. Experience since then has shown diampron as being ineffective against B. equi infection in numerous horses treated. Therefore, appropriate deletions should be made in this area of the Prospectus.

2. Recognizing these inadequacies of diampron, the experimental drug 4A65 has been employed on a limited number of B. equi infected horses at variable levels with questionable success. Notwithstanding, it is herewith recommended that continued drug trials using 4A65 be conducted inasmuch as no other therapeutic agent for sterilization of this strain of piroplasmosis is in the offing.

3. In the area of categorizing E.P. reactors, additional research has demonstrated that the minimum classification for a reactor to both B. equi and B. caballi should be at the level of 2+ in 1-5 dilution. Such a change is therefore recommended with proper amendments being applied to the Prospectus.

The following resolutions are herein included as a part of this report and will be presented to the Committee on Nominations and Resolutions for its consideration.

(Resolution recognizing CF test)  
(Resolution on international movements)
(Resolution on interstate movements)

AMERICAN HORSE COUNCIL MEMBERSHIP

A communication from the American Horse Council, Inc., requesting that the U.S. Animal Health Association consider sponsor-body membership affiliation with that organization was discussed. Your Committee wishes to direct attention of this body to its 1969 report defining the activity area and accomplishments of the American Horse Council. It is apparent that the endeavors of that organization and those of the U.S. Animal Health Association are closely akin, and it is evident that a mutual affiliation between the two groups will be of value to the equine industry. With this in mind, it is recommended that the U.S. Animal Health Association affiliate with the American Horse Council in the capacity of a sponsor-body member, and invite affiliation of that august group with the U.S. Animal Health Association.

EQUINE INFECTIOUS ANEMIA

The disease area which provoked the most vociferous response was that of equine infectious anemia. The paramount issue of consideration involved the agar gel immunodiffusion or Coggins test for the diagnosis of this disease. Diverse views were expressed by all members and the majority of the audience in attendance as to the immediacy of acclaiming recognition or official sanction to the test. It was quite apparent from the disclosure of certain precipitous incidents which had occurred in various parts of the nation in recent weeks, irrespective of sparse confirmatory evaluations, that the test is going to be used. Certainly, the data presented to your Committee—and these reports will appear in the proceedings of this meeting—are most promising albeit limited.

Your Committee acknowledges that the test may not have met the criteria to have removed it from the experimental category, and until approved by the U.S. Department of Agriculture will probably continue to be considered as experimental. Notwithstanding, and in the interest of maintaining the greatest degree of uniformity and accuracy in the application of the test, your Committee recommends that recognition be given it by this body as to its validity, and requests that the American Association of Veterinary Laboratory Diagnosticians set down the protocol and guidelines for conducting the test in laboratories throughout the nation at the most expeditious moment. Further, and probably more importantly, we realize the impact which opening this door will have upon the nation's equine population, and those of us who are the chief state animal health officials will all too quickly be deluged with the numerous problems which the availability of this test will occasion. Undoubtedly you will be faced with having to make decisions with respect to quarantine procedures, restrictions of movements of horses in intrastate and interstate traffic, the possible cancellation of shows or meets, and many other allied problems which most assuredly will occur in the wake of pressure
from uninformed and emotional equine special interest groups. In view of this, your Committee wishes to alert the President of this Association of the possible consequences which are now imminent with respect to equine infectious anemia, and the possible necessity for an early meeting of regulatory officials to develop a national plan so as to avoid a replication of developments of some four years ago when this disease assumed "sudden significance."

As the first step in this direction, your Committee also herewith recommends the initiation of long range procedures entailing an extensive educational program on equine infectious anemia, with the inclusion of a most important facet of the problem, that of developing a workable method of identification of equidae.

**RESOLUTIONS**

WHEREAS, the complement fixation test has been shown to be a practical aid to the diagnosis of Equine Piroplasmosis; and

WHEREAS, the American Association of Laboratory Diagnosticians has recommended that the C.F. test be recognized as the official diagnostic test for equine piroplasmosis whether caused by Babesia caballi or Babesia equi until such time as a more accurate test is developed; NOW, THEREFORE, BE IT

RESOLVED, That the U.S. Animal Health Association recommend that the complement fixation test be adopted as the official test for the diagnosis of equine piroplasmosis by all animal health regulatory agencies in the United States.

WHEREAS, it has been demonstrated that horses recently imported into the United States have been found affected with EP caused by Babesia caballi and/or Babesia equi; and

WHEREAS, horses offered for importation have often been found infested with tropical horse ticks (Dermacentor nitens); and

WHEREAS, during the last sixty days since the initiation of test procedures more than forty percent of horses offered for importation from Central and South American and Caribbean countries have been found affected with EP, and in some cases infested with vectors of this disease, namely Dermacentor nitens; NOW, THEREFORE, BE IT

RESOLVED, That the U.S. Animal Health Association take cognizance of and endorse action recently initiated by the U.S. Department of Agriculture in amending Title 9 of the Code of Federal Regulations on importation requirements, as follows:

1. All equidae, except those born in Canada, including zebras, be freed of ticks in the country of origin, be required to have a USDA permit (which includes a courtesy CF test for EP) prior to leaving the country of origin, and upon arrival in the United States be inspected, found free of ticks and receive a precautionary treatment with a permitted tickicide.

2. Prior to being released for entry, such equidae be subjected to the CF test for EP and handled in the following manner:
   a. Animals not classed as reactors to CF test be allowed to enter if otherwise eligible.
   b. Animals classed as reactors to the CF test be refused entry.

RESOLVED, FURTHER, That the procedure adopted by the U.S. Department of Agriculture in that interim before the foregoing amendments to the federal regulations become effective, which provides that equidae now being imported into the United States and destined for Florida meet prescribed negative EP and vector requirements, be immediately extended by the Director of the Animal Health Division to encompass all states.

WHEREAS, it has been shown that horses recently moved interstate from equine piroplasmosis endemic areas in 12 states where E.P. has not been considered to be endemic have been found to be affected with E.P.; and

WHEREAS, it has been shown that horses recently moved interstate have been found to be infested with tropical horse ticks upon arrival in four states not considered to be tick infested or endemic for E.P.; NOW, THEREFORE, BE IT

RESOLVED, That the United States Animal Health Association support a concerted effort to amend the code of federal regulations to measureably reduce the interstate spread of equine piroplasmosis, such amendments in the regulations to be made within one year.
FDA AND ANTIBIOTICS USED IN ANIMAL FEEDS

by

C. D. Van Houweling, D.V.M.
Director, Bureau of Veterinary Medicine
Food and Drug Administration

It is a pleasure to participate in your annual meeting again and tell you about some more recent developments that are of mutual interest. As you know from the communications media, consumer groups are becoming more concerned with the safety of the foods they eat. Our goal, and yours, is to protect human and animal health. We assist by ensuring that all veterinary drugs marketed are safe, effective, properly labeled, and correctly used.

An estimated 80 percent of the protein for human consumption is derived from livestock and poultry that have consumed medicated feed during part or all of their lives. In 1969, livestock and poultry consumed about 40 million tons of feed containing some kind of medication. Because of these facts, an increasing concern is being expressed by scientists and other informed persons, as to whether (1) residues of these drugs remain in the meat, and (2) whether the drug resistant bacteria which develop in animals may affect the health of persons consuming these foods.

During the past year, FDA reorganized under Commissioner C. C. Edwards, and the Bureaus were realigned under product lines. The Bureaus are: Bureau of Drugs, Bureau of Food and Pesticides, and the Bureau of Veterinary Medicine. Each Bureau now has complete responsibility for a given product area. This begins from the time that the product is approved and continues while it is used, and as a result we are more directly involved in FDA’s field compliance programs — both voluntary and regulatory.

As one part of our compliance program our Bureau is cooperating with industry associations in developing educational activities directed to livestock producers and related industries so that they will observe label directions, including withdrawal periods and other restrictions for use. These activities include educational meetings, published material, slide series, radio announcements, and informational material prepared for selected audiences. To achieve a high degree of voluntary compliance with our Federal law, we need a coordinated educational program directed specifically to about 10,000 medicated feed mixers, 25,000 veterinarians, and more than a million producers of livestock (poultry, swine, beef, dairy cattle, sheep). Obviously, it is impossible to check on every farmer to ensure his compliance with the law. It would be impractical and very costly to take a sample from every animal slaughtered to find out if there is any illegal residue present. Therefore, the educational activities are an important part of our compliance program.

Under FDA’s reorganization, our Bureau now has complete responsibility for

animal drugs and feed, including contamination by pesticides, Salmonella, mycotoxins, or other contaminants that may affect livestock. To reflect our compliance policy, a few months ago BVM told the district offices that “we will see to it that all significant, properly documented and otherwise well-supported violations of the Act receive vigorous enforcement attention.”

In fiscal year 1970, FDA’s field offices took 16 prosecution actions, five of which were seizures and 11 were recalls of medicated feed. According to our Compliance Office, the statement that we are prepared to support enforcement actions submitted by the field has resulted in more submissions of proposed legal actions by the field inspection staff — including seizures, injunctions, and prosecutions. Also more firms are receiving warning letters or citations to advise them of certain conditions or practices that are not in accord with the Federal Food, Drug, and Cosmetic Act. We adopted a more active compliance program because we felt that it was needed to support state and federal enforcement activities.

Also under this Act, FDA is responsible for approving animal drugs which are safe and effective, and which do not leave harmful residues in meat from treated animals. As reviewed with you last year (pages 236-248 of The Proceedings of the 1969 Annual Meeting) the legal restrictions of the Act pertaining to drug residues in food from animals are based upon human health considerations. In addition to the residues, as discussed last year, there is increased concern regarding another potential hazard — the development of drug-resistant bacteria and the transfer of resistance between bacteria. This concern is because drug resistance can reduce the effectiveness of drug treatment in persons or animals.

The so-called Swann Committee (chaired by Professor Swann) was assigned the task of studying the use of antibiotics in veterinary medicine and animal husbandry in Great Britain. Based on their findings, they recommended that the antibiotics be grouped into “feed” antibiotics and “therapeutic” antibiotics. The feed antibiotics are those which are not used to treat disease, do not usually develop multiple drug resistance, and are not known to cause transfer resistance. The committee recommended against the use of therapeutic antibiotics at the low levels in feed and that they be sold only on a prescription from a veterinarian. The purpose of this recommendation is to reserve the use of therapeutic antibiotics for treating diseases and to avoid the development of resistance to these drugs that are so valuable for combating disease.

Partly because of the Swann Report, and also because we have been concerned about this problem for several years, the FDA Commissioner has established a Task Force (of which I am Chairman) which includes infectious disease and animal husbandry experts from several Federal agencies as follows:

Dr. J. V. Bennett, Chief
Bacteriology Disease Branch
National Communicable Disease Center
Dr. E. Goode, Assistant Administrator  
Agricultural Research Service  
U.S. Department of Agriculture

Caro E. Luhrs, M.D.  
Medical Advisor to the Secretary  
U.S. Department of Agriculture

De. E. Seligmann, Chief, L.C.A.  
Division of Biologic Standards  
National Institutes of Health

Dr. A. L. Kolbye, Deputy Director  
Bureau of Foods and Pesticides  
Food and Drug Administration

Dr. A. E. Smith, Deputy Director  
Division of Anti-Infective Drugs  
B.D., Food and Drug Administration

Dr. W. W. Wright, Deputy Director  
Office of Pharmaceutical Sciences  
B.D., Food and Drug Administration

Dr. H. D. Mercer, Acting Director  
Division of Veterinary Research  
B.V.M., Food and Drug Administration

Dr. R. A. Baldwin, Group Leader  
Antibiotics Group, D.V.N.D.  
B.V.M., Food and Drug Administration

Dr. R. P. Lehmann, Assistant to the  
Director for Statistical and Program  
Analysis, B.V.M., Food and Drug Administration

In addition, the following people have been appointed as Consultants to the Task Force:

J. L. Krider, Ph.D., Head  
Department of Animal Sciences  
Purdue University
A. K. Saz, Ph.D., Chairman  
Department of Microbiology  
Georgetown University  
School of Medicine  

D. H. Smith, M.D., Chief  
Infectious Disease Unit of Pediatrics  
Children's Hospital Medical Center of Boston  

W. G. Huber, D.V.M., Ph.D.  
Professor of Pharmacology  
College of Veterinary Medicine  
University of Illinois  

H. L. Wilke, Ph.D., Vice President  
Director of Research  
Ralston Purina Company  

The Executive Secretary is Dr. L. C. Harold, Special Assistant to the Director of the Bureau of Veterinary Medicine.  

We are undertaking an in-depth review of the usage and actual value of low-level antibiotic feeds, the possibilities of an adverse effect on human health, the economic impact of restricting the use of antibiotic feed additives upon both the manufacturers and the users and the best method for consulting with representatives of affected industries. We have had five meetings — in Washington, D.C., Atlanta, Georgia, Denver, Colorado, Cedar Rapids, Iowa and New York City. As a result of some discussion concerning therapeutic uses of antibiotics for the treatment of animal diseases, the Task Force decided it would be helpful to state the parameters of concern relative to the levels and uses of antibiotics in feed. As a result, this statement was approved and announced:  

"The Task Force on the Use of Antibiotics in Animal Feeds is concerned with the safety and effectiveness of the antibiotics when they are used at the lower or intermediate levels for prolonged or continuous use as a means of increasing rate of gain or for disease control rather than the short-term therapeutic uses. An exception would be when the low level of antibiotic use can be shown to have an adverse effect on the subsequent therapeutic use of the same or other antibiotics."

At our first meeting held May 21 in Washington, we began our study and outlined our course for the in-depth review. As part of the study of the effectiveness of the antibiotic uses, we have had three meetings where the Task Force members have observed first hand modern poultry, cattle, and swine production practices. At each of these meetings, the Task Force has heard reports from university and industry scientists relative to the value of various antibiotic uses. On October 12-14 the Task Force attended the Conference on the Problems
of Drug Resistant Pathogenic Bacteria in New York, sponsored by the New York Academy of Sciences. The day after the conference, the Task Force met with several of the internationally-recognized scientists attending the conference to discuss in more detail transferable drug resistance in animals and the potential human health hazard involved. Our plans are to hold meetings of the Task Force during December in Washington to hear any additional presentations and to begin to draw up the conclusions and recommendations of the Task Force.

Commissioner Edwards has assured us that he is not looking forward to a lengthy report, but wants the Task Force to make specific recommendations, supported by the necessary documentation. Our charge is to undertake an in-depth review of the usage and actual value of low-level antibiotics in feeds, the acquisition by selection of the R-factor by organisms (including non-pathogens) through animal feeding practices, the possibilities of transference of the R-factor to human pathogens, the economic impact of restricting the use of antibiotic feed additives upon both the manufacturers and the users, and the best method for consulting with representatives of the affected industries.

The development of drug resistance has been a problem of great concern to the medical professions. The difficulties encountered by the medical profession in the treatment of human diseases as a result of drug resistance are well known. Problems encountered in treating drug-resistant organisms causing animal diseases are not as well documented. This may be because they have not been as extensively studied. We are hoping that the diagnostic laboratories can provide us some useful data in this regard. We do know that when antibiotics are used in feed that organisms do develop resistance. On the other hand, scarcity of studies related to resistant organisms in animals may be a reflection of the lack of problems encountered in clinical veterinary medicine. The documentation of human disease caused by drug resistant organisms which have come directly or indirectly from animals fed antibiotics in animal feed has not come to my attention. It is easy to hypothesize that this may have occurred or is occurring. However, with the millions of animals in the United States that have been fed antibiotics over the past 15 years one would expect it to be noted if human disease problems are resulting with any degree of frequency. These statements reflect the dilemma we are facing.

FDA approves new animal drug applications (NADA) when substantial evidence supporting safety and efficacy has been supplied by the sponsor. The approvals and drug performance are always under surveillance. Sponsors must supply us drug experience reports each six months after approval for the first year and annually thereafter. If experience with the drug indicates lack of effectiveness or safety, the claims and labeling may be revised or the approval may be withdrawn. Just as we require substantial evidence for approvals, drug sponsors expect us to have data which supports restrictions on drug uses or proposals to withdraw approvals. There are, therefore, limitations on the actions that FDA may take in regard to drugs that have been approved.

In our budget request for the fiscal year that started July 1st, we are asking for funds to expand our contract research program related to the safety and efficacy of
antibiotics in animals. If we receive these funds, we will be able to greatly expand our research in these areas. I hope that the drug and feed industries will also expand their support for this kind of scientific exploration. By doing so, we hope to be able to answer some questions with facts based upon solid data rather than relying on informed opinions, as we have to do now.

In carrying out FDA's responsibilities, the Bureau of Veterinary Medicine tries to strike a balance between safeguarding human health and meeting the needs of the animal food producer. But, if there is a recognizable adverse effect on human health, or if illegal residues are being detected, we must act to control or restrict their use. Therefore, we are constantly alert to development of health concerns. This is why we are carefully studying the effect of the development of drug resistance in animals to see what effect it is having on animal health and what effect it may have on human health.
ANIMAL INDUSTRY CAN PRODUCE PROTEIN FOR SEVEN BILLION BY 2000 A.D.

Robert White-Stevens  
Chairman, Bureau of Conservation and Environmental Science  
College of Agriculture and Environmental Science  
Rutgers University  
New Brunswick, New Jersey 08903

INTRODUCTION

The demand for protein in the human diet is a function of several factors including, dietary tastes and customs, physical activity, climatic environment and standard of living but the primary factor that controls the quantity demanded is population.

World protein needs by year 2000 and onward into the 21st century are therefore directly related to the size and growth rate of the human population.

As we enter the eighth decade of the 20th century world population exceeds 3300 million and at an annual increment rate of over 2%, or in excess of 8000 births over deaths per hour, will clearly pass 6.5 billion and quite probably 7 billion by 2000. In spite of the intense efforts being made to curb birth rates by persuasion in the presently over populated areas, estimates continue to be repeatedly revised upwards. As most of the effort, money, and persuasion to limit birth rate among the “emerging” nations comes from the Western nations where protein and other foods as well as all living standards are adequate if not in surplus abundance there is a natural reaction among the less favored nations to regard the efforts to reduce their population growth as an invidious scheme to keep them perpetually powerless and in penury. Such an interpretation is continually disseminated by communist propaganda to justify their philosophy of life as opposed to that of western democracy.

As these two forces essentially offset one another, it can be concluded with assurance that if the brakes of a “peoplostat” were applied today on a world wide basis — a completely unrealistic suggestion — the population of the planet would skid past 6000 million by year 2000 anyway. This means the population of the earth will increase within 30 years by as much as it has over the last 30,000 years.

This fantastic accretion of humanity constitutes the most significant biological event since life first appeared on the earth. It is a cataclysm of major proportions, and must demand, if it is to be ameliorated effectively, the concentrated efforts of all intelligent men everywhere. It should assume, immediately, priority over space exploration, nuclear arms reduction and proliferation of anti-missle protection, for it lies at the root of all domestic crises, political, economic, social or environmental, it is the basis for international tensions, jealousies, envy and conflict and it promotes ever widening gaps in technology, finance, food supply and understanding between the advanced and the emerging nations.

Within one hundred years at present rates of increase the world population will aggregate nearly 50 million humans, equivalent to 1000 people per square mile or
about the density of people in New Jersey over the entire land mass of the planet, including all the mountains all the deserts and both polar icecaps. This would reach a concentration at the outer limit of the planet to sustain at subsistence level, but is unquestionably far above that to be tolerated in a socio-economic-political civilization as now exists, albeit precariously.

There is reason to believe present technology in agricultural and food science could meet the needs of a population of 6 billion at reasonably acceptable levels of food intake and perhaps even at subsistence levels up to the 12 billion expected by 2025. Beyond this, however, the outlook is exceedingly bleak and Malthusian from the standpoint of the food supply, for a population of only 6 billion, a certainty by 2000, already shows prospects of a significant breakdown in human civilization as it exists today.

The current furor over environmental pollution is, in fact, merely an expression of “people-llution” for the classical “solution to pollution is dilution” and the concentrating populace generates pollutants faster than the available diluents of air, water and space can cope.

It has now become fashionable to seek out scapegoats upon whom to blame all the evils of pollution. The less capable such targets are of self defense the more avid the excoriation becomes. Thus the American farmer, to whom so much is owed by so many, having worked himself, by dint of his own efficiency, into a political minority is now blamed by fat, affluent and self appointed though unqualified custodians of the public welfare, for poisoning the babies of America with pesticides and fertilizers, for polluting the air with farm yard effluvia, which, though perchance arresting at times, are in fact, totally innocuous, and for polluting the streams and rivers with soil erosion, agricultural chemicals and crop detritus. Birds and other wildlife which perish from many causes, including winter wastage, starvation, insect vectored and other diseases and from such man made factors as high rise buildings, TV towers, power lines and traffic, are now reported as dying chiefly from pesticides mainly DDT. Even when the naturalist organizations own bird censuses reveal an inordinate rise in the numbers of many species of bird life, including some raptors any observed declines, in any location, under any circumstances are invariably imputed to the farmer, the forester or the parkland manager.

This evokes the natural law: “Bird numbers increase as the result of shifts in migratory habits, they decline only due to DDT.”

The current fashion that makes a travesty and a sinister conspiracy out of the application of science to modern farming threatens the lives of more humans present or as yet unborn than all the known diseases and the nuclear bomb combined. Today upwards of 20 million people starve to death annually in the world. The Paddock Brothers, in their ominous text “Famine 1975”, conclude this can easily increase five-fold to 100 million and may reach ten-fold to 200 million human deaths per year from starvation. Such a catastrophe would be unparalleled in history, and although it would relieve the “people-pressure” for a time, it would concomitantly engender socio-political-economic repercussions which would be felt
in every country in the world.

For as in the days of the hunger treks described by Pearl Buck which reached across the vast interior of China in the years of the great famines, so on a much larger scale would unnumbered millions of hungry people like plagues of locusts, wander across the face of the earth in search of food at any cost. National borders, political divisions or legal tenets would have no restraint upon them, for they would be impelled only by their hunger and their right to survive. Under such an impact civilization could not survive, for to repel the hunger of mankind with arms and death would be equally disastrous.

It has been seriously suggested that by 2000 space exploration will provide an outlet for the excess humans on this earth by emigration to other planets. Apart from the fact that it takes more energy to lift a 170 lb. man out of the Earth’s orbit than to feed him 2000 calories a day for 70 years and house him at 70°F for life, it would cost more than the present world’s annual gross product to dispose of the excess population of one day now (i.e. 200,000), let alone of one year, 30 years from now. Furthermore we are rather certain now that there is no other planet in this solar system on which man could survive indefinitely, without enormous investment in protective equipment supplied continually from Earth. Actually it would be much more humane to allocate such “surplus” (?) humans to Antarctica, the Sahara desert, the Himalaya Mountains or the bottom of the Oceans.

In any case such measures would only relieve the situation for a short period of time, when the reproduction rate would rapidly climb to offset the withdrawal. Even massive and unconventional methods of food production, particularly protein, will only be ephemeral in effect at meeting the total human demand for food, unless there is a concomitant development of a “peoplo-stat” to curb human reproduction by 2000 on a worldwide basis, unless this is accomplished either by persuasion or coercion, the future of mankind is foredoomed to oblivion as Malthus predicted nearly 200 years ago.

PROTEIN NEEDS OF MAN — PRESENT AND FUTURE

The minimal protein needs of man and his domestic livestock have been qualitatively and quantitatively established, and the variations related to ethnic groups, climatic environment and physical activity though individually important are in total terms of world protein requirements relatively insignificant.

As the qualitative-quantitative protein requirement of man corresponds rather closely to other monogastric species such as the rat, dog or hog, experimental studies on these animals paralleled with clinical studies in man himself have provided reliable estimates of the total protein needs of man. Thus it has been established that man requires eight amino acids, at specific minimal levels. These are 1-isoleucine @ 0.70 g/day, 1-leucine @ 1.10, 1-lysine @ 0.80 g, 1-methionine @ 1.10, 1-phenylalanine @ 1.10, 1-threonine @ 0.50, 1-tryptophane @ 0.25 and 1-valine @ 0.80. The hog, in addition, requires 1-histidine while the rat and the chick also require 1-arginine. It is also of note that only the leavo isomer of these amino acids is biologically effective, which means in most food sources where the
two isomers are racemic or equivalent approximately twice the total amount of protein is required to meet the minimal needs.

Actually man, birds and swine can secure all the needed protein qualities they require from vegetable sources such as cereals, pulses and vegetables, with the possible exception of l-lysine and l-methionine which tend to be marginal in vegetable diets. For these two amino acids, plus the essential animal-protein factor vitamin $B_{12}$ or cyanocobalamine man and his mono-gastric livestock turn to animal sources of protein, meat, fish, eggs and milk.

It is quite customary now to maintain many monogastric livestock such as swine and poultry on purely vegetable diets supplemented with l-lysine, l-methionine or its analogue, and vitamin $B_{12}$ from synthetic sources and such is clearly feasible with man also.

In most of the western countries dietary customs combined with opulence have induced man to become largely carnivorous, and today in such countries as the U.S., Canada and Australia man consumes more than 7 times the minimal amount of animal protein required for his maintenance. As such animal protein is produced from vegetable protein at least at a 7:1 loss, the surplus consumption of vegetable protein by the men of the west aggregates close to 50 times above their minimal needs. This is the ultimate in affluent living, and as most of the protein consumed is for energy rather than tissue growth, nitrogen balance or maintenance the bulk of the nitrogen is excreted providing not only a serious strain on the human kidneys which leads to renal disease, but places an even more difficult stress on our already overloaded sewage disposal facilities and the capacity of our aquifers and streams to dispose of the excess nitrogen. As virtually no municipal treatment plant can cope with high level nitrates in effluents, they continue to gather and encourage algal eutrophication which compounds the problem.

However it seems quite unlikely that we in the west would accept a vegetarian diet or even a return to the more balanced animal: vegetable protein diet of the early 20th century. For the present, at least, there is an increasing desire for animal proteins in all forms, but particularly beef, and it will be some time before economic pressures reverse the trend. Even those who would promote increased consumption of vegetable proteins find it necessary to disguise them under the tastes and appearances of various familiar meat proteins.

From the established minimum levels required per person per day computed into the present and prospective population values the minimal world protein* needs are:

<table>
<thead>
<tr>
<th></th>
<th>1960</th>
<th>1980</th>
<th>2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>11</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>Cereal</td>
<td>48</td>
<td>70</td>
<td>103</td>
</tr>
<tr>
<td>Pulse</td>
<td>16</td>
<td>23</td>
<td>34</td>
</tr>
<tr>
<td>Totals</td>
<td>75</td>
<td>108</td>
<td>160</td>
</tr>
</tbody>
</table>

For North America (U.S. and Canada) these values become:
<table>
<thead>
<tr>
<th>Animal</th>
<th>0.72</th>
<th>0.93</th>
<th>1.14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal</td>
<td>3.24</td>
<td>4.17</td>
<td>5.21</td>
</tr>
<tr>
<td>Pulse</td>
<td>1.08</td>
<td>1.39</td>
<td>1.71</td>
</tr>
<tr>
<td>Totals</td>
<td>5.04</td>
<td>6.49</td>
<td>8.06</td>
</tr>
</tbody>
</table>

(*Computed as pure dry weight protein)

Obviously these values are far below (perhaps as much as 66%) the real demand, which in the U.S. today is for animal protein approximately 7 times higher than actual need, i.e. Ca. 5 million metric tons vs 0.72 required.

There seems to be every reason to believe there will be little or no further significant increase in the total meat protein consumption of western man above the 70 g/person/day. There may well be further shifts in the qualities of protein consumed, but even these are probably going to be relatively minor, except possibly for beef.

At present the distribution of animal protein consumption in the U.S. is:

- Milk and milk products: 41%
- Beef and derivatives: 21%
- Pork and derivatives: 17%
- Poultry of all kinds: 9%
- Eggs and derivatives: 9%
- Veal: 1.4%
- Lamb and mutton: 1.1%

Should foreign demands for our vegetable proteins and cereals increase markedly, as hunger gathers around the world, we may well have to shift our diets back from animal to vegetable proteins, although it seems more probable that we will shift to those animal protein sources which are non-competitive with man in their diets. This would increase the emphasis on beef and even revive lamb, while decreasing it on poultry and swine.

Thus the prime increases in U.S. consumption of animal proteins will come from the enlarging population rather than enhanced appetites. Poultry consumption may increase 10 - 25% from 34.6 to 40 lbs./cap/annum, but this will be maximal as the industry has already made its major growth; similarly pork consumption may increase 10% from 65.3 to 70 lbs./cap/annum; while beef consumption can be expected to rise at least another 25% from 85 to 105 lbs./cap/diem. This should, of course, be particularly encouraged for grain feeding of beef is going to decline as marblelized finishing to meat is discouraged for nutritional and health reasons, and as finishing diets are designed with ingredients that do not compete with man, e.g. sorghum, cannery wastes, bagasse, cotton cake, controlled silages and, of course, non-protein sources of nitrogen such as urea, biuret, etc.

If the conservative estimate of prospective U.S. population of 330 million by year 2000 is accepted as the base, then the required increases in 1960 production of animal protein that will essentially maintain present standards would be:

- Dairy products as milk: 48 billion pounds
- more than 1960
ANIMAL INDUSTRY CAN PRODUCE PROTEIN FOR 7 BILLION

<table>
<thead>
<tr>
<th>Animal</th>
<th>Protein Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>20 billion pounds</td>
</tr>
<tr>
<td>Veal</td>
<td>200 million pounds</td>
</tr>
<tr>
<td>Lamb</td>
<td>800 million pounds</td>
</tr>
<tr>
<td>Pork</td>
<td>12 billion pounds</td>
</tr>
<tr>
<td>Poultry</td>
<td>7 billion pounds</td>
</tr>
<tr>
<td>All meats combined</td>
<td>40 billion pounds</td>
</tr>
<tr>
<td>Eggs</td>
<td>7.7 billion pounds</td>
</tr>
</tbody>
</table>

CAN ANIMAL INDUSTRY MEET THE DEMAND?

Even in conservative terms this comprises an enormous demand upon animal industry in the U.S. and Canada and the question arises as to what are the prospects for its fulfillment.

There is no question that such a supply can be attained by the U.S. animal industry, based upon the presently available capital inputs and technology; with anticipated future developments in breeding, nutrition, physiological and disease control and engineering in housing, feeding, waste disposal, processing, storage and distribution such increments in animal protein production can not only be achieved here in North America but also in a number of other advanced areas, such as Australia, New Zealand and Europe.

The most serious problem confronting the industry today is neither the prospective market nor the equipment to meet the demand, but the political, emotional and economic encumbrances which are being deliberately and irresponsibly thrust into its path. The American farmer has lost his political power at the ballot box, a fatal condition in a democracy, and by 1980 he may well be outvoted by his urban and suburban dependents 50:1.

Paradoxically to release so many workers from the farm he is enforced to turn increasingly to technology to meet his production quota, yet it is just such technology as the application of chemicals to promote growth efficiency and to suppress disease and predation in his livestock; and to employ mechanized housing, feeding and cleaning facilities which has become the target for such unreasonable criticism. As the farmer cannot alone hope to rebut and combat this wave of opposition to the practice of essential scientific agriculture, it is imperative that industry, including all phases of the agricultural chemical and engineering, the food processing and distribution industries combine with the land grant college and experiment station system and the U.S.D.A. to mount a massive educational and public relations program to “let the people know” the actual facts as to their utter dependency upon modern scientific farming for the continuation of their standard of living and general welfare.

It is an absolute absurdity that a relatively few vociferous critics, who clearly have no appreciation of the total needs of the people and even less for the arduous business of providing for such needs, should proceed uncontested to impair the efficiency and productivity of American agriculture through false and speculative accusations which can and have led to rash, ill-considered and inhibitory legislation. It is also exceedingly short sighted for certain aspects of agricultural industry to
ignore the plight and restrictions being laid upon others in the vain hope that by merely maintaining a low silhouette for a while maybe the whole silly business will just fade away in the morning light of reason.

If the critics of prophylactic antibiotics and medicaments in livestock feeds, or of organo chlorine pesticides, or of inorganic fertilizers or of food additives or of farm and processing machinery are wrong, and most of the time they certainly are in error, then all industry and all education and all research should unhesitatingly so tell the public. Conversely where the objective evidence supports the criticisms then we must all face up to it and either find a way to correct the hazard or simply abandon the product or function. The essential point is that unless our decisions are based on valid confirmed experimental objective evidence rather than subjective and emotional speculations skillfully propagated to sway the vox populi, then our food production industries will fail to meet the inexorable demands which lie ahead in the next thirty years and the ultimate suffering of mankind both here and abroad will be unparalleled in history.

LIVESTOCK FARMS-NUMBERS

Virtually all predictions of the shape and function of livestock farms of the future insist that animal production will increase in numbers but not necessarily proportionately in the land areas employed. Dairy herds are expected to average from 400-500 head of lactating cows for optimum efficiency in land use, equipment, labor, technical supervision and turn around operations. Similarly beef production will be centered on large feeding lots strategically located near to raising facilities, requiring a minimal movement to slaughter and further processing. Both industrial and farmer cooperative feed lots will run to 40 & 50,000 head while the surrounding pasture and range will nurture herds of 5, 10 & 15,000 beeves. New range lands will be opened up by improved verdure control through selective elimination of undesirable plant species by herbicide treatment and the introduction of productive species of nutritious forage such as the Bermuda grasses. Hog farms may well aggregate herds of 5,000 to 15,000 per year, with estrus controlled breeding and farrowing in uniform batch loads.

Laying poultry will be almost entirely on wire and completely automated, with flocks of 100 - 400,000 birds in production becoming the general procedure. There are already a number of 200,000 bird laying flocks now in operation where feeding, watering and waste removal, and the collection, cleaning, grading, candling and packing of the produced eggs is entirely mechanized. On one such farm two men supervise the flocks and an additional one man and four women supervise and operate the egg packing shed, into which the eggs are belt carried directly from the cages.

Broiler, turkey and duckling flocks will also become highly mechanized and single farm units will reach from 500,000 to 1 million per year.

Even lamb production which has suffered a sharp decline in this country, will be revived in concentrated mechanical operations, where they will move directly from weaning to the dry finishing lot.
The economic advantages of such livestock farming are overwhelming and in the face of rising labor costs without concomitant increases in prices are the only resort the livestock farmer can take to stay in business. There are, however, a number of disadvantages and problems which will also develop.

The major difficulty will be animal waste disposal. Much research is being currently directed at this problem and doubtless some ingenious and economically effective solution will be found. For the present it is essential to recycle these wastes into the soil where they can produce more crops, even though as fertilizer it is not economic to do so. The plow furrow slurry method of disposal of poultry manures from caged operations is showing promise, provided a crop is immediately sown to absorb the released nitrates, otherwise they descend into the deep aquifers and become a significant contaminant in drinking water. Again the severest opposition to such concentrated animal units is “people-llution”, which, in the industrial-urban-sub-urban states such as New Jersey, flows out and around the livestock operations and promptly commences to clamor and agitate for their removal. This is also an educational problem which we must leave to the social scientists and teachers for it is quite beyond our expertise to handle.

OPPORTUNITIES FOR ANIMAL HEALTH INDUSTRY

These massive aggregates of livestock species will provoke numerous new problems and magnify many of those familiar to current animal farmers. Nutritional, disease and parasite problems will become sharply increased, and as their prevention rather than treatment is paramount the demand for prophylactic measures and medicaments is certain to rise substantially.

As basic nutrition is the first step in disease prevention and as nutrition and disease are integral components in livestock husbandry, it is essential for the animal health industry to integrate disease prophylaxis into efficient dietary programs. With most livestock diseases the major loss is the general reduction in growth efficiency during the course of the disease; and treating sick flocks or herds therapeutically is in most cases, a salvage operation at best and is therefore economically untenable.

The grower must therefor anticipate all probable disease and parasite invasions and obviate them by a combination of preventative measures that include good management, adequate nutrition and use of prophylactic medicaments or treatments including antibiotics, anthelmintics, drugs, vaccines, etc.

FUTURE DEVELOPMENTS IN LIVESTOCK NUTRITION AND DISEASE CONTROL

There are a number of future developments in animal feeding stuffs and prophylactic disease control some of which are already in the mill and all of which will materially aid in meeting protein demands both of the western nations and of those which are presently designated as emerging. Perhaps some of these proposals are just “guesstimates”, but imagination is often the mother of invention, and today reality treads closely in the footprints of discovery.
There is probably no area in feedstuffs research which will prove to be more rewarding by year 2000 and thereafter than to develop sources of protein and energy for livestock which is non-competitive with man. As indicated before, the western world has such a present abundance of vegetable protein and energy sources that we can afford to convert them into animal protein at a 7:1 loss and to do so economically. Indeed we even go out over the world and purchase vast quantities of proteins, eg. fish meal from Peru, to convert it also at a 7:1 loss into poultry and hog proteins. These uses are, however, in constant and direct competition with humans, and tens of millions of children suffer kwashiorkor (protein deficiency) as a direct result of this policy. Sooner or later and hopefully sooner this will have to cease if only for socio-political if not for economic reasons.

Of course the ruminant is particularly suitable for such replacement sources of protein and energy. Non-protein nitrogen, vitamin, mineral fortified supplements derived from corn cobs, waste cotton, bagasse, fruit pomace, beet and citrus pulps are already in general use and should be widely encouraged. The new developments in silo equipment will encourage improvements in ensilage processes through controlled cellulytic and eventually lignolytic enzymes, minerals, other inorganic nitrogen sources, and specific microfloral cultures. Where silo temperatures, moisture and gas exchange can be controlled, highly specific liquid and semisolid fermentations of a wide variety of energy sources such as aliphatic oils and waxes from the petroleum industry, paper residues, sawdust and lumber mill detritus can be converted into suitable protein energy feeds for both ruminants and monogastric species as swine and poultry.

The gathering concern over water pollution from municipal and animal wastes could be substantially ameliorated by lagooning the wastes in algal ponds, seeded with a high protein species, harvesting, ensiling or drying the algae and incorporating it into livestock feeds, as suggested by Oswald at Berkeley. Alternatively the algae produced could be browsed by zooplankton, copepods and shrimp, thence to dace or carp and perhaps to trout which would be processed into fish meal either crude for livestock or purified into fish meal concentrate for use in human diets. Such non-conventional sources of protein-energy feeds for livestock would release vast quantities of cereals, pulses and roots for human consumption without precluding the satisfaction of the entrenched carnivorous appetites of the western world.

Metabolic control of animal growth both for reproduction control and tissue quality is an inevitable development. Estrus regulation among breeding stock combined with artificial insemination will enable the producer to utilize his rearing facilities and labor more uniformly and efficiently and also to time his marketing at the optimum. Such compounds would be applicable to the swine, the dairy and the egg producer. Furthermore out of such research there may well evolve products that will be applicable to humans and could aid in the alleviation of the population explosion.

Physiological agents which could modify gland function in the growing animal to increase growth rate, improve lean:fat ratio, modity fat composition, reduce
ANIMAL INDUSTRY CAN PRODUCE PROTEIN FOR 7 BILLION

cholesterol in the edible product, and induce disease antibodies are being sought be a number of research labs in both the experiment stations and industry. Their discovery and development will induce profound effects in the future of livestock production.

Enzyme additives in feeds, though not particularly promising to date, have already shown that considerable possibilities lie dormant. It seems probable that such enzymes will prove to be more effective in diets of monogastric livestock than with ruminants, they should be throughly investigated in both types of livestock.

Enzyme additives that enhance digestion and nutrient absorption would be an obvious advantage in short gut livestock. Their use as adjuncts to intestinal medicaments, such as coccidiostats and anthelmintics, to hasten the desquamation and dissolution of oocyst and egg membranes and the consequent enhanced penetration of the drug is an obvious possibility.

The control of digestion and intestinal absorption of nutrients, physiological agents and particularly of systemic disease control drugs is a clear target for combined research in nutrition, physiology and pathology. Not only would such agents provide better "mileage" from a ton of feed, but they would reduce the effective dose level of drugs and substantially alleviate the drug residue problem.

The percentage of total digestible nutrients absorbed from the diet by both monogastric and polygastric livestock is astonishingly low compared to other creatures, eg. insects. Growth rate in the young animal is directly correlated with feed intake and undoubtedly increased absorption of ingesta would be also. Chemical treatments and diet supplements which modify the enteric pH, redox, cellular permeability or ion transport may well profoundly affect intestinal absorption. The chemical chelation of undesirable diet components such as selenium, aluminum and fluorides would be a beneficial inverse effect of dietary supplements. An example of this is the removal of excess calcium as sulfate from the intestinal tract during tetracycline antibiotic treatment.

The use of phycho-physiologic drugs such as tranquilizers has not made the progress originally anticipated, although marked success attended the use of reserpine to reduce aortic rupture in turkeys. The problem of agitation and consequent bruising, ruptured tissues and broken bones becomes increasingly acute as the numbers in massed flocks and herds increase, and although transfer and shipping will be held to a minimum it may well be offset by congestion. The use of tranquilizers that are safe, cheap, effective and ephemeral could prove to be a valuable tool to the livestock producer and will find a ready market.

Although it is true that susceptibility to disease attack is not invariably inversely proportional to the plane of nutrition, there is sufficient evidence to indicate that immunogenesis is affected by nutrition and that diet quality does affect the rate and degree of recovery from an excursion of disease, particularly of bacterial diseases.

There is good reason to expect that future research into the biochemical and physiological basis of viral, bacterial, protozoan and helminth immunity will reveal methods to accelerate specific, and perhaps general, immunity to many livestock
diseases through the feeding of specific vitamins, amino acid complexes designed to induce antigenic reaction. Obviously such induction is "triggered" by a specific compound(s) produced by the infective agent. Employing tissue culture and gnotobiotic techniques it should be possible to isolate and hopefully, identify such compounds. If they can be isolated they can be mass produced either in a cell free system or in tissue culture and applied to livestock prophylactically. It would also be necessary to determine the precise nutritional substrate required for the optimal production of such antibodies and feed such a diet coincident to the exposure to the antigenic substance.

A substantial advance has been made in this field in England by Williams-Smith who is developing antigenic stimulators against Salmonella cholerasuis in swine. Similar studies on the induction of antigenesis to typhoid (S. gallinarum) in poultry by feeding massive levels of Vitamin A have been attained at North Carolina by Hill; and in the use of Vitamin K supplements in chicks, by Harms at Florida, to accelerate and enhance immunity to coccidiosis. This is a virtually untapped area of research into nutrition related to disease, and although admittedly an exceedingly complex field, nevertheless does have the added advantage that the disease agent is unlikely to develop resistance to the procedure as they do so often now to prophylactic or therapeutic drugs.

DRUGS AND BIOLOGICALS IN MAINTENANCE OF ANIMAL HEALTH

The past 30 years has seen the most remarkable developments in the use of drugs to control livestock diseases and parasites. Although initially the animal health drug was often a by-product or fall out from human drug research, it has now largely evolved into a field of its own. Indeed there is a powerful movement abroad in Europe, which may well appear in North America also, to preclude the common use of the same drug compounds for both humans and livestock. The rationale for this proposed dichotomy is based on exceedingly speculative and ill-supported grounds, that of the suggested hazard of infective drug resistance moving in zoonotic disease from animals to man, against which present available therapeutic drugs would be ineffective. The fact that this has never happened in North America after some 20 years, during which over 100 billion head of livestock have been fed and injected with a wide variety of antibiotics, sulfonamids and furan drugs many of which are also used in human medicine, should be sufficient assurance to allay any concern. Nevertheless the fear has become widely publicized and fulminated in the U.K., Europe and S. Africa and repressive legislation proposed. Here again it is to be hoped that industry, the land grant colleges and the U.S.D.A. will make the facts clearly known to the public before they are fanned into demanding rash legislation.

Apart from the obvious need of antibiotic and other antibacterial drugs to repel frank outbreaks of animal infections, a procedure which under practical livestock production conditions is in fact too little, too late, there is the essential need for such medicaments to reduce the intercurrent infections that invariable follow "stress". As animal intensity increases so will "stress" and the need for such
preventative measures will become essential. In Europe there is much criticism of the concept of “stress” as we consider it to be here in America. The recent much publicized Swann Report to the British Parliament even referred to “stress” as “an industrial myth” promulgated to promote the unneeded sale of drugs to the grower and thus inflate profits. Although, admittedly the term “stress” lacks either qualitative or quantitative definition and specificity, it is, however, a very real factor as will be attested to by an experienced animal husbandman. Such situations as shipping, driving, dehorning, and debeaking, exposure to excessive cold, or hot or wet weather and particularly collateral disease such as viremias and live virus vaccinations all encourage opportunity for bacterial contagion either from the ambient environment or often from agents sequestered within the animal tissues (e.g. air sacs, synovial membranes, spleen, etc.) that can readily emerge and flourish into an excursion of frank disease.

My colleague Dr. Robert Squibb and his co-workers at Rutgers University have prepared precise models of certain types of “stress”, and are able to monitor the effects as expressed in blood chemistry well in advance of the observed clinical effects. He can, therefore, actually qualify and quantitate stress in terms of amino acid composition changes in the blood. Suffice it to say that prophylactic feeding of antibiotics and other drugs in the diets of exposed animals has effectively held such intercurrent infections at a minimum if not supressed them entirely.

FUTURE TRENDS IN ANIMAL HEALTH RESEARCH

If it is accepted that livestock production will become increasingly concentrated in enlarged units, then the trend for future research will necessarily have to be directed towards prophylaxis and disease prevention rather than therapy or cure after infection flourishes. On these grounds the recommendations of the Swan Committee in England are a desperate attempt to turn the clock back. If the protein needs of man are to be met by year 2000, then such obsolete procedures will simply have to be abandoned and their adherents will be dragged kicking and screaming into the 21st century.

Virus diseases are now by far the greatest threat to the livestock husbandmen, and in spite of valiant efforts to develop therapeutic drugs they still can only be effectively combatted by prophylactic live virus vaccination. In any case the incubation period for most virus diseases is so insidious and the spread so rapid that by the time the grower recognizes the presence of a virus disease in his massed flock or herd, had it diagnosed and commenced therapy the infection has dispersed throughout virtually the entire aggregation of animals, and the only condition remaining to be treated is the intercurrent infection.

Until research determines the molecular biochemistry of viral immunogenesis and ways are found to produce antibodies to specific viruses by chemical means, reliance must remain with live virus vaccine. There are presently needs for vaccines against anaplasmosis in cattle, for various entero viruses in cattle, sheep and swine, for virus pneumonia and transmissible gastroenteritis in swine and particularly against leukosis and Marek’s disease in poultry.
It also seems probable that government standards of manufacture and control will be sharply tightened, with a concomittant increase in production costs, but also with a greater assurance to the grower that incidental reactions and contaminants will be reduced.

It is inevitable that new antibiotics and other antibacterials will be developed for animal production, although the government protocol required to secure registration may well become so severely limiting and costly as to discourage industry at least to reduce their prolificacy of the past. The toxicology and pharmacology formerly required primarily on the parent compound will be increasingly extended to the metabolites and even the metabolites of metabolites until the law of diminishing returns renders further invention both unprofitable and too risky. This philosophy is a legacy derived from the oblique and largely exaggerated fears of pollution. It can well become a major factor in preventing the livestock industry from fulfilling the animal protein demands to the people.

Antimycotic drugs to prevent fungal infections in livestock and to preclude the elaboration of mycotoxins in feeds and litter has been a serious gap in the armamentarium for animal protection. As a wider appreciation of the importance and hazards of mycotic infections and toxins develops among livestock producers and food processors, preventive chemicals and methods will gather importance. Perhaps the most potent carcinogenic compound yet discovered is aflatoxin a metabolite of *Aspergillus flavus* which is a virtually ubiquitous mold. It is a curious reflection on the general misemphasis when useful compounds which even in massive doses are non-carcinogenic, are banned as cancer inducers, while quite generally distributed substances of natural origin that are apparently useless though hazardous are essentially ignored.

The search for the perfect and perpetual coccidiostatic drug will doubtless continue in the animal health industry, even though it appears increasingly to be quite futile. The principle of inducing immune response to coccidiosis in chickens by allowing a modest exposure that is held down by a partially effective drug dosage, necessarily selects drug immune strains which eventually proliferate into the total population and render the applied drug inactive. This has led to drug mixtures and to drug switching which merely delays the point where all active species become selected for multiple drug fastness. Ultimately the continuing problem of coccidiosis in fowl will only be met successfully by immunogenesis elicited by a prophylactic vaccine preferably of a cell free composition.

Recent developments in helminth control are exceedingly promising and can be very effective for increasing protein production among the emerging nations, where parasite burdens are particularly heavy. The use of nematocides now largely employed in crop protection can be developed for use in feed lots where parasites often attain serious concentrations.

The mycoplasma infections of livestock can only be really effectively controlled by eradication through blood testing and elimination of breeder carriers. This procedure proved to be the effective control with pullorum in fowl a quarter of a century ago and was eminently successful. It can and will be applied to chronic
respiratory disease, sinusitis and synovitis in poultry and to atropic rhinitis in swine.

Finally the production of Specific Pathogen Free (SPF) stock, so effectively developed and demonstrated by Young and co-workers will be expanded to all major swine breeding operations and hopefully extended to other livestock species particularly poultry. It must, however, to be fully effective, be combined with continued protection through drug prophylaxis, vaccination, strict sanitation and adequate nutrition.

CONCLUSION

In the next thirty years the demand for animal protein, in the western world, will continue at the least at its present per capita consumption and therefor the gross amount required will approximately double with the human population.

At the same time the total world protein requirement will almost treble merely to meet subsistence levels. This will place a heavy lien upon plant proteins now fed to livestock at a 7:1 loss.

Although the agriculture technology of the western nations, plus the available capital inputs can meet these protein demands through a population of 6.5 to 7 billion humans, there is doubt that it can do so without reduction in meat protein consumption in the west. This would provoke serious economic-political repercussions.

The way in which the animal health industry can aid in the provision of present levels of animal protein consumption among the traditional meat eating peoples, without denying vegetable proteins for the rest of humanity is to develop sources of protein-energy diets for livestock that are non-competitive with man.

Reduced labor and space for livestock production will lead to increased sizes of flocks and herds with resulting intensified concentrations. Such conditions lead to a greater threat from diseases and parasites, and demand a comprehensive program of disease prevention, based upon drug and vaccine prophylaxis, specific pathogen free stock, intensified sanitation and improved nutrition adjusted to climatic conditions, and age and function of the livestock produced.

The industry can meet these obligations with their presently available inputs, and through research yet to be done, provided they are not restrained by excessive and unreasonable government restrictions.

It is imperative that the people be brought to realize that an inadequate protein supply in a world of 7 billion humans constitutes a far greater threat to mankind than the presence of a miniscule quantity of an innocuous residue in the protein or other foods they do have to eat.
REPORT OF THE PHARMACEUTICAL COMMITTEE

S. F. Scheidy, Philadelphia, Pa.; Chairman
Others who attended the Committee Meeting:

We have seen much activity during the past year in the study of and application of pharmaceuticals, especially antibiotic substances, used in the prevention, treatment, and control of animal diseases and those used in food animal production. There have been many interesting and important reports and meetings, such as the National Academy of Science/National Research Council drug efficacy reports, and the conferences of the Food and Drug Administration Antibiotic Task Force on antibiotics in animal feeds that were held with livestock producers and public health representatives.

Also, some of us are aware of the "Report of the Committee on the use of Antibiotics in Animal Husbandry and Veterinary Medicine", more often referred to as the Swann Committee Report, from Great Britain. In addition, federal regulations were published proposing the administration, by F & DA, of the New Animal Drug Amendments of 1968. It is anticipated that the regulations will be finalized in the succeeding months.

The foregoing reports, activities, and proposed regulations, we believe, should be known by the U.S. Animal Health Association, and the membership should know where to search for information about them. Thus, State veterinarians, cooperative extension service veterinarians, and public health officials should initiate statewide coordinating efforts to abstract the information in these reports and regulations, as well as the pertinent scientific experimental data, and to disseminate this information. These efforts should include not only information about antibiotics and other drugs, but also pesticides, residues in foods and drug additives in feeds. Also, pertinent affairs of within-state and out-of-state agencies (U.S. Department of Agriculture, U.S. Department of Health, Education and Welfare – F & DA – and U.S. Department of Interior) should be the subject of the coordinating efforts. Not to be overlooked is the need to become acquainted with the effects that the regulations and regulatory agencies may have on the United States livestock and poultry industries.

These statewide coordinating efforts should be implemented so as to bring to the attention of livestock and poultry industries the importance of using drugs, especially antibiotics, arsenicals, and pesticides, etc., properly, as specified on labels accompanying these items. Dosage instructions and withdrawal periods should be strictly observed to avoid residues in animal tissues and products. By ignoring the label directions, we may have to face alternative regulatory actions, and the
livestock and poultry industries will be adversely affected.

The Committee reindorses the report of the Pharmaceutical Committee of 1969 and, in our opinion, that report remains applicable.

We recommend this report to the Executive Committee for their consideration.
TWO ASPECTS OF DUCK VIRUS ENTERITIS:  
PARENTAL IMMUNITY, AND  
PERSISTENCE/EXCRETION OF VIRULENT VIRUS

Thomas E. Toth  
Cornell University  
Duck Research Laboratory  
Eastport, N.Y. 11941

PARENTAL IMMUNITY

Duck virus enteritis (WE) was considered and is still a major threat to the duck industry by its effect on the breeder-duck population, yet sizable losses may also occur among market ducks. Since the spring of 1968 there has been a continuous breeder vaccination program against DVE on Long Island utilizing a duck virus enteritis modified-live-virus (DVE-MLV) vaccine. In an experiment which will be reported elsewhere, the opportunity presented itself to investigate the theoretical question whether the phenomenon of parental immunity exists in DVE, and the practical question of whether the progeny of the breeder-ducks vaccinated with this modified-live-virus vaccine would be protected by parental immunity.

MATERIALS AND METHODS

Duck Virus Enteritis Modified-Live-Virus (DVE-MLV) Vaccines — The vaccine serials contained a chicken embryo-adapted DVE virus (DVEV), which had been passaged through 12 duck embryo and 20 chicken embryo passages in Holland. It was imported in 1967, and was cloned in tissue culture in the Plum Island Animal Disease Laboratory (PIADL). The Duck Research Laboratory obtained the virus in lyophilized form. The seed virus and the vaccine serials were 10% suspensions in phosphate-balanced saline solution (pH 7.1) of embryo, chorioallantoic membrane (CAM), and allanto-amniotic fluid (AAF) harvested from 10-day-old embryonating chicken eggs that were inoculated 3 days previously with approximately 100 ELD50 of the chicken embryo-adapted DVEV.

Duck Virus Enteritis Challenge Virus (DVECV) — This virulent virus was isolated in 1967 from an epizootic among breeder ducks in Suffolk County, Long Island. The virus was passaged 6 times in ducks (of ages 16 to 41 days) susceptible to DVE to increase its virulence in order to gain a challenge inoculum for use in immunity tests. The 6th passage of virus was used throughout this work as challenge virus, and was kept in PBS at -55 C. in the form of a 10% suspension of liver from 41-day-old ducks which had died from infection with the virulent DVECV. The virus had a titer of 10^5.58 median duck lethal doses (DLD50)/0.5 ml. (av. of 2 titrations), as determined by titration in 1-day-old susceptible ducklings.

Virus-Neutralization (VN) Test — The constant-serum variable-virus method was used. The chicken-embryo-adapted DVEV was diluted serially (10-fold) in PBS pH 7.1. All the test serums and yolk preparations were filuted 1:5. Equal amounts
of the 10-fold dilutions of the virus were mixed with sterile PBS (for virus titration) or with the 1:5 diluted sera (for VN test). These mixtures were kept in a waterbath at 37°C for one half hour. Two-tenths ml. of each mixture was then inoculated via dropped CAM into 9- to 11-day-old embryonating chicken eggs (5 per mixture). The eggs were candled on 6 consecutive days and the embryo mortality was recorded. The titer reduction of the virus in the virus-serum mixtures, as compared to the virus-PBS titer, gave the neutralization index (NI), in log 10 median embryo lethal doses (ELD50) as calculated by the Reed and Muench method. On the basis of the work of Dardiri and Hess, NI value of 1.75 or higher was accepted as sign of significant neutralizing antibody development and was designated positive test result.

_Ducks_ — Ducklings and breeders susceptible to DVE and duck virus hepatitis (DVH), hatched in the Duck Research Laboratory were used.

Materials and methods in any experiments on the two subjects discussed in this presentation were as herein described.

**RESULTS**

There were 3 groups of breeder-ducks having different vaccination status which were kept in 3 different pens. These groups were: breeders vaccinated once, 6 months prior to the beginning of this trial; breeders which were vaccinated twice, 6 months and 5 weeks prior; and the contact controls of twice-vaccinated breeders, which were not vaccinated but kept always intermingled with the twice-vaccinated breeders.

The eggs laid by these breeders were collected and marked separately, according to the grouping, and set for hatching usually in 2-week intervals. The hatched baby ducklings were challenge-inoculated with virulent DVECV, thereby tested for parental immunity. Mortality was recorded for 8 days and then totaled in each trial.

The first 8 trials are summarized in Table 1. The first column shows the number of trials. The second column shows the vaccination status of breeders from which the eggs were collected, the third and fourth columns show the egg collection periods in weeks after the first or second vaccination of the breeders. In the fifth column there are the age-limits of challenge-inoculated baby ducklings, in the sixth column the doses of virulent challenge-virus. The seventh column shows the total number of dead ducklings over the total number of challenged ducklings originating from one breeder group in the particular trials, and the last column shows the percent mortality among challenge-inoculated ducklings.

In trials No. 1 and No. 2 the eggs were collected the 27th through the 29th week post first vaccination, which weeks were the 5th through 7th week after second vaccination for the twice-vaccinated breeder group. A high dose, 10^{5.6} DLD_{50} of virulent DVECV, resulted in 100% mortality in both groups of the 1- to 5-day-old baby ducklings.

In trial No. 3 the DVECV was diluted 10-fold and inoculated in the 10^{-2}
through $10^{-7}$ dilutions into 3 ducklings per dilution from the experimental breeders as well as in ducklings known to be susceptible to DVE. This experimental design could have detected the slightest protection due to the passive parental antibody. The titers were high and almost the same in ducklings of twice-vaccinated breeders ($10^6.25 \text{ DLD}_{50}$), as in ducklings of susceptible breeders ($10^6.75 \text{ DLD}_{50}$).

In the next 5 trials, lower doses of the DVECV were injected. In trials No. 4-8, the inoculation of 6- to 10-day-old ducklings with $10^2.2$ and $10^2.5 \text{ DLD}_{50}$ resulted in very high mortality, sometimes 100%, regardless of their breeders’ vaccination status.

All these trials suggested that even two inoculations with DVE-MLV vaccine, which gave a fair protection against challenge-inoculation to the breeders (see later) and induced a low level of neutralizing antibody production in them, was not enough to provide their progeny with the slightest protection.

These experiments could not give, however, an answer for the theoretical question about existence of parental immunity in DVE. The breeders themselves had negative, or very low positive neutralization indices. Consequently they could not pass neutralizing antibody to their offspring.

After trial No. 8 the 3 groups of breeder-ducks were challenge-inoculated with virulent DVECV. The survivor-rates were as follows: (Table 2): for twice-vaccinated breeders, 90%; for the contact controls of twice-vaccinated breeders, 41.2%; and for the breeders which were vaccinated once, 85%.

The virus-neutralization test of sera of surviving breeder-ducks showed, without exception, a great increase of neutralizing capacity. Neutralization indices were usually over 4 logs.

An opportunity had presented itself to test parental immunity of ducklings from breeder-ducks which had high virus-neutralizing antibody content in their blood. Naturally the egg production (number of eggs, fertility) of the breeder-ducks was heavily affected by the challenge-inoculation. It took many weeks until the surviving breeders could lay sizable numbers of fertile eggs.

We had, however, the possibility to test the virus-neutralizing capacity of some egg-yolk preparations made from infertile eggs, laid by the breeders before and after their challenge-inoculation.

The neutralization indices of these yolk-preparations for the chicken-embryo-adapted DVEV are in Table 3.

Neutralization indices 6 weeks prior to challenge were negative. Immediately after challenge-inoculation the neutralization indices began to rise; and 6 weeks after challenge, the yolk had the same high neutralizing capacity (over 4 logs) as did sera of these surviving breeders.

From eggs laid beginning with approximately the 5th week after the challenge inoculations, we succeeded in hatching a few ducklings. Trials No. 9 and 10 are summarized in Table 4. In trial No. 9 these ducklings were challenge-inoculated with 2 different doses, 2.2 and 3.2 log 10 DLD$_{50}$ of virulent DVECV. None of them died from this challenge, which was highly lethal in the previous trials.

In trial No. 10 the dose of virulent DVECV was raised to 6.2 log 10 DLD$_{50}$.
Four-day-old ducklings fully resisted the heavy challenge; however, 13-day-old ducklings suffered a 50% mortality. In this trial there was a control group of susceptible ducklings in which 80% died.

DISCUSSION

Trials No. 9 and 10 evidenced that the progeny of breeder-ducks whose serum has high virus-neutralizing capacity are provided with passive parental immunity. This protection seems to diminish rapidly, however, considering the 50% mortality of 13-day-old ducklings.

An important conclusion could be made after the completion of these trials: even though they proved that parental-immunity as a matter of fact does exist in DVE, it is to be expected that susceptible breeder-ducks vaccinated in the current manner will produce ducklings which will always be fully susceptible to DVE.

This condition is changed, however, in progeny of vaccinated breeders which survived the exposure to the virulent field duck virus enteritis virus and in consequence of this, developed high virus-neutralizing titers.

PERSISTENCE/EXCRETION OF VIRULENT VIRUS

Since the DVEV is considered to be a herpes virus, it is possible that it remains persistent in diseased and recovered ducks, maintaining a carrier status. This would give a chance for reintroduction of the disease by excretion of pathogenic virus if stresses or poor immunity status of the duck population made it possible.

Jansen came to the conclusion that the chicken-embryo-adapted vaccine virus was not excreted by ducklings inoculated with it. With the challenge-inoculation of the previously described 3 groups of breeder-ducks, their exposure to the virulent DVECV has been established.

RESULTS

To detect the possible persistence and excretion of the virulent DVECV in the surviving breeder ducks, thirty 6-week-old, DVE- and DVH-susceptible ducks were intermingled as monitor-ducks with the breeders 65 days after those had been challenged-inoculated.

The monitor-ducks were individually marked by wingbands. They were bled on the day before intermingling and 28 days later. An equal amount of the individual sera of groups of the same 5 ducks were pooled in both instances, and these pools were tested in embryonating chicken-eggs for their capacity to neutralize the chicken-embryo-adapted DVEV. Individual sera were saved in a freezer, in the event results of pooled sera made it necessary and reasonable to test them. Known positive and known negative control sera were included in both virus-neutralization tests. Table 5 shows the system of intermingling, the individual identification of monitor ducks, the pooling of their sera, and the neutralization indices of these sera in log 10 ELD$_{50}$, before and 28 days after intermingling.
The results of virus-neutralization tests of pooled sera did not indicate in either case the testing of individual samples. Their neutralization indices and the average of their neutralization indices were clearly negative before and 28 days after intermingling. Thirty-two days after intermingling, 28 monitor-ducks were challenge-inoculated intramuscularly with the virulent DVECV. Results are in Table 6.

Twenty-one of the 28 challenge-inoculated monitor ducks died within 5 days, and the total number of dead monitors was 24, i.e., 85.7% mortality.

DISCUSSION

The experiments gave a definite “no” answer to the question whether or not the virulent DVECV was being excreted 65 days after it had been inoculated into the breeder-ducks.

Three findings substantiate this conclusion:

a) None of the serum pools of 5 identical monitor-ducks, which were together for 28 days with the challenge-inoculated breeders, showed any increase in their virus-neutralizing capacity. This was a very important evidence, because in another experiment the virulent DVECV was proven to be highly antigenic.5

b) The 85.7% mortality among challenge-inoculated monitor-ducks, which corresponds with the highest mortalities of challenge-inoculated, unvaccinated, susceptible control ducks of similar age, ever achieved in previous experiments. No sublethal or subclinical amount of the virulent DVECV was consequently excreted which could have immunized to some extent the monitor-ducks.

c) None of the monitor-ducks showed any sign of clinical manifestation of DVE or died of DVE, proving that no lethal dose of the virulent DVECV was excreted.

The different immune-status of the challenge-inoculated breeders (see different survivor-rates in Table 2) has obviously not influenced the possible subsequent excretion of the virulent DVECV.

As to the persistence of the virulent DVECV, the answer cannot be definite. Indirectly, the experiment suggested rather the lack of persistence.

If one considers, namely, the locations of lesions in DVE (oesophagus, intestines, cloaca) in direct communication with the environment, it is difficult to comprehend why the virus was not excreted, had it been persisting in the challenge-inoculated breeders.

These findings question the value of epidemiological diagnoses in DVE which would be based solely on monitor-ducks as a means to detect inapparent DVE infections in flocks of ducks.
Table 1. Mortality among challenge-inoculated baby-ducklings hatched from eggs laid by the breeder-ducks at different intervals after their 1st or 2nd vaccination.

<table>
<thead>
<tr>
<th>No. of breeders</th>
<th>Ex- peri-</th>
<th>Vaccination status</th>
<th>Egg collection periods in weeks</th>
<th>Age of ducklings in days</th>
<th>Doses of challenge virus</th>
<th>No. dead/ Percent of mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per- meter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once vaccinated</td>
<td>1,2</td>
<td></td>
<td>27-29</td>
<td>1-5</td>
<td>10^5.6 DLD50</td>
<td>34/34 100.0</td>
</tr>
<tr>
<td>Twice vaccinated</td>
<td></td>
<td></td>
<td>5* -7</td>
<td></td>
<td></td>
<td>29/29 100.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>30,31</td>
<td>1-2</td>
<td>10^-2-10^-7 dil.</td>
<td>10^6.5**</td>
</tr>
<tr>
<td>Twice vaccinated</td>
<td></td>
<td></td>
<td>8,9</td>
<td></td>
<td></td>
<td>10^6.3</td>
</tr>
<tr>
<td>Monitors of twice vacc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10^6.8</td>
</tr>
<tr>
<td>Unvacc. susceptibles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10^6.8</td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td></td>
<td>32-37</td>
<td>10-15</td>
<td>10^2.5 DLD50</td>
<td>41/41 100.0</td>
</tr>
<tr>
<td>Twice vaccinated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47/53 88.7</td>
</tr>
<tr>
<td>Monitors of twice vacc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>55/56 98.2</td>
</tr>
<tr>
<td></td>
<td>7-8</td>
<td></td>
<td>38-41</td>
<td>16-19</td>
<td>10^2.2 DLD50</td>
<td>16/16 100.0</td>
</tr>
<tr>
<td>Twice vaccinated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18/18 100.0</td>
</tr>
<tr>
<td>Monitors of twice vacc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19/22 86.4</td>
</tr>
</tbody>
</table>

*Eggs were set in two-week periods beginning with the indicated week.

**Values are titers of DVE challenge virus in log 10 DLD50/0.5 ml obtained in ducklings from the respective breeders.
Table 2. Survivor-rates among challenge-virus-inoculated breeder ducks having different vaccination status.

<table>
<thead>
<tr>
<th>Vaccination status of breeders</th>
<th>No. dead/ no. challenged</th>
<th>Percent survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twice vaccinated</td>
<td>2/20</td>
<td>90.0</td>
</tr>
<tr>
<td>Contact control of twice vaccinated</td>
<td>10/17</td>
<td>41.2</td>
</tr>
<tr>
<td>Once vaccinated</td>
<td>3/20</td>
<td>85.0</td>
</tr>
</tbody>
</table>

Table 3. Virus-neutralization indices of yolk-preparations for the chicken-embryo-adapted duck virus enteritis virus (DVEV). Yolk-preparations were made of eggs collected from breeder ducks prior and after their challenge-inoculation with virulent DVECV.

<table>
<thead>
<tr>
<th>Time of egg collection in relation to challenge</th>
<th>Vaccination status of breeder ducks</th>
<th>Neutralization index in log 10 LLd50</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 weeks prior</td>
<td>Once vaccinated</td>
<td>0.62 negative</td>
</tr>
<tr>
<td></td>
<td>Twice vaccinated</td>
<td>1.38 negative</td>
</tr>
<tr>
<td>1 week after</td>
<td>Twice vaccinated</td>
<td>2.12 positive*</td>
</tr>
<tr>
<td>6 weeks after</td>
<td>Once vaccinated</td>
<td>&gt;3.79 positive*</td>
</tr>
<tr>
<td></td>
<td>Twice vaccinated</td>
<td>&gt;4.12 positive*</td>
</tr>
</tbody>
</table>

* Based on the work of Dardiri and Hess, \(1) \) NI value of \(1.75 \log\) or higher was accepted as sign of significant neutralizing antibody development and was designated as positive test result.
Table 4. Mortality among challenge-inoculated baby-ducklings which were hatched from eggs laid by the breeder-ducks at different intervals after they were challenge-inoculated.

<table>
<thead>
<tr>
<th>Egg-collection periods in weeks after challenge of breeders</th>
<th>Age of ducklings in days when vaccinated started</th>
<th>Challenge doses</th>
<th>ELD$_{50}$</th>
<th>No. dead/ challenged</th>
<th>Percent mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 One vaccinated</td>
<td>5-6</td>
<td>2</td>
<td>2.2</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>7-8</td>
<td>4</td>
<td>6.2</td>
<td>0/9</td>
<td>0</td>
</tr>
<tr>
<td>Twice vaccinated</td>
<td>&quot;</td>
<td>13</td>
<td>&quot;</td>
<td>3/6</td>
<td>50</td>
</tr>
<tr>
<td>Unvaccinated susceptibles</td>
<td>&quot;</td>
<td>17</td>
<td>&quot;</td>
<td>8/10</td>
<td>80</td>
</tr>
</tbody>
</table>
Table 5. Neutralization indices of pooled sera of monitor ducks for the chicken-embryo-adapted duck virus enteritis virus, before and 28 days after intermingling with challenge-inoculated breeder ducks, expressed in log 10 ELD$_{50}$.

<table>
<thead>
<tr>
<th>Number and vaccination status of breeder-ducks</th>
<th>Individual No.'s of monitor ducks</th>
<th>Marks of serum pools</th>
<th>Before intermingling</th>
<th>28 days after intermingling</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 once-vaccinated</td>
<td>126-130</td>
<td>A</td>
<td>0.33</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>131-135</td>
<td>B</td>
<td>0.54</td>
<td>0.50</td>
</tr>
<tr>
<td>5 contact controls of twice-vaccinated</td>
<td>136-140</td>
<td>C</td>
<td>1.16</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>141-145</td>
<td>D</td>
<td>0.03</td>
<td>0.48</td>
</tr>
<tr>
<td>18 twice-vaccinated</td>
<td>146-150</td>
<td>E</td>
<td>0.54</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>151-155</td>
<td>F</td>
<td>0.54</td>
<td>0.50</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
<td></td>
<td>3.66</td>
<td>3.50</td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td></td>
<td>0.66</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 6. Mortality among monitor-ducks which were challenge-inoculated with virulent duck virus enteritis challenge virus 32 days after intermingling with previously challenge-inoculated breeder-ducks.

<table>
<thead>
<tr>
<th>Days after challenge-inoculation</th>
<th>No. dead/ no. challenged</th>
<th>Percent Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8</td>
<td>24/27</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No. of dead monitors: 24/27 = 85.7
REFERENCES


THE STATUS OF THE CONTROL OF MAREK'S DISEASE BY VACCINATION

by
H. G. Purchase, B. R. Burmester and W. Okazaki
USDA Poultry Research Branch
Animal Science Research Division
ARS Regional Poultry Research Laboratory
East Lansing, Michigan 48823

INTRODUCTION
Marek’s disease (MD) is one of the greatest scourges of the poultry industry. Among broilers condemnation rates have risen between 1961 and 1968 from 10 to almost 200 per 10,000 carcasses inspected. Very little mortality from MD occurs in broilers before they are slaughtered. However, heavy mortality from MD has been reported in started pullets, layers, and broiler breeders and losses of 35% from MD are probably not uncommon. Heavy condemnations and early losses from MD have been ascribed to “acute leukosis” but this is a misnomer. The disease is more correctly termed “acute Marek’s disease.” Because of the similarity in lesions they induce, lymphoid leukosis, and MD are frequently confused. However, with the knowledge that is presently available it is possible to differentiate between them with a reasonable degree of accuracy. Lymphoid leukosis is caused by an RNA virus similar to that which causes leukemia in mice and other animals whereas MD is caused by a herpesvirus. The most important means of spread of lymphoid leukosis virus is from the dam to her offspring through the egg, and this shedding can be detected by the RIF and eradicating the virus from the flock. The transovarian route is unimportant in the spread of MD however, the virus is airborne and thus highly contagious. Differentiation between lymphoid leukosis and MD is becoming increasingly important because vaccines have been developed against MD and these vaccines will not protect against lymphoid leukosis.

Churchill et al have described an attenuated MD virus which will protect chickens from disease when they are subsequently challenged with a virulent strain of MD. This vaccine is being exploited by commercial companies in Europe. The vaccine of most interest in the United States was isolated from turkeys. This herpesvirus of turkeys (HVT) is antigenically related to MD virus and has proven to be an effective vaccine against MD.

MATERIALS AND METHODS

Chickens and Management Procedures. Regional Poultry Research Laboratory Line 15 x 7 which are highly susceptible to MD were used in all laboratory experiments. They were hatched in a disinfected incubator and reared in modified Horsfall-Bauer isolators under negative pressure.
For the field trials, female chicks from eight different commercial breeders were reared in conventional poultry houses. There were 27 replicates each consisting of a group of vaccinated and unvaccinated birds in direct contact with one another. From 20 to 50% of the birds in any one pen were vaccinated. Vaccinated birds were identified by wingbanding, dubbing, clipping of toes or cutting of the web between the toes. No attempt was made to change the usual husbandry procedures.

Cell Cultures. The preparation and maintenance of chick kidney (CK), and duck embryo fibroblast (DEF) cultures have been described.\textsuperscript{21}

Vaccine Virus Stocks and Vaccination. HVT isolate FC126, propagated in CK, DEF, or chicken embryo fibroblast cultures was used in laboratory experiments\textsuperscript{7} but only DEF propagated virus was used in field studies\textsuperscript{11}. The virus used in laboratory studies had been passed 9 to 13 times in one or more of the types of cell culture described above, whereas the virus used in field trials had been passed 9 to 11 times in DEF's only. Stocks of cells infected with the vaccine virus were preserved with 15% calf serum and 5-10% dimethyl sulfoxide in liquid nitrogen.\textsuperscript{18}

Except in one field trial where chicks were vaccinated at 18 days of age, all chicks were vaccinated at one day of age by intra-abdominal inoculation of 0.2 ml of virus diluted in phosphate-buffered saline (pH 7.4) or complete cell culture medium.\textsuperscript{21} After each vaccination a portion of the vaccine was returned to the laboratory and titrated in cell culture.

Virus Re-isolation. At termination of laboratory experiments and at 10, 20, 40, and 60 weeks of age in field trials an attempt was made to re-isolate HVT and MD virus from vaccinated and un-vaccinated chickens. In most instances isolation was attempted from the kidneys using the indirect CK culture method\textsuperscript{21} but in field trials buffy coat cells separated from heparnized blood by albumin flotation were used.

Antibody determinations. Plasma or serum samples were examined for antibody by the indirect fluorescent antibody test.\textsuperscript{9} Each serum was tested on 2 antigens, namely MDV- and HVT-infected CK cultures on cover slips. Homologous antibody reacted much more strongly than heterologous antibody, in addition, antibody to MD virus stained only the nuclei in HVT infected CK cultures, whereas homologous HVT antiserum stained both the nuclei and the cytoplasmic granular and diffuse antigens. MD virus antibody could not be detected in the presence of HVT antibody, however, HVT antibody could be detected in the presence or absence of MD virus antibody.

Pathology. All chicks dying during the laboratory experiments and those that survived were examined for lesions of MD. When no lesions were observed, portions of the coeliac plexus, the left and right brachial plexuses, the left and right sciatic plexuses and the gonad were taken for histopathologic examination. In field trials, all birds dying after four weeks were necropsied, however, in some instances only those dying on 3 days of each week were examined. In the majority of experiments histologic examination was made on all birds in which an obvious cause of death was not found.
RESULTS

Purity of the Vaccine. The batches of HVT vaccine used in field trials have been examined for extraneous micro-organisms by the Veterinary Biologics Division, Ames, Iowa using standard procedures. Small numbers of saprophytic bacteria have been found in some batches. Tests in chicken embryos and chicks also demonstrated freedom from common pathogenic viruses of poultry. All batches propagated in DEFs are free of lymphoid leukemia-sarcoma viruses.

Protection Against MD. A summary of laboratory experiments is presented in Table 1. Vaccination at one day of age will prevent all signs of Marek’s disease in chicks challenged by intra-abdominal inoculation 3-5 weeks later or by contact with infected chicks 2-5 weeks later. Among the vaccinated unchallenged birds, 3 (5%) had minor microscopic lesions similar to those described for MD. In the unvaccinated birds which were challenged by intra-abdominal inoculation at 3-5 weeks of age, 44% died or had gross lesions at termination of the experiment and when microscopic lesions were also included, the response was 56%. Among those challenged by contact 55% died or had gross lesions and the total MD including microscopic lesions was 68%. Among the unvaccinated unchallenged controls one isolator became contaminated with MD as indicated by serologic examination and one bird in that isolator had microscopic lesions.

All vaccinated birds and none of the unvaccinated birds produced antibody to HVT. All the unvaccinated birds exposed to MD produced antibody to this virus. All 6 birds in one control isolator had antibody to MD virus indicating that this isolator had become contaminated with MD virus.

HVT could be isolated from 98% of the vaccinated and none of the unvaccinated birds tested. MD virus could be isolated from both the vaccinated challenged and the unvaccinated challenged groups and the rate of isolation was 36%. MD virus could not be isolated from any of the unchallenged birds.

Examples of results in field trials have been chosen from among the 27 replicates consisting of groups of vaccinated and unvaccinated birds in the same pen (Table 2). In all trials in which a large proportion of the unvaccinated birds died of MD, the HVT vaccine offered adequate protection ranging from 70 to 100% (86 & 97% in trials 1 & 2, Table 2). A much lower level of protection was obtained in trials in which only small numbers of the unvaccinated birds died of MD (40% protection in trial 3, Table 2). In none of the trials was the mortality among the vaccinated chickens significantly higher than that among the unvaccinated.

In several trials there was a low incidence of lymphoid leukemia after the birds were housed and in one trial (trial 1, Table 2) mortality from this cause was considerable. The lymphoid leukemia mortality was lower in the vaccinated chickens than in the unvaccinated ones. In 7 replicates for which records were kept the egg production on a hen/day basis averaged 62.6% among the vaccinated and 56.1% among the unvaccinated in the first ten to 18 weeks after being housed. The body weights of vaccinated and unvaccinated birds were similar.

Safety of Vaccine Virus. Groups of one-day-old chicks have been inoculated...
with large doses of the vaccine virus and maintained in large plastic isolators similar to those used for gnotobiotic work. Unvaccinated controls were also included. The first 2 experiments have been terminated at 360 days and there is no indication that the vaccine virus is pathogenic. Ten serial passages of HVT in chickens known to be susceptible to MD have been made at monthly intervals. There is no indication that HVT acquires pathogenicity on serial passage in chickens.

**Spread of the Vaccine Virus.** The vaccine virus spreads very poorly from vaccinated birds to those in direct contact with them. In the field trials HVT, or antibody to HVT, could be detected in less than 5% of the unvaccinated birds in direct contact with the vaccinated birds from the day of hatching. A low level of spread has also been detected in laboratory studies.

**Duration of Infection.** In laboratory experiments HVT could be re-isolated from over 95% of the vaccinated birds at 20 weeks. In field studies HVT could be re-isolated from 74% of the birds at a similar time. HVT can be isolated from vaccinated birds at 40 weeks but at a slightly lower rate. In both laboratory and field trials MD virus could be isolated from approximately 40% of the vaccinated birds which were challenged with MDV. Thus in most birds infection with HVT persists indefinitely and some birds also harbor MD virus for long periods of time.

**Stability of Vaccine Virus.** There is some loss of titer during freezing of the vaccine virus to liquid nitrogen temperatures but thereafter the vaccine appears to be stable indefinitely. In recent work, B. W. Calnek (personal Communication) has been able to extract virus from infected cells and to lyophylize it. Lyophylized virus should be stable for long periods.

**DISCUSSION AND CONCLUSIONS**

The HVT strain FC126 prepared at the Regional Poultry Laboratory, East Lansing, Michigan appears to meet stringent requirements for purity, safety, and potency. Thus the stocks of HVT do not contain detectable extraneous pathogenic organisms, the virus does not cause disease in susceptible laboratory chickens or in field chickens at least within the first year after inoculation, there is no difference in body weight between vaccinated and unvaccinated chickens and the former have a higher egg production than the latter, the virus does not acquire pathogenicity on serial passage in chickens and in both laboratory and field trials it is highly effective in preventing losses from MD.

There was some indication that HVT offered a small degree of protection against lymphoid leukosis. The following are some possible explanations for this observation.

1. Some of the large tumors among the unvaccinated birds diagnosed as lymphoid leukosis were in fact MD. It is sometimes difficult to differentiate between these two diseases.

2. Protection was due to killed antigens present in the vaccine. Although the vaccines are free of lymphoid leukosis viruses it is possible that oncogenes, or group-specific antigens could be present in the vaccine and thereby
immunize the recipient chickens. This is considered unlikely since attempts to immunize chickens against lymphoid leukemia with cell culture materials have generally been unsuccessful.

3. The apparent protection is due to an indirect effect resulting from the better health and liveability of the vaccinated birds. Purchase et al.\(^{10}\) demonstrated that the immunological responsiveness of birds with MD is deficient. It is possible that the immune systems of such deficient birds do not recognize the lymphoid leukemia tumor as foreign and therefore allow it to proliferate and metastasize. More of the healthy vaccinated birds would reject such tumors than unvaccinated birds.

Opinions differ on whether the best vaccine for chickens would be one that spreads from bird to bird or does not. The HVT does not spread significantly and so there is little chance of it mutating to a pathogenic form. However, because it does not spread, all chickens must be inoculated in order to protect them adequately from MD. It is also possible that if most of the birds in a flock are vaccinated, the level of MD virus in the flock may be reduced and the unvaccinated birds would receive a smaller dose of MD virus and may not succumb.

One of the major disadvantages of HVT is that it remains present within the host for long periods, however, since it does not spread from bird to bird, carriers do not form a reservoir for dissemination of the virus to other birds.

HVT strain FC126 has been made available to many commercial companies. Because viruses may behave differently when handled in other laboratories, it is important that certain of the above tests be repeated by each group producing the vaccine. Thus before a batch of vaccine can be distributed with impunity it must be thoroughly tested for purity, safety, and potency. It may not be necessary that all the tests described above be completed but at least the most important ones should have been started. Thus it is important that the final product be free of all contaminants and that the virus content be known. In addition there should be some evidence that the product is non-pathogenic and will produce immunity in short-term studies. Without these safeguards the product may be worthless and not produce an immunity or it may be highly dangerous and contain pathogenic agents. Commercial companies to whom seed HVT virus has been sent are attempting to comply with these requirements. There is an excellent possibility that in the near future sufficient quantities of well tested, effective, and inexpensive vaccine will be available on the open market.
Table 1. Protection of chickens from Marek's disease (MD) by vaccination with a herpesvirus of turkeys (HVT FC126).

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Vaccinated&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Challenged&lt;sup&gt;B&lt;/sup&gt;</th>
<th>Expt. No.</th>
<th>% Marek's disease</th>
<th>Antibody Response</th>
<th>Virus Isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dead and survivors with gross lesions</td>
<td>Total including survivors with micro. lesions</td>
<td>MDHV</td>
</tr>
<tr>
<td>1</td>
<td>1 day</td>
<td>3-5 wks IA</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>1 day</td>
<td>2-5 wks CE</td>
<td>81</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>1 day</td>
<td>None</td>
<td>55</td>
<td>0</td>
<td>5&lt;sup&gt;E&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>3-5 wks IA</td>
<td>18</td>
<td>44</td>
<td>56</td>
<td>11/11</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>2-5 wks CE</td>
<td>66</td>
<td>55</td>
<td>68</td>
<td>39/39</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>None</td>
<td>36</td>
<td>0</td>
<td>3&lt;sup&gt;F&lt;/sup&gt;</td>
<td>6&lt;sup&gt;F&lt;/sup&gt;/29</td>
</tr>
</tbody>
</table>

<sup>A</sup>Intra-abdominal (IA) inoculation with 600 to 30,000 PFU per chick.

<sup>B</sup>For IA challenge at 2-5 weeks of age, a dose of JM strain of MD-infected chick kidney culture cells sufficient to cause mortality in 90-100% of susceptible one day old chicks was administered. For contact exposure (CE) chicks from the same hatch as those in other lots were inoculated intra-abdominally with MD virus at one day and placed in a floor pen. At the respective times birds to be challenged were added to the room.

<sup>C</sup>Antibody at termination determined by the direct fluorescent antibody test using MDHV and HVT antigen. NA = not applicable, since MD antibody cannot be detected in the presence of HVT antibody. Numerator is number positive, denominator is number tested.

<sup>D</sup>Virus isolation at termination (17-20 wks) by indirect chick kidney culture.

<sup>E</sup>Represents 3 birds with minor microscopic lesions.

<sup>F</sup>In one group of 6 controls, one bird had minor microscopic lesions and all 6 birds in that isolator had antibody to MD virus indicating that the birds became contaminated with MD virus.
Table 2. Field trials of the herpesvirus of turkeys (HVT FC126) vaccine against Marek's disease (MD) under high and low MD exposure.

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Treatment</th>
<th># of birds</th>
<th>%Total Mortality&lt;sup&gt;B&lt;/sup&gt;</th>
<th>MD Mortality&lt;sup&gt;B&lt;/sup&gt;</th>
<th>LL Mortality&lt;sup&gt;BC&lt;/sup&gt;</th>
<th>%Protection against MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vaccinated</td>
<td>700</td>
<td>31.0</td>
<td>3.8</td>
<td>12.8</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Unvaccinated</td>
<td>700</td>
<td>60.2</td>
<td>28.6</td>
<td>18.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Vaccinated</td>
<td>2,000</td>
<td>5.0</td>
<td>0.2</td>
<td>0.8</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Unvaccinated</td>
<td>3,000</td>
<td>12.2</td>
<td>6.4</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Vaccinated</td>
<td>3,000</td>
<td>10.8</td>
<td>0.9</td>
<td>0.2</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Unvaccinated</td>
<td>5,000</td>
<td>9.8</td>
<td>1.5</td>
<td>0.7</td>
<td></td>
</tr>
</tbody>
</table>

<sup>A</sup>Vaccination at one day of age by intra-abdominal inoculation of 10,000 (Trials 1 & 3) or 1,000 (Trial 2) PFU of HVT FC126 per chick.

<sup>B</sup>All mortality is up to 32 to 37 weeks. MD = Marek's disease.

<sup>C</sup>Lymphoid leukemia (LL) only occurred after 20 weeks.
REFERENCES


TRANSMISSIBLE DISEASES OF POULTRY COMMITTEE

H. E. Goldstein, Columbus, Ohio, Chairman

The Transmissible Diseases of Poultry Committee met on Monday, October 19, 1970, at 1:30 P.M.

The committee was read a comminique from Dr. Ben Pomeroy, the chairman of the subcommittee for the Eradication of Pullorum Disease and Fowl Typhoid.

The report of the subcommittee was as follows:

I. Report of the Subcommittee on Pullorum Typhoid Eradication, 1970

Since the meeting of the Committee on Transmissible Diseases of Poultry on October 14, 1969, in Milwaukee considerable effort has been made to elicit industry support for an eradication program for turkeys. The proposed program (Exhibit 1) that was reviewed at the committee meeting in Milwaukee was modified by the NTF Disease Control and Eradication Committee to try to make it more palatable to the industry members who opposed the designation of the Animal Health Division as the administrator of the eradication program. This modified plan was presented to the Board of Directors of the National Turkey Federation at their annual meeting in Hot Springs, Arkansas on January 6, 1970. The proposal was defeated 34 to 28. It was then suggested that the NTF committee submit proposals to the National Plans Conference attempting to include an eradication program in the National Plans Program.

Proposals from several states concerning pullorum disease and fowl typhoid were submitted to the National Plans Conference which was held in St. Louis, July 13-15. There was a divided opinion among the delegates and by a close vote (16-13). The incorporation into NTIP of Specific Provisions for Participating States in Pullorum-Typhoid Eradication Program was defeated. Thus in the NTIP there were no changes made in the present Pullorum-Typhoid Control Program.

The Animal Health Division (Exhibit 2, March 18, 1970) stated this policy that should individual states desire to enter into an eradication program such a proposal should be sent by the appropriate state disease control authority through the ANH field station in that state to the Animal Health Division office for final consideration. Such a request if approved could be implemented by expanding the existing cooperative agreements between ANH and the appropriate state disease control agency.
At the annual meeting of the AAAP, June 24, 1970, in Las Vegas, Nevada two resolutions were passed (Exhibits 3 and 4).

1. Be it resolved that the AAAP reaffirm its position to support the pullorum-typhoid eradication program as developed by the National Turkey Federation Disease Control and Eradication Committee.

2. Be it resolved that if the National Plans Conference does not support the position of the National Turkey Federation Disease Control and Eradication Committee, or a comparable program at the meeting to be held July 13-15, that the AAAP actively support the Animal Health Division, ARS, USDA in the development of an eradication program for pullorum disease and fowl typhoid for turkeys for those states desiring such a program.

Recently, August 3, 1970, the Executive Board of the Minnesota Turkey Growers Association passed a resolution requesting the Minnesota Livestock Sanitary Board to enter into an agreement with the ANH Division to implement an eradication program for pullorum disease and fowl typhoid. The MLSB (September 18, 1970) approved the request of the MTGA and is currently pursuing the development of an agreement with ANH Division (Exhibit 5.)

There are other states who have indicated interest in the development of an eradication program.

The proposed eradication program provides for an orderly, organized reduction in the testing program and safeguards to monitor for outbreaks of the disease that ultimately will lead to complete elimination of the testing program for multiplier breeding flocks and a monitoring program for the primary breeders. This will result in the elimination of the annual cost of the pullorum-typhoid program, which annually costs the poultry and turkey industry 5 to 10 million dollars each year.

The subcommittee report was approved as read.

The committee recommends that the subcommittee be discontinued until such time as the future needs demand and that the parent committee resume the responsibilities prior to establishing the subcommittee.

Your committee has been active in pullorum disease and fowl typhoid eradication for approximately seven years. As a result of this committee and the subcommittee activities the majority of the states have adopted some or all of the criteria (6 key points) for an eradication program. For example seven states have adopted all six points in their programs. Only four states (all minor poultry producing states) have incorporated none of the key points.

Through the action, contacts, and encouragement of this committee the voluntary National Poultry and Turkey Improvement Plans have utilized and incorporated these key points into their program. (See page 285 of the U.S. AHA Proceedings October 12-17, 1969)

Your committee commends the Veterinary Biologics Division of the U.S.D.A. for its efforts in developing guidelines for the research and studies necessary for production of a Mareks Disease Vaccine.

The following report was submitted from the Veterinary Biologics Division:
II. Applications for a U.S. Veterinary Biological Product License for the production and marketing of a Marek's Disease Vaccine have been received by the Veterinary Biologics Division and are presently under review. Prospective licensees have been authorized to conduct large-scale field trials in accordance with 9 CFR Part 103, “Experimental Production, Distribution and Evaluation of Biological Products Prior to Licensing.”

Division personnel have been directed to give top priority to Marek's Disease Vaccine licensing, testing, and release, and as soon as the necessary pertinent data have been accumulated by each applicant and submitted to us, they will be promptly reviewed and evaluated for making a licensing decision.

How soon licenses are issued will be influenced by the problems which license applicants encounter in making the transition from pilot research production to large-scale commercial production and the pace at which they carry out tests and evaluations of their vaccines. If all goes well, licenses could be issued before the first of the year.

It should be kept in mind that the availability of a licensed vaccine may be some time after the first of the year because it will take time for the license applicants to build up an adequate inventory of the product for distribution and marketing.

The committee accepted the report as read.

III. Updating of the Duck Virus Enteritis (DVE) Situation in the United States.

The last case of Duck Virus Enteritis in Commercial Pekin ducks was confirmed in November 1968. The Federal quarantine for DVE was released September 25, 1969, from Suffolk County, Long Island, New York (see Proceedings USAHA, October, 1969). Following the quarantine release there was a 6-month period of close surveillance of all waterfowl in the area without evidence of the disease. The Pekin population has remained free of DVE to date.

However, outbreaks were confirmed in captive waterfowl in each of two eastern States.

A farmer at York, Pennsylvania, lost about 100 muscovy ducks over a 30-day period beginning in April, 1970; tissues from the dead muscovies disclosed lesions compatible with DVE on histopathology and virus was isolated. Other waterfowl on the premises remained apparently healthy but showed antibody to the serum neutralization test. After depopulation of the premises, replacement waterfowl were negative for antibody on two serum neutralization tests, three weeks apart.

In May, 1970, one black duck was found dead in a wildlife refuge on Long Island; New York. DVE virus was isolated from tissues of the duck. Since then, serum neutralization tests for antibody in contact waterfowl were negative. (See figures 1, 2, and 3.)
DUCK VIRUS ENTERITIS
Order of Counties Affected

COMMERCIAL, CAPTIVE & MIGRATORY: (1) JAN. 1967;
CLINICAL AND PATHOLOGICAL SIGNS, OR VIRUS ISOLATION DVE ALSO KNOWN AS DUCK PLAGUE

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
DVE CONFIRMED OUTBREAKS

OUTBREAKS

10

8

6

4

2

0

0 1 2 3 4 5 6 7 8 9 10

<table>
<thead>
<tr>
<th>Year</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
</tr>
</thead>
<tbody>
<tr>
<td>1967</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1968</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1969</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1970</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

End last outbreak (Com'l)

CLINICAL AND PATHOLOGICAL SIGNS, OR VIRUS ISOLATION DVE ALSO KNOWN AS DUCK PLAGUE

U.S. DEPARTMENT OF AGRICULTURE ANIMAL HEALTH DIVISION AGRICULTURAL RESEARCH DIVISION
# Poultry Diseases

## FIELD OUTBREAKS OF DVE

### DUCKS:

<table>
<thead>
<tr>
<th>Species</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pekin</td>
<td>16</td>
</tr>
<tr>
<td>Muscovy</td>
<td>4</td>
</tr>
<tr>
<td>Mallard</td>
<td>7</td>
</tr>
<tr>
<td>Black</td>
<td>4</td>
</tr>
<tr>
<td>Greater Scaup</td>
<td>1</td>
</tr>
<tr>
<td>Bufflehead</td>
<td>1</td>
</tr>
</tbody>
</table>

### GEESE:

<table>
<thead>
<tr>
<th>Species</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canadian</td>
<td>1</td>
</tr>
<tr>
<td>Egyptian</td>
<td>1</td>
</tr>
<tr>
<td>MUTE SWANS</td>
<td>2</td>
</tr>
</tbody>
</table>

*CLINICAL AND PATHOLOGICAL SIGNS, OR VIRUS ISOLATION DVE ALSO KNOWN AS DUCK PLAGUE*

U.S. DEPARTMENT OF AGRICULTURE  
ANIMAL HEALTH DIVISION  
AGRICULTURAL RESEARCH SERVICE
Publications and Presentations Given on Duck Virus Enteritis During 1970


IV. PROGRESS IN MYCOPLASMA CONTROL

There has been a gradual decline in positive tests for *Mycoplasma gallisepticum* in breeder chickens and turkeys as reported to the Animal Health Division by state disease control official laboratories and others. (Figure 1).

Condemnations for airsacculitis at time of slaughter for young chickens have been reduced dramatically since large-scale testing for *M. gallisepticum* was initiated in 1964-1965, and through fiscal year 1969 (Figure 2). In fiscal year 1970 there was an increase in condemnations in young chickens. Most of the increase occurred during February, March and April 1970. Many of these flocks were supposedly from *M. gallisepticum* free breeders. Investigations in several breeder flocks with severe airsacculitis in their progeny indicated: (1) serologically the incidence of *M. synoviae* was very high; (2) *M. gallisepticum* reactors were also found; and (3) many of these proved to be cross-reactions when subjected to hemagglutination inhibition tests for *M. gallisepticum* and *M. synoviae*.

Circumstantial evidence strongly suggests that *M. synoviae* is associated in some way with some cases of severe airsacculitis without showing noticeable clinical symptoms.

Varying degrees of success have been experienced in the control of Mycoplasma with the use of antibiotics by injecting breeder flocks, chicks, or embryos. Usually a combination of two or more methods is used.

Antibiotics have also been used in the feed and water of both parent stock and their progeny. Dipping of hatching eggs in antibiotic solutions has been extensively
Heat treatment of hatching eggs is being widely used by the industry to interrupt egg transmission of the infection. This is accomplished by bringing the internal temperature of the eggs to 114-114.5°F for a 12 to 14 hour preincubation period.

Dr. Harry W. Yoder, Jr., of the Southeast Poultry Research Laboratory, Athens, Georgia, reported on the preincubation heat treatment in AVIAN DISEASES, February 1970.

In turkeys, *Mycoplasma gallisepticum* infection has reached a very low level (Figure 3); however, condemnation for airsacculitis at time of slaughter has not declined accordingly (Figure 3). There are no controlled studies to indicate how important *M. meleagridis* is in the airsacculitis problem, but there is evidence it contributes to condemnations for airsaffulitis especially in fryer-roaster turkey.

The Consumer and Marketing Service supplied the following report for the committee:

<table>
<thead>
<tr>
<th></th>
<th>1967</th>
<th>1968</th>
<th>1969</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td>225,257</td>
<td>144,791</td>
<td>132,456</td>
</tr>
<tr>
<td>Leukosis</td>
<td>30,102,727</td>
<td>36,536,006</td>
<td>37,419,961</td>
</tr>
<tr>
<td>Septicemia</td>
<td>17,795,306</td>
<td>17,321,926</td>
<td>18,206,607</td>
</tr>
<tr>
<td>Airsacculitis</td>
<td>29,145,176</td>
<td>14,178,817</td>
<td>12,612,634</td>
</tr>
<tr>
<td>Synovitis</td>
<td>2,065,351</td>
<td>1,743,024</td>
<td>1,514,121</td>
</tr>
<tr>
<td>Tumor</td>
<td>2,423,117</td>
<td>2,256,546</td>
<td>2,753,004</td>
</tr>
<tr>
<td>Bruises</td>
<td>1,004,919</td>
<td>1,185,041</td>
<td>1,435,602</td>
</tr>
<tr>
<td>Cadavers</td>
<td>2,328,153</td>
<td>2,428,317</td>
<td>2,709,892</td>
</tr>
<tr>
<td>Contamination</td>
<td>4,455,312</td>
<td>4,858,073</td>
<td>6,071,463</td>
</tr>
<tr>
<td>Overscalds</td>
<td>418,267</td>
<td>427,847</td>
<td>626,223</td>
</tr>
<tr>
<td>Others</td>
<td>3,689,124</td>
<td>3,510,869</td>
<td>3,675,960</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>93,652,709</strong></td>
<td><strong>84,591,257</strong></td>
<td><strong>87,157,923</strong></td>
</tr>
</tbody>
</table>

The committee discussed the problem of environmental quality control and reflected that the poultry industry has had the most serious legal complications resulting from antipollution enforcement.

The committee recommends that the executive committee of the U.S.D.A., A.H.A. or its incoming president give serious consideration to the formation of a committee relative to environmental control. This committee could assist in providing uniformity in the approach to the problem.

The committee took action on the following resolution:
TRANSMISSIBLE DISEASES OF POULTRY

RESOLUTION

WHEREAS, Duck Virus Enteritis (Duck Dutch Plague) occurs in many foreign countries; and

WHEREAS, waterfowl infected with this disease shed the virus for at least 45 days and infection can spread by direct or indirect contact; and

WHEREAS, this disease was eradicated from commercial waterfowl in the United States only following great costs to the industry and public funds; now, therefore, be it

RESOLVED that the Code of Federal Regulations, Title 9, be amended to require all imported waterfowl be tested prior to release for use or from quarantine and found negative for Duck Virus Enteritis.

The committee referred this resolution to the resolutions committee for further action.

Publications and Presentations Given on Duck Virus Enteritis During 1970

332 REPORT OF THE COMMITTEE


Figure 1 – States Testing for *Mycoplasma Gallisepticum* in Chickens and Turkeys – Fiscal Year 1970
## FIGURE 2

**MYCOPLASMA GALLISEPTICUM**

TESTING REPORTED TO ANH

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Birds Tested</th>
<th>Reactor Rate</th>
<th>Air Sac Condemnation per*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>8,345,320</td>
<td>0.9%</td>
<td>5.57 Carcasses</td>
</tr>
<tr>
<td>1969</td>
<td>4,457,440</td>
<td>1.16%</td>
<td>4.93 &quot;</td>
</tr>
<tr>
<td>1968</td>
<td>4,161,409</td>
<td>1.44%</td>
<td>10.27 &quot;</td>
</tr>
<tr>
<td>1967</td>
<td>2,834,175</td>
<td>4.51%</td>
<td>15.50 &quot;</td>
</tr>
</tbody>
</table>

*September 30, 1970
*C & MS, U.S.D.A.*
### FIGURE 3

**MYCOPLASMA GALLISEPTICUM TESTING REPORTED TO ANH**

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Birds Tested</th>
<th>Reactor Rate M. Gallisepticum</th>
<th>-All Turkeys- Air Sac Condemnation per 1000 Carcass Inspected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>523,830</td>
<td>.20%</td>
<td>6.75 Carcasses</td>
</tr>
<tr>
<td>1969</td>
<td>583,310</td>
<td>.63%1</td>
<td>6.05 &quot;</td>
</tr>
<tr>
<td>1968</td>
<td>681,696</td>
<td>.39%</td>
<td>6.46 &quot;</td>
</tr>
<tr>
<td>1967</td>
<td>561,729</td>
<td>1.04%</td>
<td>7.96 &quot;</td>
</tr>
</tbody>
</table>

September 30, 1970
*C & MS, U.S.D.A.*
Because of my own interests, and because the principles are much the same for all of the foodborne agents, I’m going to take the liberty of directing my remarks to the salmonella problem. Of the major foodborne diseases I believe that this is the one we as veterinarians have the most interest in. It is a zoonosis that has included in its cycle animals and animal products over which we have some control.

In deference to my honored, departed colleagues, and friends, Dr. P. R. Edwards and Mrs. M. M. Galton I think an excellent starting point for this topic would be a few paragraphs from the first Salmonella Surveillance Report. I feel certain, though I can’t document it, that they were among the prime movers in instituting this very useful and valuable report, which has been published since 1962. It has probably done more to stimulate awareness of the Salmonella problem and subsequently more reporting, among public and animal health workers than any other factor. “Salmonellosis remains one of the most prevalent diseases of public health importance in the United States today; the extent of this problem is poorly defined ... The study of sporadic epidemics of salmonellosis has not lead to any significant decrease in the incidence of the disease as judged from reports of isolations from various laboratories. We believe a surveillance of salmonella infections is the most effective method for increasing our knowledge of the natural history of salmonellosis and subsequently effecting a decrease in the incidence of this disease ...”

“We hope this surveillance program will stimulate increased investigations of individual and contact cases. The concept that each reported case is, in reality, associated with additional cases which are not covered is an intriguing thought which needs further confirmation.”

“The ease with which salmonella can be isolated from non-human sources is frightening. At the present, it is not possible to answer the question as to how much human disease can be related to salmonella isolated from food destined for human consumption. We hope to be able to develop increasing associations between non-human sources of salmonella and human susceptibles through surveillance of all salmonella recoveries, associated with appropriate investigations.”

Dr. Alex Langmuir has defined surveillance as the maintenance of a close scrutiny over the trends of the incidence, geographic distribution, and other characteristics of a disease. The most important source of the data to support surveillance is the morbidity and mortality reports that originate with the private physician. These reports are collected by the local and state health departments, who in turn report weekly to the Center for Disease Control. Data from this source are supplemented by reports from epidemiologists, veterinarians, laboratories,
school and public health nurses, sanitarians, and other specialists.\textsuperscript{3} In the case of
the Salmonella Surveillance Report these other specialists are scientists working
with the USDA, FDA, and veterinary diagnostic laboratories. There is analysis and
evaluation of the data at the local and state level prior to transmission to the
national level. At the CDC the data are studied, evaluated, verified for correctness (if
numbers are unusual for a given area), compiled, and incorporated into reports.
These are sent out to those who find them useful.\textsuperscript{2,3} We admit that especially with
the foodborne diseases the number of reported cases falls short of actual
occurrence, and that some of the case reports contain imprecise (but not
inaccurate) data, but most workers concerned with animal and human health agree
with the observations of a public health statistician that the surveillance reports
generated by national, state or local government are the most effective instruments
we have for monitoring diseases in the U.S. that may threaten the public health.\textsuperscript{3}

Epidemiologists seem to be in general agreement that a good surveillance
program has at least three features as follows:
1. The collection of data;
2. an epidemiologic evaluation looking at time, place, person and
3. the regular dissemination of the data presented.\textsuperscript{2,4,5,6}

This latter feature deserves some special emphasis. The evaluated report not
only alerts the public health authorities and other interested workers to changing
patterns and disease trends, it stimulated increased and better reporting. Dissemi-
nated surveillance data also stimulates disease investigations and research.\textsuperscript{5}

Let us focus our attention on salmonella surveillance in particular. This is a
multidisciplinary cooperative effort. The reservoir of most of the 1300 or so
serotypes of the organism is in domestic and wild animal populations. For the
salmonella surveillance data to be meaningful it must include all of the information
available from reservoir animals, their sources of infection — feed and environment,
and human foods — the vehicle and culture media necessary to infect man. The
private physicians and the public health laboratory are the main sources of
information for human infection. What are our information sources for the animals
and food? These data are the results of epidemiological investigations, the results of
special surveys, and reports of diagnostic laboratory findings. Human data is for the
most part morbidity data. A word of caution. The animal data, for the most part
from diagnostic laboratories, is over represented by mortality. If we look at the
non-human portion of an annual summary of the Salmonella Surveillance Report,\textsuperscript{7}
and the graph therein we could reach the conclusion that poultry are the main
source of salmonellosis in this country. Based only on this graph, our assumption
may be in error. Birds that go to diagnostic laboratories do not go to slaughter and
become human food. Better than we base our conclusions concerning poultry on
the findings from outbreak investigations and surveys of poultry meat. I'm not
saying that this non-human data is not useful data — it is. We should use care,
however, in how we interpret it. It is also the best we have, and we encourage more
reporting in this area. We are especially interested in the results of surveys of live
animals destined for slaughter, and sources of these infections.
A special word about laboratories as a source of surveillance reports. The laboratory is a good source of data for certain diseases – one of them being salmonellosis. On some occasions a physician will submit specimens for diagnosis but will not report the disease, or at least not report it in a specific category. Until he receives the laboratory report he may not know what agent is involved. It may be, in fact, the disease is not a reportable one in a given state. The comparison of laboratory findings and reported episodes of disease is well illustrated by this table and graph4 (Table 1 and Figure 1). Experienced laboratory personnel are often the first to suspect that something unusual is going on. In 1964 when working with the Minnesota State Health Department one of my good friends in the enteric bacteriology section of the laboratory tipped me off to the occurrence of a small epidemic.8 He was used to seeing about one isolation of Salmonella enteriditis a month over the previous years. During the first 20 days of November more than 10 isolations of this serotype had been identified. I plotted them on a map and found that there was a concentration of cases in northwest St. Paul and the adjacent area of Minneapolis. I interviewed a few of these cases, but obtained no leads. Then there was a lucky break. One of the cases remembered that the food history for she and her husband, for the Saturday in question, was not complete. They had eaten a spaghetti sauce sample at a large supermarket, passed out by a man in a white chef's hat. I returned to two or three other cases, and they all remembered the man in the hat and his sample. To make a long story short, all of the cases in the cluster could remember consuming the sample, and one of the cases had consumed no other food from the store – she was shopping with a friend. One little girl took her little brother's sample away from him, and consumed it before the mother could answer his anguished cries. Big sister became ill, little brother did not, and justice triumphed. The spaghetti sauce has been manufactured in the kitchen of an old church. The food was improperly refrigerated and warmed up for the day of sampling. As far as I know the two cooks went back to work assembling washing machines, and their bagged frozen spaghetti sauce business never became a reality. An interesting sidelight – I could not readily ascertain who had jurisdiction over food served as supermarket samples. The department of agriculture had jurisdiction over meat and vegetables before they were prepared for human consumption, and the health department had jurisdiction over cooked foods served in public eating establishments. To the best of my knowledge this was the first time a supermarket food sample had been reported as a source of salmonellae.

A few recent epidemics of salmonellosis illustrate the usefulness of long term surveillance and evaluation. The first was the S. java epidemic associated with the consumption of smoked whitefish. It is well illustrated in this graph (Figure 2). This comparatively rare serotype suddenly jumped into prominence in the New York area, and when all the investigations were completed at least 300 persons at 12 separate gatherings were involved.9 Another example involving one of the rarer serotypes was the S. new-brunswick epidemic traced to consumption of powdered milk. Twenty-nine isolations of this organism during one year were clearly in excess of the usual 4 to 6
isolates from humans the previous 2 years. Extensive investigations implicated several brands of dry milk as the source, and this serotype was isolated from milk drying equipment plants.10

An example of local surveillance at work, with a common serotype involved, was the Wichita, Kansas political rally outbreak of S. typhimurium. Local practitioners notified the state and local health departments that gastroenteritis was a common complaint in their patients during the first of the week and attention was focused on a political rally attended by 1,300 adult guests. Epidemiological investigations implicated turkey rolls as the most likely source of infection.11 Other episodes, including the famous S. derby hospital epidemic12 and the S. cubana carmine dye episode13, could be included in this list. In the first mentioned, raw or uncooked eggs seemed to be the source of infection in 53 hospitals in 13 states.12 Carmine dye, used in gastrointestinal diagnostic work ups, was shown to be the vehicle of infection for 27 patients in four U.S. hospitals and a hospital in England.13

The root word of the word surveillance is survey, and cross-sectional and longitudinal surveys for food poisoning organisms are certainly an important part of this matter. Many surveys have been conducted and reported in the literature. Examples of these have been a 10 month longitudinal survey of Louisiana feed ingredients14, a national laboratory service survey of South American meat imported into the Netherlands15, surveys of imported and domestic meat supplies in Great Britain16,17,18,19, a poultry processing plant in Massachusetts20, and numerous others. They have focused our attention on vehicles and sources of contamination and suggested possible control procedures to decrease the number of human infections.

Food poisoning is a disease of many agents and salmonellosis is a disease of many serotypes. For proper surveillance we need to collect samples and identify the agent. If it can be further identified by serotype, a bacteriophage type21,22, or any other handle (e.g. an unusual biochemical reaction22, colony type, etc.) that we can grab on to, this is most useful in conducting an investigation that will ultimately suggest control measures. The type of etiological agent (biological or chemical) is information needed by the physician. It will guide his selection of a drug or antibiotic and help determine the patient's prognosis. The serotype or phage type does not help him in any way. These are epidemiological tools. However, we need to get these tools and use them whenever we can.

Two authors have suggested the importance of international surveillance because of the increasing international trade in human foods, animal feeds, and feed ingredients. Salmonellae in these products recognize no national boundaries and surveillance data is needed to access the transfer across these boundaries. With this information public health workers could try to do something to stop the introduction of these organisms into their countries.5,23

Until we can formulate and carry out an eradication scheme to eliminate salmonellae from our environment we must conduct the most complete surveillance possible, do retrospective studies to determine source when an incident occurs,
suggest possible control measures so that it will not occur the same way again, and then use surveillance to assess the adequacy of our control measures.

A most apropos closing statement was made by Dr. Alex Langmuir. I quote him. "When major health problems arise, someone must make decisions. This is not the primary responsibility of the epidemiologist. Administrative and political as well as technical considerations must also be brought to bear. It is the epidemiologist's function to get the facts to the decision makers."

"Good surveillance does not necessarily ensure the making of the right decisions, but it reduces the chances of wrong ones."
REFERENCES

REPORT OF THE COMMITTEE ON PUBLIC HEALTH
RADIOLOGICAL FALLOUT AND TOXICOLOGY

R. L. Parker, Atlanta, Georgia, Chairman

The Committee on Public Health, Radiological Fallout, and Toxicology met on October 20. The Committee heard two reports on subjects appropriate to the Committee's name and function.

Dr. John Spaulding, Consumer and Marketing Service (C&MS), United States Department of Agriculture (USDA), discussed the formation and operation of the Toxicology Group, which he heads.

The Toxicology Group was established two years ago in the Technical Service Division, C&MS, USDA; one of its purposes was to set up a foodborne disease communication network to inform the directors of the consumer protection programs of any foodborne disease outbreak that involved a meat or poultry product. The Food and Drug Administration and the Center for Disease Control, Public Health Service, volunteered to combine their resources and expertise with those of the USDA so that foodborne disease outbreaks could be traced to their source and stopped. The unique cooperative program was then proposed to the State health departments and the Armed Services. The Armed Services and over half of the states have joined this program, realizing that it would re-establish their ability to favorably affect the quality of incoming food in their area of jurisdiction and reduce outside interference in foodborne disease outbreaks.

This program recognizes that artificial barriers imposed by laws were a hindrance to safeguarding the food supply. However, these same laws provided a framework within which the agencies legally responsible for the wholesomeness of the food supply could work harmoniously. Two years' experience show this can and does happen as information about food wholesomeness is exchanged among agencies. The contributing causes of foodborne disease outbreaks and their sources are being determined.

Corrective actions are being taken by the responsible agencies on a local or national basis as the conditions warrant. When it is found that an industry procedure produces a potential public health hazard, the procedure is modified on an industry-wide basis.

The potential public health hazard of a food is the sum of the effects of all steps in the food chain. With food animals, the food chain starts on the farm and extends to the consumer's table.

The present system works well for the exchange between involved agencies of information about foodborne diseases. It needs modification to be equally effective
in the field of chemical and drug residues. The Slaughter Inspection Division, C&MS, is expanding its residue surveillance program to include preventive measures by identifying problem areas and reporting to responsible agencies at both the national and state level. This program needs to be expanded further and to be made into a communications network, so that these agencies can be alerted to problems caused by local usage of drugs and chemicals.

The need for such a network is exemplified by the current concern over environmental mercury pollution. Cases of mercury poisoning were originally reported as a possible foodborne disease outbreak. Coordinated investigation revealed the nature of this problem.

For the consumer's protection, the food chain must be regarded as a single unit. The agencies responsible must coordinate their activities and exchange information so that each agency's action contributes to the wholesomeness of the final product and neither duplicates nor negates the efforts of the processor or other agencies to supply the consumer with a nutritious, wholesome diet.

Dr. J. Aseido, of the New York City Health Department, discussed his animal control program. The program is basically directed toward dog bites; dogs are the most numerous of the estimated one million pets in the city of eight million persons. Fear has prompted many private individuals to keep dogs that are trained to protect property, and the result has been an increase in the dog-bite problem. New York City now has a good system of animal control, developed in response to the need for rabies prevention. Although there is no dog rabies in New York City, the system is continuing because of the threat of rabies introduction from neighboring areas.

Physicians who treat bite wounds must report the incident to the health department. The health department investigates to make certain the biting animal is examined initially and again after 10 days by a veterinarian, or is submitted to a laboratory for rabies examination. Actual quarantine of the animal is required in the case of face bites. The presence of many chows, bull terriers, and other breeds that bite without warning creates additional concern, regulations provide that any animal that bites humans three times within two years shall be destroyed. Forms are utilized for reporting bites (stamped pre-addressed postcard) and for bite investigations. Police officers enforce the law when necessary.

Reported bite cases have increased from 27,137 in 1960 to 35,728 in 1969 and 18,831 in the first half of 1970; the rate is about 450 humans bitten per 100,000 population. About 10 percent of the biting animals are not apprehended for observation. The ASPCA, which licenses dogs, collects nearly 16,000 strays per year and turns over more than 2,400 to approved laboratories. In 1968, the ASPCA recorded over 130,000 dog deaths; most of these dogs were submitted by their owners for euthanasia.

Other species of animals create bite problems, also. With wild animals the question of rabies becomes more exacting because of the prevalence of the disease in foxes, skunks, and raccoons in the United States. Poisonous snakes are a problem at times since they are occasionally kept as pets.
Dog bites occur more frequently in the warmer months of the year, in the late afternoon and in the after dinner hours. More than a third of the persons bitten are under 16 years of age. In the evening, bites occur in adults more frequently than in children; many bites are from dogs that associate primarily with adults. Pet shops and dog trainers are licensed, but no special license is required for the guard dogs.

Males are bitten more frequently than females, in a ratio of 1.8:1. About 350-400 persons receive anti-rabies treatment at City Health Department facilities each year, usually because the biting animal cannot be found.

Dogs create other problems in a city also. Mechanized sanitation methods are not as efficient in removing dog feces as were the old time street sweepers. Not only does dog feces constitute a nuisance problem but may be the means of spread of other diseases, notably the larvae of *Toxacara canis* and related organisms and, potentially, salmonella infection.

To reduce the problem of face bites, the Health Department has pursued an education campaign, with some positive results. The Center for Disease Control film "Animal Bites and Rabies Control" has been used in this campaign.

The Committee also discussed and noted its concern for the public health and animal health implications of Venezuelan Equine Encephalomyelitis, which has been reported in several Central American countries, southern Mexico, and Florida.
HUMAN RABIES DEATHS IN UNITED STATES – 1970
IMPACT AND SIGNIFICANCE

Richard L. Parker, D.V.M.

It seems appropriate to begin a discussion of current rabies problems in the United States with a discussion of the two cases in humans reported through September 1970 to the Center for Disease Control. These were both fatal and were reported during the same week. The following is quoted from the CDC "Morbidity and Mortality Weekly Report" for the week ending August 1, 1970.1

"Case 1: At 1 a.m. on June 29 near McNary, in the Mogollon Rim area of Arizona, a skunk entered the tent and sleeping bag of an 11-year-old boy and bit him four times on the right shoulder and left hand. The skunk was captured and on July 1 was found to be rabid by the fluorescent antibody (FRA) test. On July 2 the boy was begun on the 14-dose series of duck embryo rabies vaccine. He also received one dose of tetanus toxoid initially.

"On July 22 he developed stiff neck, sore throat, and malaise. He was admitted with these complaints to the Tucson Medical Center on July 26. One day after admission, paresthesia developed in his left hand. In the hospital his temperature fluctuated between 98 and 105°F, and progressive weakness of the left arm, fatigue, dysphagia, dysarthria and focal rhythmic motor activity developed. On July 30 he lapsed into coma, and in the early morning on July 31 he died.

"Laboratory tests of serum obtained from the patient on July 26 showed a rabies antibody titer of 1:64. Sections of brain examined postmortem were positive by the FRA technique.

"Epidemiologic investigation showed that specimens of four other skunks submitted to the Arizona laboratory this year from the same camping area where the patient had been bitten were positive for rabies. There has been a 3-fold increase in the number of animal specimens positive for rabies submitted to the laboratory this year from the Mogollon Rim area in central Arizona.

"Case 2: On June 23, 1970, a 4-year-old boy in Chamberlain, South Dakota, was severely bitten about the upper extremities by a wild skunk which had been living in his backyard and had recently had a litter. The child's mother had considerable difficulty in dislodging the skunk and finally had to hit it over the head with a pipe. The child was immediately taken to a physician who cleaned the wounds, administered equine rabies antiserum, and began the 14-day course of duck embryo vaccine. The course of therapy was complicated by the development of giant urticaria after the 10th and 11th doses, which responded to small doses of steroids and antihistamines. The subsequent doses were well tolerated. He remained well until July 26 when he developed intermittent headache. On July 28 he developed sore throat and fever and was noted to have a markedly injected posterior pharynx and swelling in the area of the left submaxillary gland when seen by his physician on the following day. He was started on antibiotics. He was hospitalized on July 30 with the
appearance of nuchal rigidity and signs of pulmonary congestion. Increasing lethargy and disorientation on the next day and increasing pulmonary congestion prompted his transfer to a hospital in Sioux Falls, South Dakota, where tracheostomy and ventilatory assistance were required. Progressively deepening coma led to death on August 2. Postmortem examination of the brain at the state laboratories revealed Negri bodies, and fluorescent antibody staining for rabies virus was also strongly positive.”

The editorial comment directed attention to the continual hazard to the general population of rabies in wild animals. In 1969 rabies in wild animals accounted for 76 percent (2,672) of all reported cases in the United States, with skunks being the most frequently reported rabid. Rabies has been reported from 49 of the 50 states during the past 10 years, the annual average number of cases was 3,990, and the range was 3,457 to 4,784 (Table 1). Perhaps the most dramatic change was in the number of cases of dog rabies, which decreased from 697 to 256 during the decade (and declined from 9,067 in 1944).5 Dogs, because of their close association with man, have traditionally been the animal associated with human exposures to rabies; however, with the present level of control of dog rabies in this country and the expansion of the problem in wild animals, the reservoir for human exposure has changed. Rabies in wild species is generally believed to be under-reported, but to what degree we do not know. For this reason, absolute numbers have limited value in discussing the problem.

In recent years, about three-quarters of all reported rabies cases have been in wild animals, and in no year in the past decade have less than half of the cases been in wild species. Nearly half of the cases in wild animals have been in skunks. In the eastern United States, foxes are an important reservoir; in South Georgia and in Florida the raccoon is the most commonly reported rabid animal. Rabies in bats has been reported from all of the contiguous 48 states, but many of the cases are in parts of the Southwest inhabited by large numbers of colonial bats.

In 1969, there were less than 10 percent as many cases of dog rabies as cases in wild animals. Over the past 25 years, control programs aimed at the reduction of stray dogs combined with effective vaccines for protecting pets have reduced the incidence of dog rabies by more than 95 percent. Five years ago, counties on our southern border reported 20-25 percent of all cases in the country; today the only major focus of dog rabies along the border is in the lower Rio Grande Valley. Control programs, such as described by Glosser et al., in El Paso, Texas2, and those developed by the Mexican Ministry of Health in cooperation with the Pan American Health Organization along the northern border of Mexico have almost removed the blight of dog rabies on both sides of the border. The techniques used were the classics of vaccination and stray dog control. Dog rabies cases in the rest of the country are generally scattered; only four states reported more than 20 cases in dogs last year (Texas 70, Kentucky 41, Missouri 28, and West Virginia 26).

The implications of this change in the reservoir of rabies from dogs to wild animals are great, both for the health of our people and for agricultural interests. As was predicted at this meeting 9 years ago when I reported on rabies in skunks,
people with increased leisure time are engaging more and more in outdoor recreation.\(^3\) An article in the October 14, 1970 issue of the Atlanta Journal stated that in 1965 approximately a tenth of the U.S. population had tried camping, in 1969, a third of the population had tried camping. The impact of this increasing exposure to the environment of wild animals that may be infected with rabies is reflected in the sources of exposure for human rabies cases in the past 10-3/4 years in this country (Table 2). Wild animals were the source of exposure more frequently than dogs; indeed, in cases in which the biting animal was positively identified, skunks were the most frequent source of human rabies infections.

Even in our home environments we may be exposed to wild animals infected with rabies, since many suburban developments are suitable habitats for skunks and even foxes. It is important to maintain our pet vaccination programs to keep dogs and cats from transmitting rabies from wild animal reservoirs to man.

While suburban development supports wild animal populations, clean farming techniques seem to inhibit these animals in many instances. However, with increasing concentrations of domestic animals in feed lots the chance for exposure of many animals by one rabid fox or skunk becomes more likely. The result of such mass exposures could be economic disaster.

Our future needs for rabies control in this country are clear. First, we must not be complacent about our currently favorable dog rabies situation in the face of a constant threat of reintroduction of rabies from sylvatic reservoirs. We must continue to protect our pets with the most potent safe vaccines that can be made; efficacy and safety of the vaccines used must be the prime considerations. We must also continue to limit our stray dog populations, since, being unprotected against rabies, they are a reservoir of susceptible hosts.

We must continue to seek improvements in our vaccines and procedures for preventing human rabies infections. In both of the cases reported this year, vaccine was given following a skunk bite; however, in one case the treatment was delayed. We still rely on vaccines whose antigenicity is so low that multiple doses are necessary to stimulate the development of antibody to significant levels. To date, our only source of passive immunity has been hyperimmune horse serum, although there is some indication that a high potency human origin immune globulin will be available soon.\(^6\)

At present our only technique for controlling rabies in wild animals is population reduction, aimed at eliminating as many as possible animals in the rabies incubation stage and reducing the contact rate between infected and susceptible animals. While the technique has been effective in stopping the spread of some outbreaks, the effort must be at a continued high level or the program considered only a temporary measure. Cost factors limit the use of this technique on a widespread geographic basis or for long periods of time (years). We must seek new and more practical methods for controlling rabies in our wild populations; the best technique should also protect the individuals of the species. Any new methods, or for that matter our older methods, must be viewed in relation to our total environment.
Education needs to play a bigger than ever role in rabies prevention. Groups at risk, because of vocation or avocation, can and should be identified and made aware of the effectiveness of preventive measures. Few people would visit an area endemic for smallpox without prior vaccination, but what portion of the rabies “at risk” population is even aware that pre-exposure rabies immunization is available and practical? Avocational groups, especially, need to be made aware not only of the desirable features of rural recreation but also of common sense relationships with our wildlife. It is as absurd to play with a “friendly fox” as it is to poke an American bison bull to his feet to “get a better picture,” but people will try either one. Continuing education programs are essential to maintain a reasonable dog control program. We must emphasize not only rabies immunization but also the nuisance problem and the hazard of animal bites as entities in themselves.

We cannot leave the subject of rabies and animal bites without a brief word on pets. Pets, or companion animals, have been a part of man’s environment since before recorded history. We select animals for those characteristics that make them desirable as companions; certainly gentle disposition toward the owner and his family has been one of the most important. Even so, our pet owning population suffers many dog and cat bites and lacerations each year. We have seen in recent years an increased interest in keeping as pets animals that have not had the advantage of countless generations of selective breeding, the so-called exotic pets. Small rodent cage pets — hamsters, mice, guinea pigs, and gerbils — are often mistreated by their well-meaning but misguided owners. All too often the basic life requirements of these species are overlooked, with a consequently low survival rate. All too often they die about two days after having bitten someone, to the concern of the family, physician, and diagnostic laboratory. The likelihood of rabies under these circumstances is very remote, primarily because of the absolute absence of opportunity for exposure of the animal to rabies.

Of much more concern are the exotic carnivores — the skunks, fox, and raccoons — that are indigenous reservoirs of rabies. Perhaps less common but even more risky as pets are the imported cats and non-human primates, for there is almost no way of knowing about possible previous exposure. These animals bite. Recently the Center for Disease Control was asked to help investigate an episode involving pet skunks, which was not unusual but really quite typical. A man supplying skunks to the pet trade had captured some kits from the wild — about 70 in all, caged together. One bit its new owner after having been sold by a pet store, it was subsequently shown to be rabid. Since its litter mates could not be identified, an attempt was made to recover all of the lot, but only about 50 were accounted for. Treatment was instituted in about 20 persons who had had contact with this group of animals; fortunately, investigation and in some cases examination of the animals for evidence of rabies infection proved that some of the people were not at risk, and treatment was discontinued for them.

In summary, rabies in wild animals has replaced rabies in dogs as the most serious threat to man. Present indications point to continued public health and animal health problems associated with the expansion of the sylvatic rabies
problem. New techniques must be found for combating the problem while we continue our campaign to eliminate dog rabies. The public must be made to understand the problem and persuaded to apply methods of rabies prevention. The problems of bites and possible rabies transmission from exotic pets is emerging as a significant part of the overall rabies prevention program.
<table>
<thead>
<tr>
<th>Year</th>
<th>Dogs</th>
<th>Cats</th>
<th>Farm Animals</th>
<th>Foxes</th>
<th>Skunks</th>
<th>Bats</th>
<th>Other Animals</th>
<th>Man</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960</td>
<td>697</td>
<td>277</td>
<td>645</td>
<td>915</td>
<td>725</td>
<td>88</td>
<td>108</td>
<td>2</td>
<td>3,457</td>
</tr>
<tr>
<td>1961</td>
<td>594</td>
<td>217</td>
<td>482</td>
<td>614</td>
<td>1,254</td>
<td>186</td>
<td>120</td>
<td>3</td>
<td>3,470</td>
</tr>
<tr>
<td>1962</td>
<td>565</td>
<td>232</td>
<td>614</td>
<td>594</td>
<td>1,449</td>
<td>157</td>
<td>114</td>
<td>2</td>
<td>3,727</td>
</tr>
<tr>
<td>1963</td>
<td>573</td>
<td>217</td>
<td>531</td>
<td>622</td>
<td>1,462</td>
<td>303</td>
<td>224</td>
<td>1</td>
<td>3,933</td>
</tr>
<tr>
<td>1964</td>
<td>409</td>
<td>220</td>
<td>594</td>
<td>1,061</td>
<td>1,909</td>
<td>352</td>
<td>238</td>
<td>1</td>
<td>4,784</td>
</tr>
<tr>
<td>1965</td>
<td>412</td>
<td>289</td>
<td>625</td>
<td>1,038</td>
<td>1,582</td>
<td>484</td>
<td>153</td>
<td>1</td>
<td>4,584</td>
</tr>
<tr>
<td>1966</td>
<td>412</td>
<td>252</td>
<td>587</td>
<td>864</td>
<td>1,522</td>
<td>377</td>
<td>183</td>
<td>1</td>
<td>4,198</td>
</tr>
<tr>
<td>1967</td>
<td>412</td>
<td>293</td>
<td>691</td>
<td>979</td>
<td>1,568</td>
<td>414</td>
<td>250</td>
<td>2</td>
<td>4,609</td>
</tr>
<tr>
<td>1968</td>
<td>296</td>
<td>157</td>
<td>457</td>
<td>801</td>
<td>1,400</td>
<td>291</td>
<td>210</td>
<td>1</td>
<td>3,613</td>
</tr>
<tr>
<td>1969</td>
<td>256</td>
<td>165</td>
<td>428</td>
<td>888</td>
<td>1,156</td>
<td>321</td>
<td>307</td>
<td>1</td>
<td>3,522</td>
</tr>
</tbody>
</table>

*Data from CDC*
### TABLE 2
HUMAN CASES OF RABIES FOLLOWING EXPOSURE WITHIN THE UNITED STATES 1960 – SEPTEMBER 1970

<table>
<thead>
<tr>
<th>Case</th>
<th>Date Died</th>
<th>Age</th>
<th>Sex</th>
<th>Biting Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5/21/60</td>
<td>9</td>
<td>M</td>
<td>dog</td>
</tr>
<tr>
<td>2</td>
<td>1/6/61</td>
<td>53</td>
<td>F</td>
<td>fox</td>
</tr>
<tr>
<td>3</td>
<td>1/20/61</td>
<td>76</td>
<td>M</td>
<td>dog</td>
</tr>
<tr>
<td>4</td>
<td>6/27/61</td>
<td>74</td>
<td>M</td>
<td>fox</td>
</tr>
<tr>
<td>5</td>
<td>7/24/62</td>
<td>4</td>
<td>M</td>
<td>probably dog</td>
</tr>
<tr>
<td>6</td>
<td>10/8/62</td>
<td>11</td>
<td>M</td>
<td>possibly bat</td>
</tr>
<tr>
<td>7</td>
<td>9/4/63</td>
<td>52</td>
<td>F</td>
<td>probably dog</td>
</tr>
<tr>
<td>8</td>
<td>9/1/64</td>
<td>10</td>
<td>M</td>
<td>skunk</td>
</tr>
<tr>
<td>9</td>
<td>5/21/65</td>
<td>60</td>
<td>M</td>
<td>dog</td>
</tr>
<tr>
<td>10</td>
<td>9/5/66</td>
<td>10</td>
<td>M</td>
<td>skunk</td>
</tr>
<tr>
<td>11</td>
<td>10/10/68</td>
<td>13</td>
<td>M</td>
<td>unknown, possibly dog</td>
</tr>
<tr>
<td>12</td>
<td>8/29/69</td>
<td>2.5</td>
<td>M</td>
<td>bobcat</td>
</tr>
<tr>
<td>13</td>
<td>7/31/70</td>
<td>11</td>
<td>M</td>
<td>skunk</td>
</tr>
<tr>
<td>14</td>
<td>8/2/70</td>
<td>4</td>
<td>M</td>
<td>skunk</td>
</tr>
</tbody>
</table>
REFERENCES

REPORT OF THE COMMITTEE ON RABIES

J. C. Shook, Harrisburg, Pa., Chairman

In 1969, 3522 laboratory-confirmed cases of rabies were reported to the National Communicable Disease Center; this is a 2.5 percent decrease from 1968 and 19 percent decrease from the average for the previous five years. Forty-six states reported animal rabies; only Delaware, Hawaii, Rhode Island, South Carolina, and the District of Columbia reported no rabies cases in 1969. Of the two U.S. territories, Guam and Puerto Rico and the Commonwealth of the Virgin Islands, only Puerto Rico reported cases of rabies. Virginia reported the largest number of cases with 386. The species accounting for the majority of laboratory-confirmed cases in 1969 were skunks (33%), foxes (25%), cattle (10%), bats (9%), dogs (7%), raccoons (7%), cats (5%), and horses and mules (1%). Only 256 cases of laboratory-confirmed rabies in dogs were reported in the United States in 1969; this is a 14 percent decrease from 1968 and a 29 percent decrease from the average for the last five years. One human rabies death occurred in 1969. A 2½ year old boy who had been bitten by a rabid bobcat on April 1 died in California on August 29.

It should be noted that for the ninth consecutive year, skunks were the most frequently rabies-infected wildlife species. They accounted for 33 percent of the total animal rabies cases and 43 percent of the wildlife rabies cases reported in 1969. Inasmuch as skunks are the wildlife species most frequently distributed for family pets, your committee recognizes the potential hazard to human health and the spread and perpetuation of rabies by this means. We recommend that measures be taken by all federal and state regulatory agencies in this field to prevent the capture, sale, and distribution of skunks. A program to educate the various professions and the general public through all available media regarding the health and safety hazard of pet skunks should be a part of this effort.

Your committee reviewed three resolutions submitted by the Association of State Public Health Veterinarians and endorses the following resolutions:

1. That each State utilize the "Guidelines for the Control of Rabies" recently developed by the Rabies Committee, Conference of Public Health Veterinarians. Although each State may presently have regulations which vary from these guidelines, they will serve to standardize rabies control practices nationally.

2. That States supply species identification and other information for each wild animal diagnosed with rabies. To accomplish this, NCDC is developing a new form which will be circulated among State Public Health Veterinarians for their approval. A completed form will be available by January 1, 1971.

3. That a form be prepared by NCDC for use by State Health Departments to provide a complete case history on each positive dog or cat. When
completed, each case history should be forwarded immediately to the Rabies Unit, NCDC.

Your committee discussed the need nationally of a uniform rabies vaccination certificate. This problem and recommendation will be referred to the appropriate committee of this organization.

In our report last year we recommended seeking sponsorship of a nationwide rabies control program from the National Academy of Science – National Research Council. Representatives of your rabies committee met with the Committee on Animal Health of that organization and cited past difficulties in achieving an effective coordinated rabies control effort. The Animal Health Committee of NAS-NRC authorized the formation of a task force to undertake a comprehensive assessment of the rabies situation in the United States. Recommendations from that task force will be forthcoming.

The statistical portion of this report, along with the several charts and maps, is provided by the National Communicable Disease Center.
## INCIDENCE OF RABIES IN THE UNITED STATES BY TYPE OF ANIMAL

### 1953–1969*

<table>
<thead>
<tr>
<th>Year</th>
<th>Dogs</th>
<th>Cats</th>
<th>Farm</th>
<th>Foxes</th>
<th>Skunks</th>
<th>Animals</th>
<th>Other Animals</th>
<th>Man</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1953</td>
<td>5,688</td>
<td>538</td>
<td>1,118</td>
<td>1,033</td>
<td>319</td>
<td>8</td>
<td>119</td>
<td>14</td>
<td>8,837</td>
</tr>
<tr>
<td>1954</td>
<td>4,083</td>
<td>462</td>
<td>1,032</td>
<td>1,028</td>
<td>547</td>
<td>4</td>
<td>118</td>
<td>8</td>
<td>7,282</td>
</tr>
<tr>
<td>1955</td>
<td>2,657</td>
<td>343</td>
<td>924</td>
<td>1,223</td>
<td>580</td>
<td>14</td>
<td>98</td>
<td>5</td>
<td>5,844</td>
</tr>
<tr>
<td>1956</td>
<td>2,592</td>
<td>371</td>
<td>794</td>
<td>1,281</td>
<td>631</td>
<td>41</td>
<td>126</td>
<td>10</td>
<td>5,846</td>
</tr>
<tr>
<td>1957</td>
<td>1,758</td>
<td>382</td>
<td>714</td>
<td>1,021</td>
<td>775</td>
<td>31</td>
<td>115</td>
<td>6</td>
<td>4,802</td>
</tr>
<tr>
<td>1958</td>
<td>1,643</td>
<td>353</td>
<td>737</td>
<td>845</td>
<td>1,005</td>
<td>68</td>
<td>157</td>
<td>6</td>
<td>4,814</td>
</tr>
<tr>
<td>1959</td>
<td>1,119</td>
<td>292</td>
<td>751</td>
<td>920</td>
<td>789</td>
<td>80</td>
<td>126</td>
<td>6</td>
<td>4,083</td>
</tr>
<tr>
<td>1960</td>
<td>697</td>
<td>277</td>
<td>645</td>
<td>915</td>
<td>725</td>
<td>88</td>
<td>108</td>
<td>2</td>
<td>3,457</td>
</tr>
<tr>
<td>1961</td>
<td>594</td>
<td>217</td>
<td>482</td>
<td>614</td>
<td>1,254</td>
<td>186</td>
<td>120</td>
<td>3</td>
<td>3,470</td>
</tr>
<tr>
<td>1962</td>
<td>565</td>
<td>232</td>
<td>614</td>
<td>594</td>
<td>1,449</td>
<td>157</td>
<td>114</td>
<td>2</td>
<td>3,727</td>
</tr>
<tr>
<td>1963</td>
<td>573</td>
<td>217</td>
<td>531</td>
<td>622</td>
<td>1,462</td>
<td>303</td>
<td>224</td>
<td>1</td>
<td>3,933</td>
</tr>
<tr>
<td>1964</td>
<td>409</td>
<td>220</td>
<td>594</td>
<td>1,061</td>
<td>1,909</td>
<td>352</td>
<td>238</td>
<td>1</td>
<td>4,784</td>
</tr>
<tr>
<td>1965</td>
<td>412</td>
<td>289</td>
<td>625</td>
<td>1,038</td>
<td>1,582</td>
<td>484</td>
<td>153</td>
<td>1</td>
<td>4,584</td>
</tr>
<tr>
<td>1966</td>
<td>412</td>
<td>252</td>
<td>587</td>
<td>864</td>
<td>1,522</td>
<td>377</td>
<td>183</td>
<td>1</td>
<td>4,198</td>
</tr>
<tr>
<td>1967</td>
<td>412</td>
<td>293</td>
<td>691</td>
<td>979</td>
<td>1,586</td>
<td>414</td>
<td>250</td>
<td>2</td>
<td>4,609</td>
</tr>
<tr>
<td>1968</td>
<td>296</td>
<td>157</td>
<td>457</td>
<td>801</td>
<td>1,400</td>
<td>291</td>
<td>210</td>
<td>1</td>
<td>3,613</td>
</tr>
<tr>
<td>1969</td>
<td>256</td>
<td>165</td>
<td>428</td>
<td>888</td>
<td>1,156</td>
<td>321</td>
<td>307</td>
<td>1</td>
<td>3,522</td>
</tr>
</tbody>
</table>

*Data prior to 1960 from USDA, ARS. Subsequent data from PHS, NCDC.
STREPTOCOCCIC LYMPHADENITIS I.
THE FEBRILE LEUKOCYTIC AND PATHOLOGIC RESPONSES
OF SWINE FED VARYING QUANTITIES OF
GROUP E STREPTOCOCCI

J. A. Schmitz and L. D. Olson*

Streptococcic lymphadenitis (jowl abscess) of swine has been recognized as a
disease of economic importance, particularly since 1963, when a national survey
was conducted by Livestock Conservation Incorporated.1,4 Based on this survey, it
was estimated that the annual loss to the swine industry was approximately $12
million.

This condition can be produced experimentally in susceptible pigs by the oral
or intranasal instillation of viable cultures of streptococci of Lancefield's Group E.

For the benefit of future studies on the pathogenesis and immunogenesis of
this disease, it was felt that a minimum infective dose should be established. Thus,
this experiment was conducted to clarify the relationship between the quantity of
oral inoculum of Group E Streptococci and the incidence and severity of abscesses.
Further, certain postinoculation responses, viz. temperature and leukocyte counts,
were recorded and examined for correlation with the presence or absence of abscess
formation.

MATERIALS AND METHODS

Animals. Fifty-five conventionally reared pigs 11 to 15 weeks of age were
equally divided into 7 groups. One group served as control and 6 groups were
inoculated orally with 6 different concentrations of inoculum. The number of
animals per group and inoculum levels are shown in Table 1.

Inoculum. A vial of freeze-dried Group E Streptococci (GES) strain 3x29a**, was
hydrated and streaked onto blood agar (5% citrated sheep blood in tryptose
phosphate agar). This media, after inoculation and incubation at 37°C for 24
hours, was used to inoculate flasks of Todd Hewitt† broth which were also
incubated for 24 hours and showed approximately 2.7x10^7 colony-forming units
(CFU) per milliliter of broth. Tenfold graduations of the broth cultures were used
to obtain the desired inoculum concentration. Inoculum levels for each group were:
2.7x10^5 CFU, 2.7x10^6 CFU, 2.7x10^7 CFU, 2.7x10^8 CFU, 2.7x10^9 CFU, and
2.7x10^10 CFU. Sterile Todd Hewitt broth was used as necessary to bring the total
volume of inoculum to 100 ml. For the 2.7x10^10 CFU inoculum level it was

*Department of Veterinary Pathology, School of Veterinary Medicine, University of Missouri,
Columbia, Missouri 65201.

Supported by Cooperative Agreement No. 12-14-100-9741 (45) from the United States De-
ment of Agriculture.

**Obtained from Dr. R.D. Shuman, National Animal Disease Laboratory, Ames, Iowa.
†Difco Laboratories, Detroit, Michigan.
necessary to centrifuge 1000 ml. of original broth culture and decant 900 ml. of supernatant fluid leaving the bacterial sediment suspended in 100 ml. of broth medium.

Method of Inoculation. The 100 ml. of inoculum was thoroughly mixed with 200 Gms. of diet, placed in a stainless steel pan and fed to each pig individually. Each pig consumed its portion completely before being returned to its pen. The pans were washed in a disinfectant solution††, rinsed in water and dried before being reused.

Parameters Measured. Commencing one day prior to inoculation and continuing for 8 days, rectal temperatures were recorded and blood samples collected* for total and differential leukocyte counts.

The general appearance, behavior and appetite of the pigs each day following inoculation were observed as a further measure of the response in different test groups.

Necropsy. Four weeks after inoculation, all pigs were sacrificed and examined for abscesses. The abscesses were classified as “intranodular” if the abscesses were small and completely contained within the lymph node. Large abscesses with rupture of the capsule of the lymph node and invasion into surrounding tissues were classified as “extranodular” abscesses.

Bacteriology and Serology. Culture swabs from all abscesses were streaked onto blood agar. From these cultures, 40 ml. tubes of Todd Hewitt broth were inoculated and used for antigenic extraction.

Confirmation of bacterial isolates as GES was based on positive precipitin tests utilizing the agar gel double-diffusion method of Collins. The formamide technique of Fuller was used to extract bacterial antigens. Antiserum was prepared by hyperimmunization of a rabbit with GES strain 3x29a according to Lancefield’s method.

RESULTS

During the first 2 to 3 days postinoculation, the pigs in all inoculated groups were anorectic and moderately depressed. The group receiving the 2.7x1010 inoculum level was noticeable more affected than the other groups and also developed a transient, mild diarrhea which did not occur in the other groups. By the fourth and fifth days postinoculation, all pigs displayed normal appetites and behaviors.

A febrile response occurred in all test groups following exposure to GES (Figure 1). This response was characterized by a peak in body temperature on day 2 postinoculation followed by a gradual decline to the preinoculation temperature, +

††Winter-Phene. James Varley and Sons, Inc., St. Louis, Mo.
*Blood samples for total leukocyte counts were collected in Unipettes, Becton-Dickinson, Rutherford, New Jersey.
F, on the fifth or sixth day postinoculation. Mean temperatures of each group were tested by analysis of variance and showed that the group receiving the 2.7x10^{10} inoculum level had a significantly greater pyrexia than other test group (P < 0.05)

All 6 groups had a leukocytosis following exposure (Figure 2). The characteristic leukocytic response was a gradual increase, maximizing on the third or fourth day postinoculation and then a gradual reduction toward the preinoculation level by the sixth day postinoculation. There appeared to be a relationship between the level of inoculum and the degree of leukocytosis, in that the leukocytic responses were greater in the groups receiving the higher dosages of inoculum. Mean total leukocytes of each group were tested by analysis of variance. The leukocytic response in the group receiving the inoculum level of 2.7x10^{10} CFU was significantly greater (P < 0.05) than other test groups.

The mean temperatures and total leukocyte counts of all abscessed and all nonabscessed animals are given in Figures 1 and 2 respectively. The abscessed animals exhibited slightly higher temperature and leukocyte values than the nonabscessed animals; however, the difference was not significant (P<0.05) as determined by analysis of variance of the means of both groups.

The postinoculation lymphocyte, mature neutrophil, immature neutrophil and eosinophil counts, expresses as means for each group of pigs are shown in Figures 3, 4, 5, and 6. In all groups a panleukocytosis developed and peaked on the third day postinoculation. The total leukocytic response as well as a neutrophilia. The group receiving 2.7x10^{10} CFU exhibited higher differential leukocyte counts in all categories than did other test groups.

Table 1 contains the necropsy findings. Abscesses were present in 47.2% of the pigs in all groups. There was no relationship between concentration of inoculum and occurrence of abscesses. Similarly, the number of lymph node abscesses per animal and the size of these abscesses was not related to the level of inoculum.

Single and multiple abscesses ranging in size from 0.3 cm. to 9 cm. in diameter were present in mandibular, cervical and parotid lymph nodes. The mandibular lymph nodes were most frequently affected. Typically the abscesses were surrounded by a thick fibrous capsule and contained a thick, greenish, odorless exudate from which GES was readily isolated. Small intranodular abscesses were contained completely within the lymph nodes which often were enlarged, while the larger abscesses ruptured the capsules of the lymph nodes and invaded the surrounding tissues. Many of the larger extranodular mandibular abscesses had stratified muscle and salivary gland tissue as well as lymphoid tissue incorporated into the capsule wall.

**DISCUSSION**

Within the limits of this experiment, the occurrence and severity of abscesses was not related to the quantity of oral inoculum. Thus, for future studies of the disease it appears that dosage of inoculum is not a critical factor although
extremely low inoculum levels might well result in complete absence of abscess formation. Possibly the streptococci establish themselves and proliferate on the mucous membranes of the oral or pharyngeal cavities or in the tonsils. If this condition does occur, the bacterial proliferation in the oral or pharyngeal cavities might ultimately result in maximal challenge of the lymphatic system of the cephalic and cervical regions, regardless of quantity of inoculum.

In evaluating this experiment, it should be stated that the quantities of organisms in the inocula may be misleading. It is not assumed that all the organisms in the inocula, administered mixed with feed as it was, actually contacted oral and pharyngeal mucosal surfaces. However it is felt that the number of organisms contacting mucosal surfaces at each inoculum level should have increased tenfold relative to the preceding dosage level.

The observation of the greatest febrile and leukocytic responses in the groups receiving the higher quantities of inocula could possibly be explained on the basis of a general systemic response to a bacterial agent. A similar graded response might be seen in pigs inoculated with varying quantities of a staphylococcal agent incapable of causing a specific disease entity in pigs. The absence of significant differences in the febrile and leukocytic responses of the abscessed versus the nonabscessed pigs further indicates that abscess formation is not a function of these bodily defensive mechanisms alone.

The leukocytic response exhibited by the control group on the day of inoculation and the first day postinoculation (Figure 2) cannot be explained definitively but is attributed to blood collection procedures. Five milliliter blood samples were collected from control and test groups alike, thus it is not felt that this factor interferes with the evaluation of these data of this experiment.

SUMMARY

Six groups of pigs fed varying quantities of viable Group E Streptococci were observed for development of streptococcic lymphadenitis (jowl abscesses). Inocula consisted of approximately $2.7 \times 10^5$, $2.7 \times 10^6$, $2.7 \times 10^7$, $2.7 \times 10^8$, $2.7 \times 10^9$, and $2.7 \times 10^{10}$ colony-forming units. Temperature and leukocytic values of all pigs were recorded following inoculation. Necropsy examination conducted 4 weeks post-inoculation indicated that occurrence and severity of abscesses was not related to quantity of oral inoculum. Febrile and panleukocytic responses occurred in all pigs following inoculation and were not related to development of abscesses.
Figure 1. — (A,B) A. Mean temperatures per group following oral inoculation with various quantities of Group E Streptococci. (Control = Control group; 2.7x10⁸ CFU, 2.7x10⁶ CFU, etc. = Quantity of oral inoculum). B. Mean temperatures of abscessed pigs of all groups and nonabscessed pigs of all groups following oral inoculation with Group E Streptococci.
Figure 2. — (A,B) A. Mean total leukocytes per group following oral inoculation with various quantities of Group E Streptococci. (Control = Control group; $2.7 \times 10^5$ CFU, $2.7 \times 10^6$ CFU, etc. = Quantity of oral inoculum). B. Mean total leukocytes of abscessed pigs of all groups and nonabscessed pigs of all groups following oral inoculation with Group E Streptococci.
Figure 3. - Mean mature neutrophils per group following oral inoculation with various quantities of Group E Streptococci. (Control = Control group; $10^5 = 2.7 \times 10^5$ Colony-forming units, $10^6 = 2.7 \times 10^6$ Colony-forming units, etc.)
Figure 4. — Mean immature neutrophils per group following oral inoculation with various quantities of Group E Streptococci. (Control = Control group; $10^5 = 2.7 \times 10^5$ Colony-forming units, $10^6 = 2.7 \times 10^6$ Colony-forming units, etc.)
Figure 5. – Mean lymphocytes per group following oral inoculation with various quantities of Group E Streptococci. (Control = Control group; $10^5 = 2.7 \times 10^5$ Colony-forming units, $10^6 = 2.7 \times 10^6$ Colony-forming units, etc.)
Figure 6. — Mean eosinophils per group following oral inoculation with various quantities of Group E Streptococci. (Control = Control group; $10^5 = 2.7 \times 10^5$ Colony-forming units, $10^6 = 2.7 \times 10^6$ Colony-forming units, etc.)
REFERENCES

STREPTOCCIC LYMPHADENITIS II.
SUSCEPTIBILITY OF VARIOUS AGES OF SWINE FOLLOWING ORAL INOCULATION WITH GROUP E STREPTOCOCCI

J. A. Schmitz and L. D. Olson*

It has been interpreted from field and experimental observations that swine of different ages vary in their susceptibility to streptococcic lymphadenitis.1,2 Under field conditions, this disease is most commonly seen in pigs 4 to 5 months of age. Personal observations of experimental animals have also been interpreted to indicate an increased susceptibility of pigs 10 weeks and older.

Based on this information an experiment was conducted to determine the age related susceptibility of swine to streptococcic lymphadenitis.

MATERIALS AND METHODS

The materials and methods of this experiment were similar to those reported in the previous paper.3 The swine used in this study were from a specific-pathogen-free (SPF) herd. The inoculum consisted of Group E Streptococcus, strain 3x29a†, sprayed onto the pharyngeal and tonsillar surfaces of the oral cavity. The approximate dosage of inoculum for all age groups was 9x10^8 colony-forming units (CFU) as determined by serial dilutions and plate counts. Rectal temperatures were recorded and blood samples were collected on 8 consecutive days commencing 1 day prior to inoculation. Two noninoculated control animals of each age tested had rectal temperatures recorded and blood samples taken as for inoculated animals.

The various ages of pigs inoculated and the number inoculated per age group are presented in Table 1.

SUMMARY OF RESULTS

I. Pigs ages 10 days and 6 weeks did not develop abscesses following oral inoculation of Group E Streptococci.

II. Pigs 14 weeks, 1 year and 2.5 years of age developed abscesses following oral inoculation with Group E Streptococci (Table 1). The 14 week old group had the highest rate (5 of 6), the 1 year old group was next (4 of 6) and the 2.5 year old group had the lowest rate (1 of 6).

III. Febrile and leukocytic responses were greatest in the 14 week old group followed by the 1 year old group. The 6 week old group demonstrated an initial marked leukocytosis on the first day postinoculation followed by a depression in the total number of leukocytes on postinoculation days 2, 3, 4,

*Department of Veterinary Pathology, School of Veterinary Medicine, University of Missouri, Columbia, Missouri 65201.
Supported by Cooperative Agreement No. 12-14-100-9741 (45) from the United States Department of Agriculture.
†Obtained from Dr. R. D. Shuman, National Animal Disease Laboratory, Ames, Iowa.
and 5. There was little febrile response in the 6 week old group following inoculation. The febrile and leukocytic responses in the 2.5 year old and 10 day old groups were much less than those of the other aged groups.

IV. These data indicate that an age factor does exist as to susceptibility of swine to streptococcic lymphadenitis. The cause of this apparent age related susceptibility factor is not known at this time.

REFERENCES

## TABLE 1

INCIDENCE AND SEVERITY OF ABScesses IN SWINE OF VARIOUS AGES
FOLLOWING ORAL ADMINISTRATION OF GROUP E STREPTOCOCCI

<table>
<thead>
<tr>
<th>AGE</th>
<th>NUMBER PIGS IN GROUPS</th>
<th>NUMBER PIGS WITH ABSCESSES</th>
<th>SINGLE LYMPH NODE ABSCESSED</th>
<th>MULTIPLE LYMPH NODES ABSCESSED</th>
<th>PIGS WITH &quot;INTRANODULAR&quot; ABSCESSES</th>
<th>PIGS WITH &quot;EXTRANODULAR&quot; ABSCESSES</th>
<th>LYMPH NODES AFFECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 da.</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6 wks.</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14 wks.</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>MANDIBULAR CERVICAL PAROTID</td>
</tr>
<tr>
<td>1 yr.</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>MANDIBULAR POPLITEAL</td>
</tr>
<tr>
<td>2.5 yr.</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>MANDIBULAR</td>
</tr>
</tbody>
</table>
RESPONSE OF SWINE TO VIRULENT AND MODIFIED PSEUDORABIES VIRUSES

J. A. Howarth, D.V.M., Ph.D.

The recognition and interpretation of clinical signs is one of the more reliable means of detecting the presence of a specific disease, as well as being a useful indicator of the prevalence of latent infection. Pseudorabies is primarily a latent subclinical infection, but the overt infection that has been recognized in swine and other livestock species has been responsible for acute deaths and serious economic loss.

The occurrence of pseudorabies in most animal species is often the result of contact with swine; and, therefore, recognition of the disease in this reservoir host is most important. The relatively few documented instances of clinical disease in swine in the United States may not be an accurate reflection of its incidence. The limited nature of serologic surveys conducted to date does little to indicate the geographical distribution of porcine pseudorabies; and, until a continuous serologic surveillance can be instituted, the detection of pseudorabies will be dependent on the recognition of clinical signs.

Pseudorabies in the neonatal pig is characterized by abortion, stillbirth, and high mortality in the newborn. It is unlikely that clinical disease would reappear in such breeding herds because the colostrum of latently-infected sows would protect subsequent litters during their most susceptible period of life. In contrast, in the intensive type of hog-fattening system where aggregation of pigs from many sources is common, pseudorabies is likely to be a recurrent problem. Epizootics of pseudorabies encephalitis in post-weanling pigs being fattened under assembly-line types of management place this disease among the more important emerging diseases of swine.

The purpose of the present study was to compare the acute and mild clinical syndromes produced in weanling pigs by virulent and modified pseudorabies viruses and correlate them with virus isolation and serologic studies where applicable.

MATERIALS AND METHODS

Swine. Thirty-six Duroc pigs, 8 weeks old and weighing 19 kg. each, were obtained from the swine herd of the University of California. These pigs were vigorous, of uniform size, and showed no evidence of internal or external parasitism. Repeated serologic testing had shown the breeding herd from which they were obtained to be free of brucellosis, leptospirosis, and pseudorabies.

Viruses. The virulent pseudorabies virus used as the challenge inoculum was isolated in California in October 1966 from the brain of a pig with clinical signs of pseudorabies. The challenge inoculum used in experiment 1 had undergone a total
of 5 passages in tissue culture and that used in experiment 2 had undergone 6 passages.

The pseudorabies virus used to vaccinate pigs in experiment 2 had been modified by Dr. R. Skoda of Czechoslovakia. Virus strain Bucharest (BUK)* had been modified by passage in chick embryo chorio-allantoic membranes, passage in chick embryo cell tissue cultures, and plaque purification. The BUK virus was in primary rabbit kidney cells when received. It was passaged once in second-passage porcine kidney cells and twice in procine kidney line cells. The virus used as a vaccine was harvested from the second passage in porcine kidney line cells.

All serum neutralization tests were performed with a reference pseudorabies virus (VR 135) obtained from the American Type Culture Collection.

**Vaccination and challenge inoculation.** The intranasal inoculations of virulent virus in experiment 1 and BUK vaccine virus in experiment 2 were done in the same manner. The inoculum was drawn into a 5 ml syringe and the needle removed. The contents were introduced into the nasal cavity while the pig was restrained in a sitting-dog position with the head held upright. The pig was held in this position with the syringe occluding the nares until the inoculum had flowed caudally along the ventral surface of the nasal cavity to the pharynx.

The virulent virus used as a challenge inoculum for vaccinated and control pigs in experiment 2 was introduced into the left nasal cavity while the pigs were restrained horizontally in dorsal recumbency. In this instance, the inoculum flowed caudally along the dorsal surface of the nasal cavity rather than in the ventral portion, as was the case in the previous inoculations.

The BUK vaccine virus administered intramuscularly was injected into the muscles of the medial surface of the ham.

**Observations.** The 6 pigs in experiment 1 were maintained in a single enclosure during the experiment. In experiment 2, the intranasally vaccinated group, the intramuscularly vaccinated group, and the control group were maintained in 3 separate enclosures to prevent contact among the groups. The pre-challenge isolation was used to prevent possible spread of the BUK vaccine virus among the groups.

Rectal temperatures were recorded each morning for 3 days before challenge and for 7 days post-challenge. The pigs were examined 3 times daily and clinical signs noted.

**Specimens.** From each pig that died, samples were taken from the following organs: cerebrum, cerebellum, midbrain, medulla, tonsil, spleen, and kidney.

Except for the tonsil, suspensions of each tissue were used for direct inoculation of tissue culture in virus isolation attempts. Suspensions of tonsillar tissue were pretreated overnight at 4 C. in a medium containing 5000 units of penicillin, 2000 ug of streptomycin, and 300 units of mycostatin per ml. before inoculation of tissue cultures.

In experiment 1, blood for virus isolation attempts was collected twice daily from each pig from the day of inoculation until death occurred. Ethylenediaminetetraacetic acid (EDTA) was used to prevent clotting so that the buffy coat cells

*Obtained from Dr. R. Skoda of Czechoslovakia by Dr. D. Berger of Washington State University, Pullman, Wash.
could be separated by centrifugation. Each buffy coat was frozen and thawed twice to disrupt cells for virus isolation attempts.

Blood for virus isolation attempts and serologic studies was obtained by anterior vena cava venipuncture.

**Virus isolation and serology.** The virus isolation and serum neutralization test procedures were the same as those used previously.8,9

**Experimental plan.** In experiment 1, the 6 pigs were inoculated intranasally with 2 ml. of virulent virus containing $10^{5.24}$ TCID$_{50}$/0.2 ml. Post inoculation blood samples were obtained from each pig twice daily until death occurred. The buffy coat from the twice daily blood samples and tissues collected during necropsy were used in virus isolation attempts.

In experiment 2, the 30 pigs were allocated to 3 groups: one in which the pigs were inoculated intranasally with 1 ml. of BUK vaccine virus containing $10^{7.5}$ TCID$_{50}$/0.2 ml.; one in which the pigs were inoculated intramuscularly with 2 ml. of BUK vaccine virus containing $10^{7.5}$ TCID$_{50}$/0.2 ml.; and a final group which were unvaccinated controls. Twenty-one days after vaccination, the pigs in all groups were challenged intranasally with 2 ml. of virulent virus containing $10^{7.48}$ TCID$_{50}$/0.2 ml. Blood samples were collected at frequent intervals for serum neutralization testing and tissues were collected during necropsy for virus isolation attempts.

**RESULTS**

**Experiment 1.** Clinical signs were readily noticed on the second day post-inoculation, and all 6 pigs were dead by the seventh day (Table 1). Fever (up to 107 F.), loss of appetite, and extreme depression were constant signs in all pigs. Feed placed on the floor of the pen was left untouched, and all pigs remained "piled up" when left undisturbed. When aroused, the pigs would stand and move a few steps but were oblivious to all but extreme stimuli. When the pigs were restrained to obtain the rectal temperature, they were hypersensitive to touch on post-inoculation day 2; and, from post-inoculation day 4 until death, there was complete loss of voice. Epileptiform convulsions preceded death in 3 animals.

The results of virus isolation attempts with tissues obtained at necropsy are recorded in Table 2. Pseudorabies virus was isolated from all the brain tissues tested and from the tonsil of each pig. Virus was isolated from the spleen and kidney of only one (pig 326) of the 6 pigs. Virus was isolated from one of the 68 buffy coat samples collected twice daily from these pigs. This single isolation was obtained from the blood sample collected on the afternoon of post-inoculation day 2 from pig 323.

**Experiment 2.** There was no clinical response during the observation period following intramuscular administration of the BUK vaccine virus to 10 pigs or to the intranasal instillation of the same virus to an additional 10 pigs. The body temperatures of both groups of vaccinated pigs and those of the control pigs remained unchanged throughout the prechallenge period. There was no evidence of lameness in the pigs which received BUK vaccine virus intramuscularly and no
evidence of rhinitis in the group inoculated intranasally.

After challenge with virulent pseudorabies virus, all 10 control pigs sickened and died within 6 days (Table 3). The response of these pigs was similar to that which occurred in experiment 1: fever (Fig. 1), loss of appetite, extreme depression, aphonia, epileptiform convulsions, coma, and death.

In addition, however, a variety of striking clinical signs not seen in experiment 1 also became evident. On the first day post-challenge, the pigs were afebrile and alert but each one sneezed continuously. The sneezing persisted until the fourth day post-challenge, at which time all pigs showed some evidence of central nervous derangement. Pruritus was the predominant clinical sign on the third and fourth days post-challenge. In spite of their depression, these pigs were restless and moved about without obvious reason. Much of their time was spent rubbing their snout and left eye against other pigs, the fence, and the concrete floor. At the onset of pruritus, the cornea and conjunctiva of each pig were grossly normal and lacrimation was not excessive. As the rubbing and scratching activity continued, the left eyelids of each pig became progressively more abraded and leather-like in appearance. Corneal laceration and extensive subconjunctival edema were common sequela. The right eye of each pig appeared normal and exhibited corneal reflex. When the cornea of the left eye, however, was touched with an object, such as a pencil tip, there was no reflex action. This corneal anesthesia persisted until death.

After challenge with virulent pseudorabies virus, the 10 pigs which had received BUK vaccine virus intramuscularly 21 days previously underwent a mild illness but all survived (Table 4). Sneezing on the first day post-challenge preceded a 3-day period during which fever (Fig. 1), reduced appetite, and depression were recorded. On the fourth through sixth day post-challenge, a conjunctivitis largely confined to the left eye was the predominant clinical sign. The conjunctivae were diffusely reddened, and purulent material tended to glue the eyelids together. A discrete, centrally-located corneal lesion developed in the left eye of one pig 7 days post-challenge and in the left of another pig 10 days post-challenge. In each case, the corneal lesion consisted of a 3 mm. diameter opaque plaque which was a vesicle when first examined but subsequently became a shallow ulcer. Although the ulcers did not increase appreciably in size, the keratitis persisted for approximately 3 weeks.

After challenge with virulent pseudorabies virus, the 10 pigs which had received BUK vaccine virus intranasally 21 days previously underwent a minimal clinical reaction (Table 3). Sneezing, a mild 2-day febrile response, and diarrhea were the principal signs recorded. One pig, however, on the tenth day post-challenge, responded with a corneal lesion as described previously.

The results of virus isolation from tissues obtained at necropsy are recorded in Table 4. Since all pigs of the 2 vaccinated groups survived challenge exposure, the results refer only to the 10 control pigs that died. Virus was isolated from 19 of 20 brain tissues from pigs that died on the fourth and fifty day post-challenge. A lower rate of 10 isolations from 20 brain tissues was obtained when pigs died on the sixth day post-challenge. Virus was isolated from the tonsil of every pig that died. The
high rate of virus recovery from the spleen (4/10) and kidney (2/10) was again correlated with death early in the course of the disease.

Gross lesions detected during necropsy of the 10 control pigs were confined to the nasopharynx. Superficial or deep necroses were found in the mucous membranes of the nasal cavity and turbinates. The nasal meatuses of the left side of each pig were completely occluded with a fibrinous, purulent exudate. In 2 pigs, the process also extended to the right nasal cavity. Deep confluent necrosis of the pharyngeal cavity extended to the epiglottis, larynx, and tonsils in all instances.

The results of serum neutralization studies in experiment 2 are contained in Table 5. Pseudorabies antibody was not detected in the serum of control pigs before challenge or on the day of their death. Both groups of pigs which received the BUK vaccine virus, whether by intramuscular or intranasal route, had similar antibody titers during the pre-challenge period. Neither the range nor the mean of the titers differed significantly at 7, 14 and 21 days after vaccination. After challenge, however, the pigs vaccinated intramuscularly responded with antibody titers consistently higher than those of the intranasally vaccinated group. The mean titers of the intramuscularly vaccinated group at 7, 14 and 21 days post-challenge were approximately 2-fold higher.

DISCUSSION

Except for the higher mortality, the disease produced in the 6 pigs of experiment 1 was equivalent to that produced in similar aged pigs by previous workers. Fever, anorexia, depression, aphonia, epileptiform convulsions, and coma were the common clinical signs recognized in naturally infected swine and in those where the disease was reproduced experimentally by intranasal inoculation (Fig. 1)

While a progressively severe localized pruritus is a constant sign in pseudorabies of cattle, it has seldom been reported as occurring in swine. In one published report, a pig rubbed its snout on a wall until the flesh was raw and bleeding, while another pig appeared to try to rub the sides if its mouth with its fore legs. In other reports, pigs were noticed to have scratched themselves frequently because of a skin irritability that lasted for about 2 weeks and one pig rubbed one of its ears raw. In the present study, after challenge with virulent virus, all of the control pigs in experiment 2 developed a severe localized pruritus as evidenced by their persistent rubbing and scratching of the left side of the face, particularly the eyelids. The restriction of pruritus to the left facial area was associated with an intranasal challenge inoculation which was placed only in the left nasal cavity. Two factors may have been responsible for occurrence of pruritus and corneal anesthesia in the control pigs of experiment 2 while these signs were absent in experiment 1. The higher virus content of the challenge inoculum in experiment 2 (10^7.48 TCID<sub>50</sub> versus 10^5.24 TCID<sub>50</sub>) may have overwhelmed the nasopharyngeal mucosa and hastened virus entry into a greater number of peripheral nerve endings. The sneezing and extensive necrosis noted in the nasal cavity of the control pigs in
experiment 2 support this viewpoint. The different positioning of the pigs in dorsal recumbency during challenge inoculation in experiment 2 may have allowed the virus more access to the maxillary branch of the trigeminal nerve. With the pigs restrained in this position, the challenge inoculum may also have passed through the lacrimal duct into the left conjunctival sac. Involvement of the maxillary rather than the ophthalmic branch of the trigeminal nerve was suggested because of the absence of pruritus in the area of the ears and top of the head. The anesthesia of the cornea may have resulted from local invasion of nerves in the conjunctival sac rather than by involvement of the ophthalmic nerve. The left-sided conjunctivitis and keratitis which occurred in the vaccinated pigs of experiment 2 indicates the probable presence of the virus in the conjunctival sac.

In experiment 1 where minimal post-inoculation damage to the nasopharyngeal mucosa resulted, virus was isolated from the spleen and kidney of only one pig and from one of 68 buffy coat samples tested. It would appear that, in those instances where the virus causes minimal epithelial damage, it replicates in surface cells and travels to the central nervous system by extension along nerve pathways with viremia being a transient occurrence. In a similar study where specific lesions were confined to the central nervous system, virus was isolated on only 2 occasions from a total of well over 500 blood samples tested. In experiment 2 where the control pigs had extensive post-challenge necrosis of the nasopharyngeal mucosa, virus was isolated from nearly all brain tissues of pigs dying 4 and 5 days post-challenge but in only half of those dying on the sixth day. Virus was isolated from the spleen or kidney of 5 of the 10 pigs in this group. It is likely that the presence of virus in spleen and kidneys was due to a viremia in which leukocytes attracted to the damaged nasopharyngeal mucosa were carrying the virus. It is less likely that virus in the spleen and kidneys was the result of neural spread from the central nervous system. These results are in accord with those of Hungarian workers who reported extensive nasopharyngeal lesions and 100% virus isolation rates from the spleen and kidneys but only 85% isolation rate from the brain of 12 pigs dead of the natural disease.

Inoculation of BUK vaccine virus, either by the intramuscular or intranasal route, did not elicit an ontoward reaction in 8-week old pigs as judged by the lack of febrile response and absence of recognizable clinical signs. The nearly equal 21-day post-vaccinal serologic response obtained with each route of inoculation was comparable to that obtained with the same virus in eastern Europe. The mild post-challenge response of both vaccinated groups indicated that previous exposure to BUK virus confers a degree of resistance to infection with virulent pseudorabies virus. The sneezing that occurred post-challenge in both vaccinated groups would suggest invasion of the nasal mucosa by the virulent virus. The post-challenge fever, anorexia, and depression noticed in the intramuscularly vaccinated pigs was evidence of extension of virulent virus beyond the nasopharynx but with minimal invasion of the nervous system. Previous exposure of both groups to BUK virus afforded protection against the neural damage which was characterized by pruritus and corneal anesthesia in the control pigs.
Although the lesions produced in the conjunctiva and cornea of the vaccinated pigs were of a mild and essentially transient nature, they do point out that pseudorabies virus may have a predilection for tissues of the eye as does *Herpes simplex* virus in man.\(^{17}\) Experimental pseudorabies infection in the pig could serve as a model for the study of herpetic kerato-conjunctivitis of man. Blindness without recognizable gross eye change has been reported in naturally occurring pseudorabies of swine.\(^{10,4,8}\) In those instances, however, blindness was probably due to retinal and optic nerve damage rather than to lesions of the anterior eye.

The more pronounced clinical signs and greater anamnestic antibody response after challenge indicated that intramuscular administration of BUK vaccine virus conferred protection inferior to that provided by intranasal inoculation of the same virus. Studies with parainfluenza-3 vaccine have shown that intranasally vaccinated calves were better protected from infection than were intramuscularly vaccinated animals.\(^{19}\)
# TABLE 1

Clinical Response of 8-week Old Pigs to Intranasal Inoculation with Virulent Pseudorabies Virus.

<table>
<thead>
<tr>
<th>1*</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>101.9**</td>
<td>104.8</td>
<td>106.1</td>
<td>105.0</td>
<td>103.6</td>
<td>100.1</td>
<td></td>
</tr>
<tr>
<td>6/6 Anorexia</td>
<td>6/6 Anorexia</td>
<td>6/6 Anorexia</td>
<td>5/5 Anorexia</td>
<td>5/5 Anorexia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/6 Depression</td>
<td>6/6 Depression</td>
<td>6/6 Depression</td>
<td>5/5 Depression</td>
<td>5/5 Depression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/6 Aphonie</td>
<td>5/5 Aphonie</td>
<td>5/5 Aphonie</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/6 Hypersensitive</td>
<td>1/6 Diarrhea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/5 Convulsions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Dead</td>
<td>3 Dead</td>
<td>2 Dead</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Days post-inoculation.
** Days post-inoculation.

Mean temperature response, degrees F.
### TABLE 2

Distribution of Pseudorabies Virus in Tissues Obtained at Necropsy from Experimentally Infected Pigs.

<table>
<thead>
<tr>
<th>Pig #</th>
<th>Days post-inoculation</th>
<th>Cerebrum</th>
<th>Cerebellum</th>
<th>Midbrain</th>
<th>Medulla</th>
<th>Tonsil</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>321</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>322</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>324</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>326</td>
<td>6</td>
<td>+</td>
<td>N.T.</td>
<td>N.T.</td>
<td>N.T.</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>323</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>325</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Pseudorabies virus isolated.
- = Isolation attempted but unsuccessful.
N.T. = Not tested.
Clinical Response of 11-week Old BUK-vaccinated and Control Pigs to Intranasal Challenge with Virulent Pseudorabies Virus.

<table>
<thead>
<tr>
<th>Control Group</th>
<th>Intra-muscular Vaccination BUK</th>
<th>Intranasal Vaccination BUK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>102.8**</td>
<td>105.8</td>
</tr>
<tr>
<td></td>
<td>9/10 Anorexia</td>
<td>10/10 Anorexia</td>
</tr>
<tr>
<td></td>
<td>10/10 Sneezing</td>
<td>5/10 Sneezing</td>
</tr>
<tr>
<td></td>
<td>10/10 Depression</td>
<td>10/10 Depression</td>
</tr>
<tr>
<td></td>
<td>9/10 Fruiritus</td>
<td>9/10 Fruiritus</td>
</tr>
<tr>
<td></td>
<td>1/10 Aphonis</td>
<td>1/10 Aphonis</td>
</tr>
<tr>
<td></td>
<td>1/10 Circling</td>
<td>2/10 Circling</td>
</tr>
<tr>
<td></td>
<td>1/10 Diarrhea</td>
<td>1/10 Diarrhea</td>
</tr>
<tr>
<td></td>
<td>1/10 Convulsions</td>
<td>2/9 Convulsions</td>
</tr>
<tr>
<td></td>
<td>9/10 No corneal reflex</td>
<td>9/10 No corneal reflex</td>
</tr>
<tr>
<td></td>
<td>1 Dead</td>
<td>4 Dead</td>
</tr>
<tr>
<td></td>
<td>4 Dead</td>
<td>5 Dead</td>
</tr>
<tr>
<td></td>
<td>104.5**</td>
<td>104.2</td>
</tr>
<tr>
<td></td>
<td>4/4 Anorexia</td>
<td>4/4 Depression</td>
</tr>
<tr>
<td></td>
<td>2/10 Sneezing</td>
<td>2/10 Sneezing</td>
</tr>
<tr>
<td></td>
<td>9/9 Depression</td>
<td>9/9 Depression</td>
</tr>
<tr>
<td></td>
<td>1/9 Fruiritus</td>
<td>1/9 Fruiritus</td>
</tr>
<tr>
<td></td>
<td>9/9 Aphonis</td>
<td>9/9 Aphonis</td>
</tr>
<tr>
<td></td>
<td>1/9 Circling</td>
<td>1/9 Circling</td>
</tr>
<tr>
<td></td>
<td>1/9 Diarrhea</td>
<td>1/9 Diarrhea</td>
</tr>
<tr>
<td></td>
<td>2/9 Convulsions</td>
<td>2/9 Convulsions</td>
</tr>
<tr>
<td></td>
<td>9/9 No corneal reflex</td>
<td>9/9 No corneal reflex</td>
</tr>
<tr>
<td></td>
<td>4/4 No corneal reflex</td>
<td>4/4 No corneal reflex</td>
</tr>
<tr>
<td></td>
<td>1 Dead</td>
<td>4 Dead</td>
</tr>
<tr>
<td></td>
<td>4 Dead</td>
<td>5 Dead</td>
</tr>
<tr>
<td></td>
<td>104.2**</td>
<td>104.0</td>
</tr>
<tr>
<td></td>
<td>4/4 Anorexia</td>
<td>4/4 Depression</td>
</tr>
<tr>
<td></td>
<td>2/10 Sneezing</td>
<td>2/10 Sneezing</td>
</tr>
<tr>
<td></td>
<td>9/9 Depression</td>
<td>9/9 Depression</td>
</tr>
<tr>
<td></td>
<td>1/9 Fruiritus</td>
<td>1/9 Fruiritus</td>
</tr>
<tr>
<td></td>
<td>9/9 Aphonis</td>
<td>9/9 Aphonis</td>
</tr>
<tr>
<td></td>
<td>1/9 Circling</td>
<td>1/9 Circling</td>
</tr>
<tr>
<td></td>
<td>1/9 Diarrhea</td>
<td>1/9 Diarrhea</td>
</tr>
<tr>
<td></td>
<td>2/9 Convulsions</td>
<td>2/9 Convulsions</td>
</tr>
<tr>
<td></td>
<td>9/9 No corneal reflex</td>
<td>9/9 No corneal reflex</td>
</tr>
<tr>
<td></td>
<td>4/4 No corneal reflex</td>
<td>4/4 No corneal reflex</td>
</tr>
<tr>
<td></td>
<td>1 Dead</td>
<td>4 Dead</td>
</tr>
<tr>
<td></td>
<td>4 Dead</td>
<td>5 Dead</td>
</tr>
<tr>
<td></td>
<td>103.2**</td>
<td>102.7</td>
</tr>
<tr>
<td></td>
<td>10/10 Depression</td>
<td>7/10 Diarrhea</td>
</tr>
<tr>
<td></td>
<td>4/10 Diarrhea</td>
<td>3/10 Conunctivitis</td>
</tr>
<tr>
<td></td>
<td>4/10 Convulsions</td>
<td>4/10 Conunctivitis</td>
</tr>
<tr>
<td></td>
<td>110 Keratitis</td>
<td>110 Keratitis</td>
</tr>
<tr>
<td></td>
<td>102.7**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/4 Anorexia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/10 Sneezing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 Depression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Fruiritus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 Aphonis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Circling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Diarrhea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/9 Convulsions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 No corneal reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/4 No corneal reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 Dead</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 Dead</td>
<td></td>
</tr>
<tr>
<td></td>
<td>103.2**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10/10 Depression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/10 Diarrhea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/10 Convulsions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>110 Keratitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>102.7**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/4 Anorexia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/10 Sneezing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 Depression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Fruiritus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 Aphonis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Circling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Diarrhea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/9 Convulsions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 No corneal reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/4 No corneal reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 Dead</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 Dead</td>
<td></td>
</tr>
<tr>
<td></td>
<td>103.2**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10/10 Depression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/10 Diarrhea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/10 Convulsions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>110 Keratitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>102.7**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/4 Anorexia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/10 Sneezing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 Depression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Fruiritus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 Aphonis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Circling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Diarrhea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/9 Convulsions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 No corneal reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/4 No corneal reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 Dead</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 Dead</td>
<td></td>
</tr>
<tr>
<td></td>
<td>103.2**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10/10 Depression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/10 Diarrhea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/10 Convulsions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>110 Keratitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>102.7**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/4 Anorexia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/10 Sneezing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 Depression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Fruiritus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 Aphonis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Circling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Diarrhea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/9 Convulsions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 No corneal reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/4 No corneal reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 Dead</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 Dead</td>
<td></td>
</tr>
<tr>
<td></td>
<td>103.2**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10/10 Depression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/10 Diarrhea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/10 Convulsions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>110 Keratitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>102.7**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/4 Anorexia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/10 Sneezing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 Depression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Fruiritus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 Aphonis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Circling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/9 Diarrhea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/9 Convulsions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/9 No corneal reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/4 No corneal reflex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 Dead</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 Dead</td>
<td></td>
</tr>
</tbody>
</table>

*Days post-challenge.
**Mean temperature response, degrees F.
TABLE 4
Distribution of Pseudorabies Virus in Tissues Obtained at Necropsy from Control Pigs in Vaccination Challenge Experiment.

<table>
<thead>
<tr>
<th>Pig #</th>
<th>Days post-challenge</th>
<th>Cerebrum</th>
<th>Cerebellum</th>
<th>Midbrain</th>
<th>Medulla</th>
<th>Tonsil</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>996</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>998</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>999</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>995</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>994</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>993</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1000</td>
<td>6</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>992</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>997</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>991</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Pseudorabies virus isolated.
- = Isolation attempted but unsuccessful.
TABLE 5

Serum Neutralization Titers of Pigs Vaccinated with Modified BUK Strain of Pseudorabies Virus and Challenged with Virulent Pseudorabies Virus.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Strain of Virus</th>
<th>Route of Inoc.</th>
<th>Antibody Titers* at Day after Vaccination</th>
<th>Antibody Titers* at Day after Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>121</td>
<td>BUK</td>
<td>I.M.</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>122</td>
<td>BUK</td>
<td>I.M.</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>123</td>
<td>BUK</td>
<td>I.M.</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>124</td>
<td>BUK</td>
<td>I.M.</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>125</td>
<td>BUK</td>
<td>I.M.</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>126</td>
<td>BUK</td>
<td>I.M.</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>127</td>
<td>BUK</td>
<td>I.M.</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>128</td>
<td>BUK</td>
<td>I.M.</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>129</td>
<td>BUK</td>
<td>I.M.</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>130</td>
<td>BUK</td>
<td>I.M.</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
</tbody>
</table>

| 142     | BUK             | I.N.           | Neg. | Neg. | 4  | 8  | 8  | 8  | 8  |
| 143     | BUK             | I.N.           | Neg. | Neg. | 4  | 8  | 16 | 16 | 16 |
| 144     | BUK             | I.N.           | Neg. | Neg. | 2  | 8  | 128 | 64 | 32 |
| 145     | BUK             | I.N.           | Neg. | Neg. | 4  | 8  | 128 | 64 | 64 |
| 146     | BUK             | I.N.           | Neg. | Neg. | 4  | 8  | 32 | 32 | 16 |
| 147     | BUK             | I.N.           | Neg. | Neg. | 2  | 8  | 64 | 128 | 64 |
| 148     | BUK             | I.N.           | Neg. | Neg. | 2  | 4  | 64 | 64 | 32 |
| 149     | BUK             | I.N.           | Neg. | Neg. | 2  | 8  | 64 | 64 | 64 |
| 150     | BUK             | I.N.           | Neg. | Neg. | 2  | 8  | 64 | 64 | 64 |
| 151     | BUK             | I.N.           | Neg. | Neg. | 2  | 8  | 128 | 128 | 64 |
| Mean    |                 |                | Neg. | Neg. | 3  | 8  | 70 | 63 | 42 |

10 control pigs Neg. Neg. Neg. Neg.  ...  ...  ...  

*Reciprocal of the serum dilution neutralizing 100 TCID50 of virus.
**All 10 control pigs dead before 7th day post-challenge.

I.M. = Intramuscular
I.N. = Intranasal
Figure 1 – Post-challenge rectal temperature response of pigs inoculated with virulent pseudorabies virus 21 days after vaccination with BUK virus. Each point is the average response of pigs alive on that day.
REFERENCES

INt. D. H. Ferris  
Department of Veterinary Pathology and Hygiene,  
College of Veterinary Medicine  
University of Illinois at Champaign-Urbana

Statements similar to the following have been quoted or expressed frequently in regard to TGE: "Transmissible gastroenteritis probably is not the most important cause of baby pig mortality, but in individual herds it has caused losses as high as 80 to 100 per cent of the pigs farrowed." Writing after this, early in the decade of the 1950's, Bay found the highest incidence of TGE during the Spring farrowing season in March. Both he and Doyle expressed the opinion that over the past decade or more, TGE had increased in incidence and severity and had changed from a sporadic to a widespread invader. Haelterman in his classic paper on the epidemiology of TGE reported that over 90 per cent of the TGE outbreaks in Indiana which he observed occurred between late December and April with a peak in March. The mortality observed by these and other pioneer workers, of 90 to 100 per cent in pigs under one week of age, has been widely accepted.

Veterinarians and extension workers have expressed a variety of opinions in regard to nearly every aspect of the epizootiology and economic impact of TGE. Recently one veterinary practitioner with a large clientele among pork producers wrote that he had observed a "new" TGE with a lower mortality in baby pigs than the "old" form, which he sometimes saw concurrently on different farms. Most epidemic diseases have exhibited long term variations in incidence, mortality, morbidity and related characteristics. The observations of workers in TGE two decades ago indicated that this is probably true of this disease also, but, unfortunately, no long term investigations were carried out.

This investigation was started two years ago in an effort to collect data on the incidence of overt TGE and relate this to other epidemiologic factors, such as weather, temperature, precipitation and management. Leman has well documented the need for the continuation of epidemiologic investigations of TGE.

MATERIALS AND METHODS

The materials and methods used in this research have been published in detail elsewhere. It should be emphasized that efforts were made in this study to obtain reports on all diseases causing gastroenteritis in swine herds as well as all diseases resulting in a high mortality in baby pigs. Only those reports having to do with TGE are discussed in this paper. The determination of what constitutes TGE is important. All outbreaks included in this report were diagnosed as TGE by veterinarians employing the classic criteria recommended by Doyle, Doyle and Hutchings, Haelterman as well as criteria on differential diagnosis.
tests were employed in a few instances, as well as viral isolations. As will be noted later, the classic and differential diagnostic criteria are often adequate to diagnose epizootic TGE. Any objection to their use must also be applied to the classic work done previously without the benefit of serologic or viral identification procedures. Certain aspects of TGE at present cannot be associated with any other disease.

RESULTS AND DISCUSSION

Tables 1 and 2 summarize morbidity and mortality data on TGE reported from 297 outbreaks in 18 states and Canada during the winter of 1967-70. Table 1 has data on baby pigs under 10 days of age as well as their dams. Morbidity in this study was defined as those baby pigs which were sick, but survived, since all that died also were morbid. Baby pig morbidity varied from less than 1% to 60%, the average for 17,867 survivors out of a total of 59,439 baby pigs farrowed was 30%. Mortality in this cohort varied from 40% to 100%; the average for the season was 63%. It should be noted in this connection that some very large and modern pork production operations suffered baby pig losses at or near 100%. Two farms in Michigan, for example, reported a total loss of 3,200 baby pigs from 400 sows, all of which were sick.

Transmissible gastroenteritis in the das and other swine on a farm is of importance in differentiating TGE from colibacillosis and other diseases. From 43% to 100% of the dams involved were sick, with an average of 75%. Figure 1 shows the geographic distribution of the TGE outbreaks summarized in Table 1. Table 2 summarizes the incidence of TGE in older swine during the same period of observation as Table 1. The majority of these animals were on the same farms as the baby pigs the losses of which were noted in the preceding table. However, as Figure 2 shows, some farms had no baby pigs, but suffered TGE in larger animals. Morbidity in these larger swine tended to be considerably higher than in the baby pigs and their dams; the morbidity varied from 56% to 100%, with an average of 92%. This morbidity over such a wide geographical area shows a remarkable uniformity, regardless of the size of swine population. For example, 92% of 66 swine on a Canadian farm and 87% of 61,906 swine on scores of Illinois farms were sick with TGE during this epizootic.

Intensive work has been done on the epizootiology of TGE in Illinois which is borne out by the larger amount of data compiled. During the period of September, 1968 to August 1969, 211 swine gastroenteritis outbreaks were diagnosed by veterinarians as TGE. These producers lost 41% of 54,315 baby pigs with a morbidity of 56%. The ages of these baby pigs were not carefully defined in the initial work, which may account for the lower average mortality. Some reports may have included pigs beyond 10 days of age. This defect in the reporting system was corrected. During this same period a year later, 1969-70, reports of 202 outbreaks were similarly diagnosed. Losses were somewhat higher with 55% of 41,474 baby pigs dying while morbidity in survivors was 35%. The 8,549 sows which farrowed the pigs had a morbidity of 74%. A still larger number of TGE outbreaks in larger
swine was reported the second year; 70,978 gilts, boars, shoats, older pigs and other sows suffered a morbidity of 87%.

Geographic and secular correlations of outbreaks with environmental and management factors are underway. Figures 3 and 4 show the geographic distribution of TGE outbreaks in Illinois in 1968-69. These may be compared with Figures 5 and 6 which show the distribution of farms and breeding swine in the state. As might be expected, the largest number of reports came from those counties with the largest number of swine. However, there was not complete correlation between the number of swine and outbreaks. The initial outbreaks reported the first year occurred simultaneously in September in a county of high swine density in the north and in one of low swine density in the south. In October and November a few other outbreaks took place in counties with low swine density. In December scattered outbreaks around the state took place simultaneously over a wide geographic area with no correlation between swine population and the number of outbreaks. Focal groupings of outbreaks did not appear until January and February. So far, it has not been possible to relate these foci to any definite factors such as common sources of feed, movement of trucks between farms, sale of animals and similar ones. Many of the reporting producers were veterinarians or individuals with a degree in agriculture. Most of the producers were well informed regarding swine management and practiced a high state of sanitation. There were some specific pathogen free (SPF) swine operations included and many which practiced strict isolation procedures, such as not allowing trucks on their farm, disinfection their truck when it returned, requiring all personnel to change clothing and boots when attending to swine, and restricting access of other persons to their swine. Many producers raised their own food.

A good example of TGE attacking swine under superb management is that of our own swine herd at the University of Illinois. Champaign county and our general region have few swine. The isolation facilities of our swine are located in the midst of what is now suburban housing developments and thus isolated from any private swine herds. The buildings are well designed for isolation, with disinfectant bootbaths and control devices regulating flow of air, humidity and temperature. Research on TGE is carried on here and our ambulatory and extension veterinarians are unusually well informed on swine diseases. Nevertheless, TGE has been diagnosed in the herd for the past three years and losses were substantial.

TGE appeared in the above herd in January at the same time the largest incidence was reported. Figure 7 summarized the distribution in time of 243 outbreaks during the year from September, 1968 to September, 1969. Plotted on the graph are also the average monthly farrowings (in thousands) for the past 10 years. It will be noted that the low period in Illinois farrowings has been from October to February. The peak of the state-wide TGE epizootic took place in the first week in January, 1969. Figure 8 shows a similar peak the following year. This figure was prepared from reports received to the middle of February, but when all reports were in (254), the peak remained at this same time. Statistically, the similarity of the two peaks was significant at the 0.01 level.
This investigation supports the observations of the original investigators that the majority of outbreaks occur between November and April, but not that the peak occurs in March. So far as we know, this is the first report of such a peak; veterinarians in the state who have worked with TGE for several years were previously of the opinion that the peak coincided with Spring farrowing in March. Whether there is a similar peak elsewhere needs to be determined. This concentration of outbreaks may indicate that factors other than population density are important in the initiation and spread of farm epizootics.

Only about half of the outbreaks were characterized by the traditional losses of 90% to 100% of baby pigs under 10 days of age: 45% in the first year and 48% in the second reported such losses. Various hypotheses may be formulated to explain the lower mortality in the other half of the producers: variation in viral strains, variation in dosage, variations in the immunizing and protective potential of colostrum and milk of dams are some which come to mind.

Although baby pigs and their sows are highly susceptible, as shown by the morbidity and mortality data, there were not found to be the first animals on a farm to come down. Only 4 respondents reported TGE first in baby pigs in 1968-69; fat hogs were first to show signs in most instances, followed by gilts and pregnant sows.

It is obvious from the statistics in this report and previous ones that it will probably be difficult to evaluate vaccines and controlled infection of pregnant sows. Regardless of the type of management and prophylaxis employed, TGE seldom struck the same farm twice in consecutive years. A very large majority of the nearly 500 producers who reported in Illinois reported that this was their first outbreak of TGE. Less than 10% reported TGE in any two previous consecutive years. About 20% reported TGE from two to four times in a 10 year period.

As stated earlier, most, if not all, epidemic diseases exhibit natural secular trends in incidence, severity of illness, mortality (if characteristic) and other parameters. We know almost nothing of these swings in TGE. This study supports, in a small way, at least, the judgement of the first observers that TGE had the potential for such changes. We do not know what the effects may be of greatly increasing the amount of virulent TGE virus in the environment at times other than the natural increases, through controlled infection and possibly by live vaccines. It would appear highly desirable to continue to monitor TGE on as wide a scale as possible. It is highly probably that we may expect changes in the nature of the host-parasite relationship and we should be ready to measure them. Final judgement on the effectiveness of control measures will take time.

ACKNOWLEDGEMENTS

Acknowledgement is made of the generous support by Dr. Paul Doby, Superintendent of the State Division of Livestock Industry, Paul R. Schnurrenberger, Assistant Epidemiologist, State of Illinois, Mr. Charles Bloomberg, Executive Vice-President, Illinois Pork Producers Association, Mr. C. R. Mitchell,
OVERT TGE OF SWINE

Editor, the National Hog Farmer, county Extension advisers, veterinarians of Illinois, Iowa and other states and especially to Drs. Al Leman, J. R. Pickard, H. N. Becker and M. Ristic of the College of Veterinary Medicine.

SUMMARY

Overt transmissible gastroenteritis (TGE) of swine was studied in Illinois through volunteer reporting by veterinarians and pork producers from 1968 to 1970. From 1969 to 1970 other states were included. During the first year, 243 reports from Illinois were analyzed and during the second year, 254 reports from Illinois plus 96 reports from 17 other states and Canada. Baby pig losses in Illinois reported the first year were 41% of 54,315; morbidity in survivors was 56%. Losses were still higher this past winter; 63% of 59,439 baby pigs were lost with 30% morbidity in survivors. An even higher proportion of dams and larger swine were sick. Most of the respondents both years had their first experience with TGE. Very few had TGE in their swine on any tow consecutive years but many reported having from two to four TGE episodes during a 10 year period. The peak of the epizootic occurred early in January both the first year of the study and the second, in Illinois, where adequate reports were received.

"This investigation was supported by General Research Support Grant RR-05460 from the General Research Support Branch, Division of Research Resources, National Institutes of Health."
Figure 1. Incidence of TGE in Baby Pigs by States. (See Table 1)
Figure 2. Incidence of TGE in Older Swine by States. (See Table 2)
Figure 3. Incidence of TGE in Baby Pigs by Counties in Illinois, 1968-69.
Figure 4. Incidence of TGE in Older Swine by Counties in Illinois, 1968-69.
Figure 5. Distribution of Pork Producing Farms in Illinois by Counties, 1968.
Figure 6. Distribution of Swine Breeding Stock by Counties in Illinois, 1968.
Figure 7. Incidence of TGE Outbreaks by Months in Illinois, 1968-69. The solid line indicates the trend of the TGE epizootic; numbers identify the number of outbreaks during the month. The dash line indicated farrowing practice in the state. Smaller numbers identify number of farrowings (in thousands) each month; these are averages of the past 10 farrowings during the month indicated.
Figure 8. Incidence of TGE by Weeks in Illinois, Winter of 1969-70, until February. Graph based upon slightly more than half of the reports received. Peak remained when all data were in.
<table>
<thead>
<tr>
<th>STATE</th>
<th>Total No. Baby Pigs</th>
<th>No. Sick, %</th>
<th>No. Died, %</th>
<th>No. Dams, %</th>
<th>No. Sick, %</th>
<th>No. Farms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas</td>
<td>435</td>
<td>20, 5%</td>
<td>386, 89%</td>
<td>80, 6%</td>
<td>50, 63%</td>
<td>2</td>
</tr>
<tr>
<td>N. &amp; S. Dakota</td>
<td>551</td>
<td>255, 47%</td>
<td>276, 88%</td>
<td>96, 6%</td>
<td>96, 100%</td>
<td>3</td>
</tr>
<tr>
<td>Delaware</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Georgia</td>
<td>140</td>
<td>37, 26%</td>
<td>103, 74%</td>
<td>56, 24%</td>
<td>24, 43%</td>
<td>1</td>
</tr>
<tr>
<td>Illinois</td>
<td>41,474</td>
<td>14,536, 35%</td>
<td>23,148, 55%</td>
<td>8,549, 74%</td>
<td>6,378, 74%</td>
<td>202</td>
</tr>
<tr>
<td>Indiana</td>
<td>1,482</td>
<td>290, 20%</td>
<td>1,192, 80%</td>
<td>468, 65%</td>
<td>304, 65%</td>
<td>12</td>
</tr>
<tr>
<td>Iowa</td>
<td>6,956</td>
<td>1,985, 29%</td>
<td>4,959, 71%</td>
<td>1,351, 84%</td>
<td>1,132, 84%</td>
<td>40</td>
</tr>
<tr>
<td>Kansas</td>
<td>160</td>
<td></td>
<td>160, 100%</td>
<td>60, 100%</td>
<td>60, 100%</td>
<td>1</td>
</tr>
<tr>
<td>Kentucky</td>
<td>320</td>
<td>1, 0.03%</td>
<td>319, 99.7%</td>
<td>90, 47%</td>
<td>42, 47%</td>
<td>2</td>
</tr>
<tr>
<td>Michigan</td>
<td>3,200</td>
<td>3,200, 100%</td>
<td>200, 100%</td>
<td>400, 100%</td>
<td>400, 100%</td>
<td>2</td>
</tr>
<tr>
<td>Minnesota</td>
<td>658</td>
<td>231, 20%</td>
<td>477, 72%</td>
<td>362, 79%</td>
<td>287, 79%</td>
<td>8</td>
</tr>
<tr>
<td>Missouri</td>
<td>2,613</td>
<td>168, 6%</td>
<td>2,445, 94%</td>
<td>649, 67%</td>
<td>433, 67%</td>
<td>11</td>
</tr>
<tr>
<td>Montana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohio</td>
<td>655</td>
<td>147, 22%</td>
<td>508, 78%</td>
<td>163, 72%</td>
<td>118, 72%</td>
<td>8</td>
</tr>
<tr>
<td>Texas</td>
<td>350</td>
<td>210, 60%</td>
<td>140, 40%</td>
<td>38, 35%</td>
<td>13, 35%</td>
<td>1</td>
</tr>
<tr>
<td>Virginia</td>
<td>45</td>
<td>8, 18%</td>
<td>37, 82%</td>
<td>75, 93%</td>
<td>70, 93%</td>
<td>1</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>290</td>
<td>55, 19%</td>
<td>235, 81%</td>
<td>157, 62%</td>
<td>97, 62%</td>
<td>1</td>
</tr>
<tr>
<td>Canada</td>
<td>110</td>
<td>24, 21%</td>
<td>86, 78%</td>
<td>11, 100%</td>
<td>11, 100%</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>59,439</td>
<td>17,867, 30%</td>
<td>37,585, 63%</td>
<td>12,633, 75%</td>
<td>9,533, 75%</td>
<td>297</td>
</tr>
</tbody>
</table>
## Table 2
TGE Reported in Swine Other Than Baby Pigs and Their Dams

<table>
<thead>
<tr>
<th>State</th>
<th>Class of Swine</th>
<th>No. Sick</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas</td>
<td>Gilts</td>
<td>130</td>
<td>205</td>
</tr>
<tr>
<td>N. &amp; S. Dakota</td>
<td>Boars</td>
<td>5</td>
<td>103</td>
</tr>
<tr>
<td>Delaware</td>
<td></td>
<td>90</td>
<td>600</td>
</tr>
<tr>
<td>Georgia</td>
<td></td>
<td>180</td>
<td>87</td>
</tr>
<tr>
<td>Indiana</td>
<td></td>
<td>601</td>
<td>2,778</td>
</tr>
<tr>
<td>Illinois</td>
<td></td>
<td>4,912</td>
<td>35,273</td>
</tr>
<tr>
<td>Iowa</td>
<td></td>
<td>402</td>
<td>2,058</td>
</tr>
<tr>
<td>Kansas</td>
<td></td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Kentucky</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Michigan</td>
<td></td>
<td>16</td>
<td>2,100</td>
</tr>
<tr>
<td>Minnesota</td>
<td></td>
<td>9</td>
<td>1,025</td>
</tr>
<tr>
<td>Missouri</td>
<td></td>
<td>50</td>
<td>3,046</td>
</tr>
<tr>
<td>Montana</td>
<td></td>
<td>2</td>
<td>81</td>
</tr>
<tr>
<td>Ohio</td>
<td></td>
<td>19</td>
<td>1,323</td>
</tr>
<tr>
<td>Virginia</td>
<td></td>
<td>350</td>
<td>350</td>
</tr>
<tr>
<td>Wisconsin</td>
<td></td>
<td>26</td>
<td>683</td>
</tr>
<tr>
<td>Canada</td>
<td></td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>2,112</td>
<td>523</td>
</tr>
</tbody>
</table>
REFERENCES

REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE

D. P. Gustafson, Lafayette, Indiana, Chairman
(*absent members)

The Committee gave attention to five important diseases of swine; trichinosis, pseudorabies, streptococci lymphadenitis, "vibrionic dysentery," and transmissible gastroenteritis.

In a progress report on a proposed Swine Trichinosis Eradication Program it was reported that the program has been favorably reviewed by the U.S. Department of Agriculture which is currently studying possible methods of implementation.

A film on the Trichinosis Pilot Project at Fort Dodge was shown. New methods of technique improvement and possible cost reductions were outlined. Lively discussion brought out that implementation of this program would:
1. Protect the consumer from the possibility of trichina infection.
2. Improve the image of pork and thus stimulate domestic consumption.
3. Decrease industry pork production costs by removing costly trichina control procedures.
4. Remove a major discriminatory barrier to U.S. pork in foreign trade. Reports were received on current trichina incidence in the human and swine populations. It was reported that, to date, no trichina infected swine have been found in a pooled sample technique survey of over 7,000 swine from approximately 2,000 premises in Wisconsin.

Continued interest in a Trichinosis Eradication Program was expressed by representatives of swine producers and industry groups. Officials of Livestock Conservation Incorporated, reported on plans to implement a swine identification project in cooperation with the U.S. Department of Agriculture.

Your committee recommends the formation of a subcommittee or task force to stimulate and coordinate activity in the field of Trichinosis Eradication.

A workable swine identification system is vital to the success of a Trichinosis Eradication Program. Therefore, your committee urges that an Animal Identification Committee be established within USAHA to foster this objective.

A resolution in support of a Trichinosis Eradication Program has been prepared by this committee and submitted to the Executive Committee for consideration.

The disease known as "vibrionic dysentery" was the subject of discussion again this year following the publication of the results of a survey in the proceedings of the 73rd Annual Meeting. It continues to be a major problem in at least all of the
swine producing states in the midwestern area. Two research groups have developed limited programs to seek the cause of this very costly disease and subsequently to find means of suppressing or elimination it as an important factor in livestock losses.

In recognition of the broad importance of "vibrionic dysentery" the Committee submits a resolution urging the U.S.D.A. to develop support for research necessary to provide the basis for control and prevention measures.

The Sub-Committee on Transmissible Gastroenteritis (TGE—has entered a report which is submitted as a part of the report of this Committee. In addition a report of this Committee was presented concerning a study of some epidemiological aspects of TGE between 1968 and 1970. It appears in the Proceedings elsewhere as presented to the Committee.

Pseudorabies infection is likely to be a continuing problem in herds composed of swine from many sources. Epizootics have been observed in systems in which there is a continuous removal of some swine for sale followed by the addition of new individuals for fattening. Epizootics are likely to continue as long as the practice survives. A study was presented, (the text appears elsewhere as a separate manuscript in the Proceedings of the Annual Meeting) comparing the syndromes in pigs exposed to virulent and to artifically modified strains of pseudorabies virus (PRV) Exposure to modified virus intramuscularly resulted in no observable signs of the disease. Subsequent exposure to virulent PRV resulted in signs of the disease which were mild and all survived. Suggestion was made that the modified virus might be of value in the measure of alternatives in these rather specialized circumstances. It was not and is not recommended as a proper course in other production systems. The disease continues to be a sporadic one causing losses among cattle, sheep, dogs, and cats that become infected through various types of relationships with asymptomatic swine which shed the virus or with swine that are sickened by the infection. Virus isolates with marked virulence for swine have been found in widely separated sections of the United States suggesting that losses from this disease will continue.

The Committee heard a report on the economic losses due to streptococci lymphadenitis based on a survey of condemnations and trimming losses of swine heads in five packing houses. The total loss to the entire industry was extrapolated from the data obtained by a rate of loss related to the total number of swine killed in USDA inspected plants in the United States in the calendar year of 1970. The cost in dollars is based on the value of various parts of the head to the packer and is not the retail value nor necessarily the value to the swine producer. Consequently it is rather clearly a conservative dollar loss in total. It does not include losses caused by lymphadenitis caused condemnations of other carcass parts or total carcass condemnation losses due to pyemia which may or may not be related to streptococci lymphadenitis.

The Committee commends the continued support of efforts to develop information which is the basis for control measures of management and immunization.
STREPTOCOCCIC LYMPHADENITIS OF SWINE AND ITS ECONOMIC SIGNIFICANCE

DR. M. J. BARTA

DATA FROM FIVE FEDERALLY INSPECTED SWINE SLAUGHTERING ESTABLISHMENTS
WHAT IS THE VALUE OF A SWINE HEAD?

<table>
<thead>
<tr>
<th>Item</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>.02</td>
</tr>
<tr>
<td>Cheek Meat</td>
<td>.35</td>
</tr>
<tr>
<td>Head Meat</td>
<td>.15</td>
</tr>
<tr>
<td>Ears</td>
<td>.06</td>
</tr>
<tr>
<td>Lips</td>
<td>.02</td>
</tr>
<tr>
<td>Snout</td>
<td>.09</td>
</tr>
<tr>
<td>Tongue</td>
<td>.10</td>
</tr>
<tr>
<td>Jowls</td>
<td>.22</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$1.01</strong></td>
</tr>
</tbody>
</table>

No significant variation throughout year

Information courtesy of M.G., Swift & Co., Chicago
TRANSMISSIBLE DISEASES OF SWINE

<table>
<thead>
<tr>
<th>Month</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
</tr>
</thead>
<tbody>
<tr>
<td>110 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>86 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>76 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No. Sold: 745,595
Hogs. Cond: 9,395 1.26% Value
Hogs. Traded: 4,130 0.55%

EST. A
**No. 5110** 182,566

**Hrs. Cond.** 4,926 2.69% Value $4,926

**Hrs. TAMD.** 1,284 .70%

<table>
<thead>
<tr>
<th></th>
<th>JAN</th>
<th>FEB</th>
<th>MAR</th>
<th>APR</th>
<th>MAY</th>
<th>JUN</th>
<th>JUL</th>
<th>AUG</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 M</td>
<td>3745</td>
<td>2745</td>
<td>2752</td>
<td>2731</td>
<td>2296</td>
<td>1303</td>
<td>17400</td>
<td>23251</td>
</tr>
<tr>
<td>30 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>900</td>
<td>2917</td>
<td>514</td>
<td>713</td>
<td>741</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>700</td>
<td>2.39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>600</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>393</td>
<td>1.61</td>
<td>2.58</td>
<td>3.31</td>
<td>4.40</td>
<td>4.03</td>
<td>2.74</td>
<td>3.13</td>
</tr>
<tr>
<td>400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>1.02</td>
<td>.28</td>
<td>.78</td>
<td>.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>.31</td>
<td>.56</td>
<td>.91</td>
<td>.57</td>
<td>.42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*EST. B*
<table>
<thead>
<tr>
<th>Month</th>
<th>1974</th>
<th>1975</th>
<th>1976</th>
<th>1977</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jun</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jul</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aug</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dec</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ESTC**

No. Sin. 426,381

Hog. Cond. 15,383

24.0% Value #15,383
No. Sh. 227,595
Hos. Cond. 8,159 3.58% Value $8,159
Hos. Trmd. 2,349 1.03%
EST. "E"

No. Sla. 342,509
Hds. Cond. 8480 2.47 % Value $8480
Hds. TMD. *

* This establishment elected not to trim heads.
For economic reasons, therefore, all affected heads were, in effect, condemned.
Seasonal Fluctuation of Involvement

- Extensive Involvement
- Slight Involvement
**ECONOMIC STATISTICS**

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine Slaughtered At Plants</td>
<td>1,582,137</td>
</tr>
<tr>
<td>A, B, C &amp; D - Jan to Aug, '70</td>
<td></td>
</tr>
<tr>
<td>Heads Condemned At Plants</td>
<td>37,863</td>
</tr>
<tr>
<td>A, B, C &amp; D - Jan to Aug, '70</td>
<td></td>
</tr>
<tr>
<td>Per Cent Condemnation</td>
<td>2.39</td>
</tr>
<tr>
<td>A, B, C &amp; D - Jan to Aug, '70</td>
<td></td>
</tr>
<tr>
<td>Total Swine Slaughtered In All F.I. Ests. - Jan-Aug, '70</td>
<td>48,150,602</td>
</tr>
<tr>
<td>Anticipated Number Of Heads Condensed At 2.39% Rate</td>
<td>1,150,800</td>
</tr>
<tr>
<td>Estimated Value Of Heads At $1.00 per Head</td>
<td>1,150,800</td>
</tr>
<tr>
<td>Value Projection For CY-70 Based On Jan-Aug Figure</td>
<td>1,726,200</td>
</tr>
</tbody>
</table>
**ALTERNATE CONSIDERATION**

Assume that all slaughterers decline salvage of heads, slightly involved, for economic reasons:

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine slaughtered at plants A, B, C, D, E; Jan-Aug, 1970</td>
<td>1,924,646</td>
</tr>
<tr>
<td>Heads involved at plants A, B, C, D, E; Jan-Aug, 1970</td>
<td>65,129</td>
</tr>
<tr>
<td>Percent involvement, plants A, B, C, D, E; Jan-Aug, 1970</td>
<td>3.39%</td>
</tr>
<tr>
<td>Total swine slaughtered in all P.I. Ests.; Jan-Aug, 1970</td>
<td>48,150,602</td>
</tr>
<tr>
<td>Anticipated involvement at 3.39% rate</td>
<td>1,632,300</td>
</tr>
<tr>
<td>Estimated value of involved heads at $1.00; Jan-Aug</td>
<td>$1,632,300</td>
</tr>
<tr>
<td>Value projection for CY-70 based on Jan-Aug value</td>
<td>$2,448,450</td>
</tr>
</tbody>
</table>
A special license for a TGE vaccine was issued to Diamond Laboratories, Inc., Des Moines, Iowa, on August 13, 1970. The vaccine is composed of live, modified virus propagated in procine cell cultures, and is to be administered to pregnant swine by two intramuscular injections at approximately six and two weeks before expected farrowing, for the purpose of providing passive immunity to suckling pigs. Three qualifications associated with the issuance of this license were: (1) the vaccine is to be sold only to veterinarians, (2) sales are to be made only in states where State livestock health authorities have given official authorization, and (3) the term of this license is for one year during which time Diamond Laboratories must investigate the efficacy of the vaccine under field conditions. Tests on this vaccine conducted by the Division of Veterinary Biologics (ARS) resulted in a mortality of 38% in pigs nursing vaccinated sows in contrast to 71% in pigs nursing non-vaccinated sows, following challenge of 3-day-old pigs with virulent TGE virus. Present evidence indicates that this type of TGE vaccine (live, cell-cultured modified virus injected by the intramuscular route) as used on pregnant swine is of limited value in preventing infection and clinical signs of TGE in suckling pigs, but that it will tend to reduce the high mortality normally associated with the occurrence of the disease in very young pigs. Thus, the temporary licensing of such a product appears warranted so that further data can be collected on its efficacy under field conditions.

Progress has been made in standardizing the procedure of evaluating the passive immunity of suckling pigs, mainly through the cooperative efforts of the North Central Regional Research Project**, NC-62 (Enteric Diseases of Young Pigs), and the Division of Veterinary Biologics†. However, because of the many variables associated with the passive immune mechanism in TGE, a single practical procedure, that is entirely satisfactory, is difficult to devise.

There is increasing evidence that passive immunity in this disease is associated with adequate levels of neutralizing antibodies in the secretory immunoglobulin (Ig)A of milk. This, in turn, may well be determined by the type of viral

---

*This is a sub-committee of the Committee on Transmissible Diseases of Swine in the United States Animal Health Association.

**Supported by the State Agricultural Experiment Stations in cooperation with the Cooperative State Research Service, USDA.

†A division of the Agricultural Research Service, USDA.
preparation used and by its route of administration. Future research is needed to clarify these points, the knowledge of which should be most helpful in selecting and evaluating immunization procedures not only for TGE but, probably, also for other intestinal infections of the newborn. The prevalence of the disease in the Midwest is probably greater than generally thought, as judged by the increased awareness of its occurrence in feeder and fattening hogs, and by serologic surveys.
A REPORT ON THE INVOLVEMENT
OF MARKETS IN THE SPREAD OF HOG CHOLERA

by
Terry Beals, D.V.M.; William Downey, D.V.M.;
William Cowart, D.V.M. and Sam H. Young, D.V.M.

At the beginning of the Hog Cholera Eradication Program, the swine marketing
practices existing in the United States were favorable for dissemination of highly
contagious diseases such as Hog Cholera, African Swine Fever, and vesicular
diseases. We are now eight years into the Hog Cholera Eradication Program. Even
after considerable effort to improve marketing practices, in the calendar year 1970,
the following States have experienced sizable outbreaks because of marketing of
infected swine. In the Southeastern Region, these were Alabama, Mississippi, and
Virginia; in the Northeastern Region, Pennsylvania, and Ohio; in the Midwest,
Illinois, Indiana, Minnesota, and Missouri; and in the West, Oklahoma and Texas.

Despite continuing efforts to improve market standards, it becomes clear that
when an infected pig enters market channels, it may expose not only the herd to
which it is added, but other herds receiving pigs it contacted enroute to its
destination. This is well demonstrated by a case this year wherein 52 exposed
feeder pigs entering the market channels resulted in the depopulation of about
10,000 pigs on ten farms in five counties of two other States for hog cholera.

The swine industry has developed in a manner whereby feeder pigs move into
the Corn Belt for grain feeding, and into the Northeast near large population
centers for garbage feeding. (More than 2½ million feeder pigs move interstate each
year.) During this process, pigs are commonly assembled, graded, sorted, and
shipped by pig dealers. Often the above steps are repeated one or more times before
the pigs reach their final destination. Swine husbandry practices differ markedly
between regions of the United States. However, a surprising similarity in marketing
practices exists throughout the nation. The average producer of feeder pigs does not
have the volume to move his pigs directly to the feeder, whether it be intrastate or
interstate. Therefore, he must take advantage of the marketing system which
includes markets and dealers.

The dealers can be roughly divided into two categories: The Order Buyer and
the Speculator. An Order Buyer acts as a direct agent between the supply and the
demand. The speculator assembles pigs without a definite market at the time of
Purchase. By the process of assembling, grading, and moving to other geographic
areas, he is able to realize a profit. The more successful dealers operate in both
categories.

Dealer operations may result in other situations that multiply the possibilities
of swine being exposed to numerous diseases during the marketing process, e.g., the
individual operating as both producer and dealer complicates the identification of
swine to the farm of origin. Some of these have falsely represented purchased swine as
“farm fresh pigs.” The conditioning of swine for resale to garbage feeders, the
market-to-market movements of swine, the exposing of feeder swine to slaughter
swine, and the holding over of swine in concentration points, are other marketing practices that are not compatible with the eradication effort. Another problem arises when pigs are purchased by an order buyer, or speculator but are not accepted by the feeder because of substandard health, quality, or some other reason. These pigs are returned to the dealer and identity is lost. Present-day cost of transportation dictates that pigs be hauled by the truckload. In cases where a buyer cannot use the entire shipment, more than one order is filled by swine in one truckload. Thus, several premises may be involved in one shipment.

Disposition of swine for which there is no ready market still constitutes a hazard to hog cholera eradication. This class of swine includes thin sows, pregnant sows, "wet sows", boars, light slaughter hogs, and cull feeder pigs. Due to lack of slaughter facilities and the cost of transportation, these hogs must be congregated and held at numerous concentration points during the marketing process. Also, these swine are a great temptation to the speculator, and they may be diverted into feedlots. This magnifies the possibility of spreading hog cholera.

Several types of markets have evolved to serve the industry. These are public stockyards specifically approved and nonapproved auction markets, organized feeder pig sales and slaughter buying stations.

Basically, there are two types of swine markets, those approved under Part 76, CFR, and those non-approved. Public stockyards also must qualify under marketing standards similar to those that apply to approved markets operating under Part 76, CFR.

The latest innovation in marketing is the organized feeder pig sales. These sales strive to provide buyers with farm-of-origin feeder pigs originating from herds that have been inspected, found free of disease, or otherwise thrifty. Hundreds of sales involving thousands of pigs have demonstrated remarkable success in supplying healthy swine. Only a few can be implicated in the spread of hog cholera. These few are characterized by misrepresentation of swine as to the herd of origin.

Slaughter buying stations have low potential for the spread of hog cholera, providing swine are not diverted back to the farm.

Prior to the hog cholera eradication program, efforts to assure healthy swine in the marketing channels were dependent on market inspection by a veterinarian at the time of sale. Swine which were obviously sick were prevented from returning to farms and feedlots. This reduced the potential for exposing the total swine population to many disease conditions.

Inspection methods can only detect clinically sick animals. Exposed animals or animals in the early stages of infection cannot be detected by any method of inspection in the market. The presently recognized, more chronic forms of hog cholera are most difficult to detect in the markets. With the discontinuance of vaccines, this potential for spread to other susceptible swine in the market is markedly increased.

The danger of spreading hog cholera by the accumulation of swine from many sources in the marketing process has long been recognized. Hog Cholera vaccination was the accepted method of control prior to and in the early eradication efforts. As
a control agent, vaccination with simultaneous serum administration undoubtedly aided in the prevention of market spread to other susceptible swine in the market when infection was introduced. However, inspection and vaccination does not eliminate infection from entering the marketing system. When evidence of the capability of vaccines to produce hog cholera was known, it became obvious that eradication could not be achieved with the use of vaccines. And as the use of vaccines was prohibited, this aid to control was denied to the marketing system.

The results of serum only, at least at the old recommended dosage, have been disappointing in preventing spread to susceptible animals during normal market exposure. It is difficult to measure the benefits of serum usage. Occasionally, it appears that no benefit was obtained. Sometimes it has worked in reverse by delaying the reporting and diagnosis of herds suspicious of hog cholera. Swine moving from markets are now going into an almost totally susceptible swine population. Vaccine formerly used as a buffer in established endemic areas is no longer available. As a result, there is an increased problem of spread in areas where infected swine are moved. The potential for area spread increases where there is delay in reporting hog cholera in the swine that have moved through the markets. This compounds the total results from market movements in the form of related spread.

The eradication program has not developed to the point where all producers are willing to report the suspicion of hog cholera and rely on indemnity provisions to cover the potential loss. Sometimes the possibility of hog cholera is not considered, or the owner believes the problem to be some other condition and fails to report. During fiscal year 1970, about 25 percent of the hog cholera cases had produced illness and/or deaths for three or more weeks before being reported. A small percentage of owners continue to sort off the apparently healthy animals and market them. Speculators whose prime interest is the profit motive, tend to unload apparently healthy animals from a questionable group. It is a poor swine man that cannot sort off animals capable of passing market inspection shortly after the sorting is completed.

Vaccine and inspection did not prevent all market-related infections. How can market inspection alone be expected to be successful? Some answers are available in that some States recognized the inadequacy of market inspection early in the eradication effort. Michigan and Wisconsin are two examples of major swine producing States that are now Hog Cholera Free. Michigan did not permit the sale of swine through auction markets, except through authorized feeder pig sales where the origin of pigs was known, and farm to farm movements. There were some sales conducted under inspection where swine moved directly from the seller's truck to that of the buyer. In principal, this was a farm-to-farm movement. These measures are undoubtedly a little restrictive for the major feeder pig producing states highly dependent on interstate markets.

Wisconsin introduced a method of farm-of-origin identification early in their program. Records of tags were retained by the seller, the marketing agent, and supplied to the State. These measures, in addition to being a swine export state,
probably contributed to eradication in Wisconsin.

The Animal Health Division has recognized the need to upgrade market standards in an attempt to safeguard against market spread of hog cholera. These standards, although recommended by this Association, have been resisted by some states that have been fortunate enough to avoid excessive market involvements. Some have accepted the measures for markets involved in interstate shipment because it is a requirement, but resist such measures in markets involved in intrastate shipment. Several states that have experienced serious market related hog cholera outbreaks have recognized the need to go beyond the present market standards.

Nearly every southeastern State has experienced considerable trouble from market spread of hog cholera. Nationwide, this method of spread has been responsible for more outbreaks than any other single cause. The first serious problem since the discontinuance of vaccines occurred in Georgia in 1968. Market movements involving infected and exposed pigs began in February of that year. Outbreaks resulting from these movements continued throughout the summer in that state. From January through August 16, 1968, there were 135 cases of hog cholera. Of these, 54 percent were due to market movement of feeder pigs. A Statewide quarantine on feeder pig movements was imposed on April 11, 1968. This produced a dramatic reduction of hog cholera cases. However, soon after the release of quarantine on June 10, infected pigs again found their way into market channels, and case numbers rose. This quarantine was reimposed on August 25. Since then, such a quarantine on feeder pig movements has been a necessary step in several states to stop outbreaks.

Marked changes in swine marketing practices were instituted in Georgia and they proved to be highly successful.

Beginning in the fall of 1968, Georgia sellers were required to consign pigs several days prior to the day of the sale. The herd of origin is inspected by State Livestock Inspectors prior to movement of the pigs to the sale. Certificates of inspection are issued to the seller at the time of inspection, and these documents are turned in to the market operator when the pigs are unloaded at the sale. All pigs are eartagged as they are unloaded so that the individual pigs may be identified to the seller.

After experiencing similar outbreaks to that described above in Georgia, four other southern States have adopted similar methods of feeder pig sales. They are: Florida, South Carolina, North Carolina and Mississippi.

The effectiveness of this method of selling pigs in preventing cholera is evident in that no cases of cholera have been attributed to market movements in any of the above mentioned States since initiation of this type of sale. An attempt is made to inspect herds of origin for farm-to-farm-movements in these states also. This effort is partially successful in that it is not always possible to learn of this type movement unless purchasers or sellers request this service.

Oklahoma, Tennessee, Arkansas, and Louisiana have a modified method similar to that described above, except that herds of origin are not inspected. Sellers in
these states are required to sign a statement that the pigs offered for sale were farrowed on their farm, or have been on their farm for at least 30 days. This has been a distinct help in controlling market movements of infected and exposed pigs. However, some outbreaks have occurred from market movements in these States since these regulations have been in effect.

With the exception of outbreaks in Virginia and North Carolina due primarily to area spread, the southeastern States have had the lowest incidence of hog cholera in 1970 of any time previously. There is little question that this is because of prevention of movement of infected pigs through markets, and by closer supervision of such movements as well as the upgrading of marketing facilities.

Missouri after a rather severe outbreak over a year ago, found it necessary to stop movement of swine for a period. When movements were resumed, farm-of-origin identification was required and a system was initiated whereby the responsibility was placed on the market management and market veterinarian to know the consignors and the health status of the herds of origin. The use of serum in the market was denied and all consignments quarantined to the purchasers' premises for 30 days.

Illinois has experienced several hog cholera outbreaks associated with marketing practices in calendar year 1970. Farm-of-origin identification was strengthened. In addition, regulations were changed to permit only one market movement—farm-to-market-to-farm—followed by quarantine.

The northeastern States are primarily importing States receiving most of their feeder pigs from the south and midwest. There has been very little market spread within any of these states. However, Pennsylvania and some other states have experienced several outbreaks of hog cholera involving movement of swine from out-of-state markets. The northeast then, is particularly vulnerable to the marketing practices utilized in the south and midwest. Adoption by exporting states of any of the various methods for providing healthy pigs cited elsewhere in this report provide valuable protection to the swine producers of the northeast.

If the hog cholera program is to be successful, it is imperative that regulations be effected: (1) to better control market-to-market movement of swine; (2) to prevent holdover of swine from one week to the next; (3) to assure that feeder pigs are not exposed to slaughter swine during the marketing process; (4) that cull swine consigned to slaughter are not diverted, and, in addition, every effort must be made to prevent infected swine from entering the market system.

All systems employing some form of voluntary surveillance insuring farm-of-origin identification and the sale of farm-raised pigs are a marked improvement over the old market inspection system. The obvious fallacy in any voluntary system is the weak link where a limited number of market operators and swine owners become careless or elect to take some risk for the monetary returns they may receive and become lax in conforming with the requirements.

Therefore, State and Federal agencies cooperating must be alert to initiate regulatory changes necessary to augment any voluntary procedure. Such changes must be made with appropriate speed to be of maximum benefit to hog cholera eradication.
SURVEY OF DIAGNOSTIC LABORATORIES' FLUORESCENT ANTIBODY EXAMINATIONS FOR HOG CHOLERA


INTRODUCTION

Two fluorescent antibody procedures for the laboratory diagnosis of hog cholera (HC) have been reported, the fluorescent antibody tissue section technique (FATST) by Stair et al⁵ and the fluorescent antibody cell culture technique (FACCT) by Mengeling et al⁴. Both procedures have been compared²,³. Minimal standards for conducting each type of fluorescent antibody examination for HC have been approved by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and published in the proceedings of the United States Animal Health Association¹,⁶.

During the 1969 AAVLD Annual Meeting, the chairman appointed a HC standard diagnostic techniques committee composed of Drs. L. W. Turner, chairman; H. A. McDaniel, secretary; H. D. Anthony, F. E. Mitchell, L. G. Morehouse, H. L. Rubin, T. B. Ryan, V. A. Seaton, M. J. Twiehaus and W. W. Worcester. This committee was instructed to design and implement a program that would enable each laboratory to compare its findings with those from other laboratories on similar samples. Twenty-six laboratories voluntarily participated in the program.

PROCEDURE

Specific Pathogen Free (SPF) pigs inoculated with HC virus were the source of positive tissue specimens. A block of tissue from each organ was examined with FATST to make sure it was similar to field specimens. Normal SPF pigs were the source of negative tissue except in one instance when normal tissue from SPF pigs was not available. In this case, tonsillar tissue submitted as part of a routine laboratory submission and found to be negative for evidence of HC was sent to one laboratory.

Each participating laboratory received ten blocks of tissue preserved by freezing and identified only by a code number. Personnel in the participating laboratories examined each tissue block using either the tissue section or cell culture fluorescent antibody technique, and reported their results to the committee. The results from all the laboratories were compiled and a copy returned to each laboratory. Personnel in each laboratory were then able to compare their findings with findings from other laboratories.

---

¹H. A. McDaniel, Chief, Diagnostic Pathology and Toxicology, Animal Health Division, Diagnostic Services at NADL, P.O. Box 70, Ames, Iowa 50010
²L. W. Turner, In Charge, Tennessee Veterinary Diagnostic Laboratory, P.O. Box 9039, Melrose Station, Nashville, Tennessee 37204
One problem encountered in selecting survey specimens was amount of tissue available from a single organ such as tonsil. Pig tonsil could be divided into only 6 to 9 tissue blocks big enough for routine laboratory processing. Other organs such as the mesenteric lymph node chain could be divided into 15 to 18 blocks. Some organs such as kidney, spleen, or tonsil from a 500 pound sow were large enough to divide into a sufficient number of blocks so all 26 laboratories received similar blocks from the same organ. Therefore, some specimens were examined by only 6 or 7 laboratories, others by 13 or 14 laboratories and still others by all 26 participating laboratories.

A total of nineteen specimens were selected for the survey. They included 11 tonsils, 3 lymph nodes (mesenteric chain), 2 spleens, 2 pancreata, and 1 kidney.

RESULTS

Findings were returned by 25 laboratories. One laboratory reported 2 findings as suspicious; for tabulation these results were counted as negatives. One laboratory reported one tissue block unsatisfactory. The findings are summarized in Table 1.

DISCUSSION

Personnel in each participating laboratory demonstrated the necessary ability to find evidence of HC virus in at least some of the infected tissue blocks. If the 10 survey specimens sent to each laboratory could be thought of as a regular laboratory submission from one herd consisting of tonsil, spleen, and lymph nodes from 3 or 4 pigs, then all of the 25 participating diagnostic laboratories would have found the submission positive for HC and a diagnosis could have been made. However, the fact that 22.9 percent of the tissue blocks from infected pigs were found negative indicates the need for submitting specimens from at least three pigs per herd when HC is suspected.

The lack of uniformity among the findings may be due to (1) All laboratories did not follow the recommended standard procedure; (2) Some participating laboratories were located in areas relatively free of HC; and therefore, the personnel had limited experience using fluorescent antibody techniques to detect HC; and (3) The virus or viral antigen could have been destroyed during shipment.

Some laboratories used the FATST, others used the FACCT and some used both on the evaluation samples. However, this should not significantly alter the results. Extensive data from Diagnostic Services, Animal Health Division, National Animal Disease Laboratory indicates no significant difference exists between results obtained when the two techniques were compared.
## TABLE 1
Survey of Diagnostic Laboratories’ Fluorescent Antibody Examinations for Hog Cholera

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Findings</th>
<th>Status of Source Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Lymph Node</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>Kidney</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Spleen</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Spleen</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Pancreas</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Lymph Node</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Tonsil</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Tonsil</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Tonsil</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Tonsil</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Tonsil</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Tonsil</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Lymph Node</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Pancreas</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Tonsil</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Tonsil</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Tonsil</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>Tonsil</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Tonsil</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
REFERENCES


3. Henry, Ellen J. and McDaniel, H. A. Examination of Specimens from hog cholera suspected cases by the fluorescent antibody tissue section and cell culture technique. (To be published)


THE STATUS OF THE STATE-FEDERAL
HOG CHOLERA ERADICATION PROGRAM

M. J. Tillery, D.V.M.
Hyattsville, Maryland

INTRODUCTION

With completion of eight years activity, fiscal year 1970 was a significant period in the State-Federal Hog Cholera Eradication Program. Almost all States had reached and maintained the final phases of the four-phase program. Additional States became hog cholera free. On the other hand, hog cholera incidence increased in some localities. With vaccines being removed from general usage in FY 1970, some feared that outbreaks would spread beyond control in the increasingly susceptible population. While some increase in outbreaks occurred, this increase never approached the disasters anticipated by some.

During fiscal year 1970, the Animal Health Division assigned hog cholera eradication first priority among all programs. Therefore, this effort was intensified nationally; a desired effect on hog cholera incidence resulted. However, some old problems remained, and some new ones emerged. Both the progress achieved and the problems remaining will be the basis for this report.

PROGRAM STATUS

By July 1970, 47 States had moved from hog cholera control, represented by Phase II, to hog cholera eradication represented by Phase III and Phase IV.
One of these States operated in Phase III until an introduced outbreak, which
was not depopulated, forced a return to Phase II.

Hog cholera free States increased from 12 a year ago to 15 by July 1970. Including the Free States, the number of Phase IV States increased from 18 to 25 by year's end.

Significant advances have occurred since July 1970, such as Iowa advancing to Phase IV, Kansas becoming the 26th Phase IV State, and California's qualifying as hog cholera Free.

There are several things important to this effort which should be noted here. The large western area which is hog cholera free was expanded by the addition of California and South Dakota. Major inroads on hog cholera incidence have been made in the North Central States, so that hog cholera is not known to exist in a region stretching from Michigan in the east to the Pacific Coast. Populous swine States, such as Wisconsin, Minnesota, South Dakota, and Iowa are included in these Phase IV or hog cholera Free States. Next, with 26 of the States being in Phase IV, about 50 percent of the swine population is located in States where hog cholera is not known to exist.

The program situation in the Southeast has a bearing on the maintenance of Phase IV in the Hog Belt due to movement of feeder pigs from the Southeast to the North Central States. Again, advancement occurred with Tennessee, Alabama, Georgia, and Florida moving to Phase IV or hog cholera Free during the year.

While such advancement is expected, a program disappointment continues to be those States which have not moved ahead as fast as possible. In some instances, hog cholera is apparently non-existent in those States. In others, much longer periods of hog cholera freedom than the required three months for Phase IV, as stipulated by this Association, have passed. Then, the problems to program advancement appear to be non-technical ones, and in some cases they have yet to be solved.

Probably, one reason for dallying in Phase II or Phase III after the objectives of those steps have been satisfied is problems with financing later phases. Probably, salvage is being retained by these States as a means to stretch available funds to cover potential outbreaks. This approach is short-sighted, and salvage may cost more money rather than achieve any hoped-for savings.

Because the Animal Health Division has certain responsibilities to curtail interstate spread of the disease, regulations were amended to permit total indemnity payment from Federal funds on outbreaks when the disease spread interstate with introduced swine, provided such swine were quarantined at destination. Therefore, the States should not be saddled with indemnity costs when such spread was experienced. Some hog cholera spread interstate, and in these instances where the introduced swine were quarantined from other swine, total indemnities were paid from Division funds. Therefore, funds in those States affected were conserved.

It continues to be important to this program that funding crises be handled without delay. There is danger of the effort becoming static. Such stasis with
accompanying apathy must be avoided at all costs, for any slackening of the effort will surely delay hog cholera eradication. A more insidious danger is the adoption of less than adequate measures. Acceptance of inadequate procedures, with the excuse for their adoption being funding problems, has proven unsatisfactory in the past. Such inadequacies cannot be tolerated in the future, if this effort is to proceed to completion in 1972 as predicted by this Association in earlier years. Therefore, any problem to the acceptance of the measures laid down by this Association, including any funding difficulties, must be overcome so that all States move ahead as rapidly as possible.

Incidence

Hog cholera incidence increased in FY 1970 from 1969.

Reports of suspicious outbreaks have increased from the outset of the program. Therefore, reporting systems were probably better in 1970 than the preceding years. In some areas, searches for additional infection by regulatory veterinarians and inspectors became an active program procedure. Hog cholera was sometimes disclosed by these methods which had not been reported earlier. Such factors as
these which were not developed to the extent utilized in 1970, must be considered when comparing incidence to earlier years.

Other actions appeared to have an effect on hog cholera incidence. In June 1969, the Animal Health Division placed hog cholera eradication in first priority among the Divisions' activities, thereby giving the effort first call upon Division personnel and funds. Many of those activities reserved for emergency action were used for the first time in this program, and this intensity of operation continues.

In July 1969, Federal quarantines were first utilized in hog cholera eradication with the area quarantined being the Delmarva peninsula. After November 1969, Federal quarantines were utilized routinely in areas which became infected. In October 1970, 12 counties were under quarantine because of hog cholera, as well as portions of 48 additional counties. These quarantines involved 15 States, and in most instances, States have imposed similar restrictions.

The combination of first priority in activity and joint quarantine apparently had a desirable effect on hog cholera incidence. Of the 1,231 outbreaks occurring in FY 1970, 870 were during the first six months, and 361 were during the last six months. This desired situation continues, in that 223 outbreaks have occurred from July through September 1970, compared with 589 outbreaks in the same period of 1969.

Some predicted that uncontrollable epidemics of hog cholera would ravage the swine population of this country when vaccines could not be used. With general usage of vaccines prohibited in January 1970, many program officials anticipated some increase in outbreaks. However, the storms forecast by others have not occurred. For example, Iowa has not had a hog cholera infected herd since March 1970. Nebraska has had but two outbreaks since last October. Other examples of progress are abundant. (Figure 3)

The numbers of counties in which hog cholera occurred in FY 1970 increased by 30. The numbers of States experiencing the disease increased by two. (Figure 4)

Again, a general increase in outbreaks did not occur. A closer look at incidence reveals that 85 percent of the infected herds were confined to 11 States. Actually, hog cholera incidence declined in many States, but the decline was offset by heavier infection in these few States.

The disease incidence has been sharply reduced in most of these 11 States. The stepped-up Division activity, the Federal quarantines, and the accompanying intense action by these States practically eliminated the infected herds in most cases. Time-proven methods of program operation were once more tested and effective. Therefore, hog cholera continued to be susceptible to these steps; little change in procedure appears to be necessary; but more intense application of these procedures will likely be needed to carry this effort to a speedy conclusion.

Earlier experience by some States proved valuable here. During FY 1970, Mississippi and Oklahoma experienced explosive outbreaks involving marketing of swine. Swine movement was halted in both States and spread was quickly brought to a halt and the infection appears to be eliminated. Probably, such procedures are adopted quicker today than earlier, and more skilled application is available. These
Figure 3 - HOG CHOLERA REPORTED Fiscal Year 1970

Figure 4 - 85% OF CONFIRMED HOG CHOLERA CASES FOUND IN ELEVEN STATES
factors, while difficult to assess, also appear to have a desired influence on hog cholera outbreaks.

By years' end, the only region in which hog cholera infection seemed to persist was the Dismal Swamp area of North Carolina and Virginia. This section had accounted for as much as one half of the infected herds in certain periods since March 1970. In September 1970, the area was quarantined and regulatory veterinarians, inspectors, laboratory personnel, and other support were detailed to the area to supplement the available force and thereby bring as intense pressure as possible upon this endemic area. Similar procedures will likely be used in any area where hog cholera appears to be regaining a foothold.

EPIDEMIOLOGY

One of the most significant events in this effort was the general prohibition of vaccine usage with the federal action becoming fully effective in January 1970.

Vaccine-associated outbreaks dropped from a high of about one-third of all infected herds in 1967 to less than one percent in 1970. However, this decline is not the major impact of this action. The long-range effect is the swine industry of
this country together with the veterinary profession finally turning from hog cholera control to hog cholera eradication. The decision to forego vaccination appears timely in that no problems of consequence have resulted. Rather, the significant change is more intense program activity. Such a step-up may not have been possible without the more courageous decision to abandon vaccination having been made initially.

Other problems remained. The spread of hog cholera through swine movement, primarily marketing, continued. This movement, with the closely related area spread, is the major technical obstacle to hog cholera eradication. The market standards established in 1962 required change to reckon with an increasingly susceptible population, and this Association formulated necessary amendments. However, all States were not prepared to adopt these major changes, even though each had been utilized earlier by other States. Therefore, the standards recommended by this Association were modified to some extent when they were placed in Federal regulations concerning markets and stockyards early in FY 1971.

The major problem appeared to be initiation of swine identification in some States. Therefore, identification recommendations were relieved to some extent. This was done despite nationally known industry leaders asking for continuing identification of swine.

Longstanding concerns came into sharp focus. Some States appear unwilling to adopt uniform requirements for swine marketing. Uniformity of such requirements must remain a long range objective. Yet, swine identification is jeopardized by regulatory differences. Swine identification, if it continues, must be free of any dependence upon one program. Program success stimulates some to seek relief from requirements. Identification is costly and little justification to continue this burden in Phase IV States remains if identification for hog cholera is the sole reason. Obviously other justifications are available, and there must be utilized to continue swine identification nationally. Therefore, removing the dependence of identification from hog cholera eradication and initiation of a swine identification program adequate to support any disease eradication effort appears timely. Unless the industry acts toward this objective, swine identification may be lost when hog cholera eradication is achieved.

Another continuing epidemiological problem is hog cholera associated with garbage feeding. The temporary program procedure permitting the salvage of swine, adopted in 1966, appears to aggravate this problem. Hog cholera associated with feeding meat scraps is common. It is well known, and demonstrated again in 1970, that at least one percent of the salvaged carcasses contain hog cholera virus. In some instances, epidemiological investigation provided strong circumstantial evidence that salvaged pork had been fed to swine and hog cholera resulted. The hoped-for savings in program funds through salvage has not been realized in all instances. Experiences in Georgia, Tennessee, Oklahoma, North Carolina, Maryland, Massachusetts, Rhode Island, and even other States indicates that salvage is a costly proposition.

In adopting salvage as a program procedure in 1966, this Association called for
such operation to stop if it interfered with program advancement. Salvage was initially utilized to permit four large midwestern swine producing States to move to Phase III as a unit. Today, 26 States are in Phase IV, where salvage is not utilized.

The Animal Health Division attempted to withdraw from salvage operations in January 1970. That proposal was defeated. In view of the current interference with program advancement, the stated objective of salvage having been accomplished long ago, the nearness of the 1972 goal for eradication, and the obvious expense of salvage, this procedure cannot be tolerated. The time to have discontinued salvage passed long ago from a technical standpoint. Therefore, with over half the States in Phase IV, and others qualifying soon, it appears unreasonable for a few States to continue to resort to salvage and thereby risk setbacks in the majority of the States' efforts. Such risk must be eliminated with all possible speed.

The ability to determine means of hog cholera spread continues at a high level, despite the epidemiology of hog cholera becoming more obscure. The highly skilled activity by regulatory veterinarians and others involved in such investigations is obvious. These skills developed during the eight years of this activity, represent an intangible benefit gained through hog cholera eradication. No doubt, advancement of the art in this respect will be profitable to future animal disease eradication programs.

OUTLOOK

It appears that the States will continue to advance toward hog cholera eradication. The last apparent endemic infection is expected to be eliminated without adverse consequence. Future outbreaks in this country will likely be sporadic affairs introduced to the area through waste feeding or shipped-in swine, and these outbreaks are expected to be promptly stamped out. If signs of spread indicate a need, available resources will be utilized to the extent necessary to search out and destroy any infection present. Such operations will likely be similar to those employed today in the Dismal Swamp area of North Carolina and Virginia.

Judging by experiences of the past eight years, hog cholera eradication continues to be an excellent possibility. No veterinary evidence has arisen so far to support an opinion to the contrary.
REPORT OF THE COMMITTEE ON NATIONWIDE ERADICATION OF HOG CHOLERA

Paul B. Doby, Springfield, Illinois, Chairman


Your Committee notes that the Hog Cholera Eradication Program continues to progress satisfactorily. More than 50 percent of the Nation's swine are in states which have reached the final phase of the Program. Currently, hog cholera incidence is about one-half the level of a year ago. Most of the present infection is confined to areas within a few states, despite cessation of vaccination nationally last January.

Your Committee heard a report of a stepped-up eradication effort to confine and eliminate hog cholera from the Dismal Swamp area of southeastern Virginia and northeastern North Carolina. This area had accounted for much of the hog cholera throughout the Nation since early 1970. Your Committee is impressed by the action of these states in requesting the assistance of the United States Department of Agriculture in eliminating this problem. It appears that this operation may serve as a model for eliminating similar outbreak situations in the future. By acting promptly, such outbreaks may be eliminated before they reach the magnitude of the Dismal Swamp problem.

Your Committee accepted a resolution previously approved by the American Association of Veterinary Laboratory Diagnosticians on "Evaluation of Diagnostic Laboratories' Fluorescent Antibody Examinations for Hog Cholera" as a portion of the 1970 report (copy of this resolution attached). This provides for uniform evaluation of diagnostic laboratories conducting fluorescent antibody examinations for hog cholera. The recommended procedure would require self-management by the laboratories; cooperating officials and your Committee believe it to be worthwhile.

Your Committee is concerned that identification of swine moving in trade channels could be lost as progress is made toward the goal of a "hog cholera free" United States. Expressions were heard from industry leaders that identification of swine to farm of origin is a valuable tool in controlling animal disease. Your Committee encourages all Phase IV and "free" states not now identifying swine in this manner to do so. Further, an identification system for swine should be developed independent of the hog cholera or any other eradication program and this system should be adopted throughout the Country. It was felt that action on this matter might be more appropriately handled by another committee of this Association.
Your Committee recommends that the program adopt a 90-10 Federal-State sharing of indemnities in Phase IV and "free" states, providing that these states require identification of swine for both intra and interstate shipments.

It is obvious that we have reached the point where the salvage provision of Phase III should be abolished. Therefore, your Committee strongly recommends that immediate steps be taken to end salvage in Phase III.

With respect to the Voluntary Isolation Plan recommended last year for adoption in swine-raising operations, your Committee recommends that Federal regulations remain in effect which prevent interstate movement of feeding and breeding swine originating from V.I.P. herds in a federally-quarantined area.

In the event that it becomes necessary to depopulate units within a V.I.P. herd because of hog cholera, and it was determined that the disease probably spread through interstate shipment of swine, the present restriction of payment of 100 percent Federal indemnity during only the first 60 days of quarantine should be relieved. Thus, such swine would be eligible for 100 percent Federal indemnity so long as the herd qualified as a V.I.P. herd.

Your Committee recommends that methods for processing edible food wastes for recycling by the subject of a presentation before the Committee next year.

APPROVED RESOLUTION

The American Association of Veterinary Laboratory Diagnosticians' Committee for Evaluation of Diagnostic Laboratories' Fluorescent Antibody Examinations for Hog Cholera recommends the following revised program to assure a continued high degree of competency within diagnostic laboratories for detecting evidence of hog cholera.

1. Laboratories wishing to participate in this evaluation program should notify the secretary of the AAVLD Committee for Evaluation of Diagnostic Laboratories' Fluorescent Antibody Examinations for Hog Cholera.

2. The Diagnostic Services Animal Health Division, U.S.D.A., is requested to furnish to diagnostic laboratories the specimen needed to evaluate the laboratories and also is requested to compile the results of the examination.

Fifteen specimens consisting of tonsil, spleen, and lymph nodes from each of 5 pigs will be submitted to each participating laboratory at least annually. The specimens will be collected from either normal healthy pigs or pigs inoculated with hog cholera virus. A member of the Committee will examine tonsil, spleen, and lymph node sections from each pig to make sure they simulate field cases before they are included in the evaluation samples.

3. Each participating laboratory will examine each specimen and indicate whether each specimen is positive or negative and then classify each pig as positive or negative.
4. Each laboratory will have five working days to examine the sample and report the results to the Committee secretary.

5. Either the frozen tissue section or cell culture fluorescent antibody procedure may be used to examine the specimen. Each laboratory should use the same procedure routinely used on field specimens.

6. Each laboratory must correctly identify at least 4 out of the 5 pigs as positive or negative for evidence of hog cholera to be classified as “satisfactory”. Laboratories incorrectly identifying 2 or more of the 5 pigs will be classified as “needs improvement”.

7. The AAVLD Committee secretary will compile findings on individual samples reported by participating laboratories and furnish this composite information to each laboratory examining the samples. He will also furnish a written statement to both state and federal regulatory persons accountable for expenditure of public funds for indemnity payments on hog destroyed due to hog cholera. This statement will indicate whether each participating laboratory was “satisfactory” or “needs improvement” for conducting fluorescent antibody examinations for hog cholera. Additional information pertaining to individual situation will be furnished upon request.

8. For a laboratory in the “needs improvement” category to receive “satisfactory” status, the problem(s) must be identified and corrected; subsequently, the laboratory must correctly identify at least 4 out of 5 pigs as positive or negative for evidence of hog cholera. Special samples will be assembled and forwarded to these laboratories as soon as possible after the problem has been corrected. Personnel for Diagnostic Services, NADL, will be available for consultation and visitation when requested by the veterinarian in charge of ANH activities for the state and approved by the ANH Division.
STATUS OF THE COOPERATIVE
STATE-FEDERAL SALMONELLA PROGRAM

by
Saul T. Wilson, Jr., D.V.M., M.P.H.*
John W. Walker, D.V.M.**
Claude J. Pflow, D.V.M.***

The cooperative State-Federal Salmonella Program is designed to prevent and minimize Salmonella contamination in feed supplements of animal and marine origin while working to eliminate it from these two ingredients of livestock and poultry feeds. The accomplishment of this objective is greatly dependent upon a management operated Salmonella control program being developed by each rendering plant and each industrial fish plant that is participating in the program. The plan of work calls for the program to be conducted in three phases on an individual plant basis: Phase I “Evaluation,” Phase II “Clean-up,” and Phase III “Approved.”

The Evaluation phase of the program is now completed. The objective of this phase of the program was to identify those plants that are and are not producing a finished product in which Salmonellae can be detected by the sampling and laboratory procedures specified for the program.

It has taken us three years to accomplish this objective in terms of all plants producing feed supplement of animal or marine origin. While this may appear to have been a long time, it is a reflection of the availability of resources and priority given to the program.

Accomplishments during the Evaluation period includes considerably more than identifying positive and negative plants. I would like to call your attention to a few of those which I feel are of major significance to the accomplishment of the final goals of this program. These include:

1. Adoption of Uniform Methods and Rules for the conduct of the program.
2. Adoption of the laboratory procedures recommended by the American Association of Veterinary Laboratory Diagnosticians for the isolation of Salmonella from animal feeds.
3. Eliminated the duplications in inspection efforts of State and Federal regulatory agencies.
4. Conducted a five day Salmonella seminar and workshop in each of the four Administrative Regions of the Animal Regions of the Animal Health Division.

---

*Chief Staff Veterinarian, Poultry Diseases, Animal Health Division Agricultural Research Service, U.S. Department of Agriculture.
**Senior Staff Veterinarian, Poultry Diseases, Animal Health Division, Agricultural Research Division, U.S. Department of Agriculture.
***Staff Veterinarian, Poultry Diseases, Animal Health Division Agricultural Research Service, U.S. Department of Agriculture.
for State and Federal personnel who supervise the program.

(5) Developed a cadre of State and Federal personnel with special knowledge and understanding of the procedures for locating and identifying sources and potential sources for product contamination within rendering plants and in industrial fish establishments.

(6) Assigned a specially trained Veterinary Epidemiologist to each of the four Administrative Regions of the Animal Health Division to provide special assistance to the states in the training of field personnel and in the conduct of contamination search studies in problem plants.

(7) Established a working relationship with the various segments of the rendering industry and the industrial fish industry.

A major objective of the Evaluation phase was to obtain baseline data for future program planning and the allocation of resources (personnel and laboratory facilities) to accomplish the goals of the program. *Salmonellae* was detected in finished product samples of 71 percent of the 899 plants that had received three or more inspections at the close of Fiscal Year 1970. *Salmonella* contamination of the finished products of rendering plants and industrial fish establishments may be categorized as resulting from either of two sources:

(1) Operational practices and the movement of personnel and equipment which results in cross contamination between raw and finished product.

(2) Foci of *Salmonella* maintaining itself in the processing equipment; conveyors, bucket elevators, hammer mills, shaker screens, and storage areas.

Contamination resulting from operational practices and the movement of personnel and equipment can be eliminated relatively easy when management commits itself to the adoption of the procedures outlined in the Sanitation Guidelines. These procedures will not eliminate contamination that is resulting from foci of *Salmonella* that has established itself in the processing line, equipment, and storage areas. The task here is to locate or “search out” these foci and decontaminate the area. Locating the foci or areas where Salmonella is maintaining itself will not be an especially difficult job. Decontamination; however, will be quite a formidable task due to the nature of the equipment and structural arrangements in many of the plants. Improvement in design of equipment to facilitate cleaning and sanitization will provide a long term solution to many of the current implant decontamination problems.

The “Cleanup” phase of the program which we are now about to enter is equivalent to the disease reduction phase of an eradication program. It is during this phase that the plant owners are expected to plan and systematically eliminate those operational procedures, sanitary practices, structural arrangements, and even equipment that may contribute to contamination between raw and finished product. The most important feature of Phase II or “Cleanup” is the goals management set establishing a timetable to eliminate specified deficiencies and to initiate his own self-monitoring program.

Phase II or “Cleanup” is intended to zero in on the heart of the problem of *Salmonella* in animal by-products and fish meal. Namely, identifying the areas
where contamination is occurring in the contaminated plant. When these areas have been eliminated the operational procedures brought into adequate compliance with the Sanitation Guidelines, a self-monitoring program implemented, and the plant has achieved the required number of negative tests of official samples, the plant may be designated as an “Approved” Establishment by local State and Federal officials. After this designation, maintaining “Approved Status” is the program’s objective. This is the final goal of the program for the individual plant. As this is a voluntary program, the owner of each plant should establish the target date for his plant to achieve approval. With these goals, a national target date can be established.

Control programs for complex diseases are usually phased when the program is first initiated to facilitate administration and planning for their ultimate completion. Phase changes indicate those points in the program where a new course can be conveniently charted, where distinct changes will be made in methods and procedures, objectives to be accomplished and resources allocated (personnel, laboratory facilities, and funds). In the Evaluation phase, we were interested in identifying the positive plants. Emphasis was on finished product sampling. In the Cleanup phase, we will be interested in identifying and eliminating the areas and practices where contamination is occurring in the positive plants. Emphasis will be on inline and environmental sampling. Personnel changes should include increased use of livestock inspectors for routine inspections and sample collection.

As we embark on the course charted for Phase II, perhaps we should consider two of the frequently asked questions about this program.

1. **Is the technical knowledge available to decontaminate the plant that is producing a product in which *Salmonella* is detected under our program procedures?**

2. **What will be the impact on animal and human Salmonellosis of eliminating *Salmonella* from animal and marine proteins and hence in animal feeds?**

   In answer to the first question, the technical knowledge required to decontaminate a rendering plant is available. Increased attention must be given to developing the means and techniques for the application of this knowledge in such a manner that it causes a minimum amount of interference with good commercial practices and the operational procedures in individual plants. In this regard, a distinction should be made between the role of the State and Federal agencies and that of the plant management. Determining where and how contamination is occurring is a responsibility of the cooperating government agencies. Determining how and when decontamination will be carried out is a responsibility of plant management. Determining the adequacy and effectiveness of the decontamination effort is a shared responsibility of management and the cooperating government agencies.

   In considering the second question, one should remember that *Salmonella* infection in man and animal is the sum of the forces that have contributed to the contamination of human foods and animal feeds at various points in the production, processing, distribution, and final preparation as well as the physical
condition, age, and stress of the host at time of exposure. Animal feeds is just one of the sources for the introduction of *Salmonella* into a flock or herd. Eliminating *Salmonella* from animal feed will be only mitigating as far as the total problem of Salmonellosis is concerned. Current information indicates the clinically ill animal, the infected breeder flock or hatchery, and the contaminated environment are major sources for *Salmonella* infections in livestock and poultry. Any program designed to have a significant impact on animal and human Salmonellosis must include efforts directed at each of these sources.

We might ask, is the objective of eliminating *Salmonella* from animal feeds a desirable goal? *Salmonella* infections in our food animals are frequently subclinical. They often occur concurrently with other infections especially in the age groups most susceptible to Salmonellosis. It is the apparently healthy animal that is carrying *Salmonella* in its intestinal tract at time of slaughter that is most often responsible for introducing *Salmonella* into food processing plants. Prior to the initiation of this program, it was generally felt that little progress would be made in reducing *Salmonella* infections in livestock and poultry if they were constantly receiving *Salmonella* through their feed. Since that time, nothing has occurred to change that opinion. We are currently unable to fully assess the impact that removing Salmonella from animal feeds will have on the total Salmonella problem. Efforts are now being made to assess this from a system analysis approach. Much of the data desired for this purpose is not available. If it is agreed that eliminating Salmonella from animal feeds is desirable, what priority should this program have in relation to other approaches to Salmonella control at the producer level?

An even more pertinent question is what priority Salmonella control should be given in relation to other disease control activities? The program received a low priority during FY 1970. This is reflected by the number of states (30) that made the equivalent of three inspections of each of their plants. You will note from Figure 1 that many of the states that made less than three inspections per plant are the ones that experienced a considerable amount of Hog Cholera. These states gave first priority in the allocation of their resources to the cholera program.

The following statistics summarizes the results of Salmonella testing conducted for program purposes and the status of the program at the close of Fiscal Year 1970:

Field stations reported the examination of 27,666 samples of which 24,329 were finished product and 3,337 were inline or environmental samples. Of the finished product samples, 3,892 or 16% were positive. A comparison in the number of samples tested for program purposes in Fiscal Years 1969 and 1970 is given in Table 1.

The 1970 increase (17% to 28%) in the percentage of inline and environmental samples that were positive may be attributed to the additional year of experience our field personnel have had in locating potential sources of product contamination.
TABLE 1
Number of samples for Salmonella in Fiscal Years 1969 and 1970 by type of sample, number, and per cent positive.

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Fiscal Year 1969 Samples Tested</th>
<th>Fiscal Year 1970 Samples Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Number</td>
</tr>
<tr>
<td>In line and</td>
<td>7,821</td>
<td>1,353</td>
</tr>
<tr>
<td>Environmental</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finished Product</td>
<td>22,824</td>
<td>3,835</td>
</tr>
<tr>
<td>TOTAL</td>
<td>30,645</td>
<td></td>
</tr>
</tbody>
</table>

The number of finished product samples tested and the number and per cent positive by plant category for Fiscal Year 1970 is presented in Figure 2 and for FY 1969 in Figure 3. A total of 3,124 Salmonella isolates were reported on ANH Form 9-10 as being serotyped for program purposes. These serotypes are listed in descending order of frequency in Appendix 1 and in alphabetical order in Appendix II of this report. There were 90 different serotypes represented in these isolates as compared to 93 different serotypes represented in the 3,996 isolates serotyped for program purposes in FY 1969.

The five most frequent serotypes were S. senftenberg (10.4%), S. montenido (9.5%), S. eimsbuettel (7.4%), S. anatum (6.9%), S. oranienburg (4.7%). In 1969, S. derby representing 4.58% of the total serotypes was ranked number 5 while this year S. derby ranks number 8 and represents 4.00% of the total serotypes. The serotypes found and the rank they occupy are essentially the same as last year. The decrease in the total number of isolates serotyped is a result of the administrative decision to discontinue serotyping isolates from plants that had been evaluated unless the information was requested by the field veterinarian. This decision will permit us to provide the serotyping needed for epidemiological or inplant contamination search purposes.

A comparison of the number and per cent of plants that met the Uniform Methods and Rules definition for a negative plant based on their last three tests is given in Table 2. The increase in number of plants represented in 1970 is due to the increase in number of plants that have had at least three or more inspections.
TABLE 2
Number and per cent of plants that met the Salmonella Uniform Methods and Rules definition for a negative plant based upon the last three inspections at close of FY 1969 and FY 1970:

<table>
<thead>
<tr>
<th>Type of Plant</th>
<th>FY 1969 Plants</th>
<th>FY 1970 Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. re-presented</td>
<td>No. re-presented</td>
</tr>
<tr>
<td>Protein Blender</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Livestock Slaughterer</td>
<td>286</td>
<td>380</td>
</tr>
<tr>
<td>Poultry Slaughtener</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>Independent Renderer</td>
<td>342</td>
<td>377</td>
</tr>
<tr>
<td>Feather Meal</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Marine Product</td>
<td>63</td>
<td>81</td>
</tr>
<tr>
<td>TOTAL</td>
<td>728</td>
<td>899</td>
</tr>
</tbody>
</table>

*A negative plant is one that has had three consecutive negative tests of 10 sample units (total 30 negative sample units) of an official sample of finished product. The sample units are collected from the shipping or storage area at an interval of not less than 30 days apart within a 12 month period.

The objective of the Evaluation phase was to identify the positive and negative plants and to obtain baseline data for future program planning. This objective was achieved. The consistency of the data for FY 1969 and 1970 firmly establishes the current level of contamination in the finished product of various segments of the rendering industry, the per cent of plants in the negative category, and the serotypes that are being isolated from protein supplements of animal and marine origin.

A prerequisite to the success of any disease program is a clear understanding of the objectives of the program by all who are involved in either its conduct, support, or administration. The objective of this program is to eliminate Salmonella from feed supplements which are of animal and marine origin. The methods, procedures, and resources required to achieve this objective are markedly different from those that will be required to produce food animals free of Salmonella or free of specific serotypes of *Salmonella*.

The following criteria were proposed in 1968 as measures of the effectiveness of the program:

1. Number of Plants Approved
2. Number of Plants that Maintain their Approved Status
3. Prevalence of Salmonella in Animal By-Product and Fish Meal

At the request of industry representatives, it was agreed to not designate any plants as Approved until all plants had an opportunity to qualify for Approved status. Therefore, we have no data for the first two criteria. However, there are a
number of plants that have met all of the requirements for Approved status. The number of plants in the negative category (Table 2) also gives us some indication of the progress that is being made in achieving Approved status. We do have data for the third criteria. The prevalence rate for Salmonella in animal by-products and fish meal was 16.8% in 1969 and 15.9% in 1970.

Up to this point, I have not mentioned anything about what is being done to control Salmonella at the producers level. State and University Animal Disease Diagnostic Laboratories have continued to provide diagnostic support to the livestock and poultry industry in the form of Salmonella isolations, examinations, and identifications. There were 14,074 Salmonella isolates serotyped at ANH and State Cooperating Laboratories during FY 1970. This compares with 12,388 isolates serotyped at these same laboratories in FY 1969. The increase probably represents increased control efforts rather than increased incidence. The location of the serotyping laboratories are indicated in Figure 4. The State Cooperating Laboratories are located at the University of Massachusetts, University of Maine, the Wisconsin State Aniaml Health Laboratories and the Utah Department of Agriculture Laboratories. The Regional Serotyping Laboratories of the Animal Health Division are located at Atlanta, Georgia; Phoenix, Arizona; Orono, Maine; and Ames, Iowa.

Most of the major poultry breeders have developed or are developing various types of Salmonella control programs tailored to operations on an individual flock basis. Relatively little difficulty has been reported in preventing the introduction of outside infections in closed flocks. This is especially true where comprehensive control measures directed at preventing access to wild birds and rodents, egg transmission, and environmental contamination have been initiated. The major problem has been to eliminate resident serotypes. This may have some future implication for this organization, as it focuses on the importance of the contaminated environment in the perpetuation of Salmonella infections in succeeding flocks of poultry raised on the same premises.
FIGURE 1 –

**STATES THAT COMPLETED 3 OR MORE INSPECTIONS AT EACH PLANT DURING FISCAL YEAR 1970**

*UNDER THE COOPERATIVE STATE-FEDERAL SALMONELLA PROGRAM*

U. S. DEPARTMENT OF AGRICULTURE  
ANIMAL HEALTH DIVISION  
AGRICULTURAL RESEARCH SERVICE
### Salmonella Program

**TEST RESULTS BY TYPE & NUMBER PLANTS - FY 1970**

<table>
<thead>
<tr>
<th>TYPE PLANT AND NO.</th>
<th>PERCENT POSITIVE SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLENDER [16] PLANTS</td>
<td><strong>52%</strong> <em>(433 Samples Tested)</em></td>
</tr>
<tr>
<td>LIVESTOCK SL. [378]</td>
<td>16% <em>(10,390)</em></td>
</tr>
<tr>
<td>POULTRY SL. [41]</td>
<td>16% <em>(1,078)</em></td>
</tr>
<tr>
<td>INDEPENDENT [352]</td>
<td>16% <em>(10,421)</em></td>
</tr>
<tr>
<td>FEATHER MEAL [8]</td>
<td>10% <em>(281)</em></td>
</tr>
<tr>
<td>MARINE [77]</td>
<td>8% <em>(1,726)</em></td>
</tr>
</tbody>
</table>

**Total samples tested:** 24,329 (16% pos.)  
**Total plants represented:** 872

*Finished product samples only

**SOURCE:** STATE ANNUAL SUMMARY REPORTS

---

U.S. DEPARTMENT OF AGRICULTURE  
ANIMAL HEALTH DIVISION  
AGRICULTURAL RESEARCH SERVICE
**Salmonella Program**

**TESTING ANIMAL & MARINE PROTEIN - FY 1969**

<table>
<thead>
<tr>
<th>TYPE PLANT AND NO.</th>
<th>PERCENT POSITIVE SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLENDER 15 PLANTS (381 Samples Tested)</td>
<td>40%</td>
</tr>
<tr>
<td>LIVESTOCK SL. [373] (9,156)</td>
<td>18%</td>
</tr>
<tr>
<td>POULTRY SL. [35] (730)</td>
<td>17%</td>
</tr>
<tr>
<td>INDEPENDENT [362] (10,407)</td>
<td>16%</td>
</tr>
<tr>
<td>MARINE [86] (1,991)</td>
<td>11%</td>
</tr>
<tr>
<td>FEATHER MEAL [9] (159)</td>
<td>3%</td>
</tr>
</tbody>
</table>

Total samples tested: 22,824 (17% pos.)
Total plants represented: 880

*Finished product samples only

SOURCE: STATE ANNUAL SUMMARY REPORTS
FIGURE 4 -

SALMONELLA SEROTYPING LABORATORIES

U.S. DEPARTMENT OF AGRICULTURE - ANIMAL HEALTH DIVISION - AGRICULTURAL RESEARCH SERVICE - AUGUST 1970
APPENDIX I
Salmonella Serotypes Isolated From Animal and Marine Byproducts During Fiscal Year 1970 (Listed in Descending Frequency)

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number</th>
<th>Percent of Total Serotyped</th>
<th>Serotype</th>
<th>Number</th>
<th>Percent of Total Serotyped</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td></td>
<td></td>
<td><em>Salmonella</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>senftenberg</td>
<td>326</td>
<td>10.44</td>
<td>westhampton</td>
<td>7</td>
<td>0.22</td>
</tr>
<tr>
<td>montevideo</td>
<td>296</td>
<td>9.48</td>
<td>Untypable-Group C1</td>
<td>7</td>
<td>0.22</td>
</tr>
<tr>
<td>eimsbuettel</td>
<td>233</td>
<td>7.43</td>
<td>blockley</td>
<td>6</td>
<td>0.19</td>
</tr>
<tr>
<td>anatum</td>
<td>216</td>
<td>6.91</td>
<td>manhattan</td>
<td>6</td>
<td>0.19</td>
</tr>
<tr>
<td>oranienburg</td>
<td>148</td>
<td>4.74</td>
<td>minneapolis</td>
<td>6</td>
<td>0.19</td>
</tr>
<tr>
<td>binza</td>
<td>135</td>
<td>4.32</td>
<td>panama</td>
<td>6</td>
<td>0.19</td>
</tr>
<tr>
<td>bredeney</td>
<td>130</td>
<td>4.16</td>
<td>madelia</td>
<td>5</td>
<td>0.16</td>
</tr>
<tr>
<td>derby</td>
<td>125</td>
<td>4.00</td>
<td>taksony</td>
<td>5</td>
<td>0.16</td>
</tr>
<tr>
<td>infantis</td>
<td>98</td>
<td>3.14</td>
<td>bornum</td>
<td>4</td>
<td>0.13</td>
</tr>
<tr>
<td>thomasville</td>
<td>98</td>
<td>3.14</td>
<td>london</td>
<td>4</td>
<td>0.13</td>
</tr>
<tr>
<td>livingstone</td>
<td>97</td>
<td>3.10</td>
<td>orion</td>
<td>4</td>
<td>0.13</td>
</tr>
<tr>
<td>schwarzengrund</td>
<td>97</td>
<td>3.10</td>
<td>simsbury</td>
<td>4</td>
<td>0.13</td>
</tr>
<tr>
<td>tennessee</td>
<td>93</td>
<td>2.98</td>
<td>canoga</td>
<td>3</td>
<td>0.10</td>
</tr>
<tr>
<td>worthington</td>
<td>75</td>
<td>2.40</td>
<td>nienstedten</td>
<td>3</td>
<td>0.10</td>
</tr>
<tr>
<td>cerro</td>
<td>71</td>
<td>2.27</td>
<td>reading</td>
<td>3</td>
<td>0.10</td>
</tr>
<tr>
<td>cubana</td>
<td>57</td>
<td>1.82</td>
<td>san-diego</td>
<td>3</td>
<td>0.10</td>
</tr>
<tr>
<td>minnesota</td>
<td>57</td>
<td>1.82</td>
<td>3, 10: e,h,z48</td>
<td>3</td>
<td>0.10</td>
</tr>
<tr>
<td>drypool</td>
<td>52</td>
<td>1.66</td>
<td>braenderup</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>alachua</td>
<td>47</td>
<td>1.50</td>
<td>brandenburg</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>bareilly</td>
<td>43</td>
<td>1.38</td>
<td>corvallis</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>typhimurium</td>
<td>43</td>
<td>1.38</td>
<td>eppendorf</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>kentucky</td>
<td>40</td>
<td>1.28</td>
<td>javiana</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>heidelberg</td>
<td>38</td>
<td>1.22</td>
<td>pensacola</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>siegburg</td>
<td>35</td>
<td>1.12</td>
<td>poona</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>give</td>
<td>30</td>
<td>0.96</td>
<td>putten</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>johannesburg</td>
<td>28</td>
<td>0.90</td>
<td>thielallee</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>muenster</td>
<td>25</td>
<td>0.80</td>
<td>Untypable</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>manila</td>
<td>22</td>
<td>0.70</td>
<td>Untypable-Group C2</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>illinois</td>
<td>21</td>
<td>0.67</td>
<td>assen</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>california</td>
<td>20</td>
<td>0.64</td>
<td>ardwick</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>lexington</td>
<td>20</td>
<td>0.64</td>
<td>bareilly var 14</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>habana</td>
<td>19</td>
<td>0.61</td>
<td>bere</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>saint-paul</td>
<td>19</td>
<td>0.61</td>
<td>colorado</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>newington</td>
<td>17</td>
<td>0.54</td>
<td>dublin</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>indiana</td>
<td>14</td>
<td>0.45</td>
<td>essen</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>newport</td>
<td>13</td>
<td>0.42</td>
<td>godesberg</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>thompson</td>
<td>12</td>
<td>0.38</td>
<td>grunty</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>muenchen</td>
<td>10</td>
<td>0.32</td>
<td>halmstad</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>enteritidis</td>
<td>10</td>
<td>0.32</td>
<td>litchfield</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>new-brunswick</td>
<td>9</td>
<td>0.29</td>
<td>molade</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>albany</td>
<td>8</td>
<td>0.26</td>
<td>rubislaw</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>arkansas</td>
<td>8</td>
<td>0.26</td>
<td>ruiri</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>illite</td>
<td>8</td>
<td>0.26</td>
<td>uganda</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>urbana</td>
<td>8</td>
<td>0.26</td>
<td>uno</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>amager</td>
<td>7</td>
<td>0.22</td>
<td>Untypable-Group E</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>amsterdam</td>
<td>7</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>grumpensis</td>
<td>7</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>meleagridis</td>
<td>7</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>typhimurium</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>var copenhagen</td>
<td>7</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total Isolates</strong></td>
<td><strong>3,124</strong></td>
<td><strong>99.87</strong></td>
<td><strong>Serotyped</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## APPENDIX II

Salmonella Serotypes Isolated From Animal and Marine Byproducts During Fiscal Year 1970 (Listed Alphabetically)

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number Isolates</th>
<th>Percent of Total Serotyped</th>
<th>Serotype</th>
<th>Number Isolates</th>
<th>Percent of Total Serotyped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td></td>
<td></td>
<td>Salmonella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alachua</td>
<td>47</td>
<td>1.50</td>
<td>manhattan</td>
<td>6</td>
<td>0.19</td>
</tr>
<tr>
<td>albany</td>
<td>8</td>
<td>0.26</td>
<td>manila</td>
<td>22</td>
<td>0.70</td>
</tr>
<tr>
<td>amager</td>
<td>7</td>
<td>0.22</td>
<td>meleagridis</td>
<td>7</td>
<td>0.22</td>
</tr>
<tr>
<td>amsterdam</td>
<td>7</td>
<td>0.22</td>
<td>minneapolis</td>
<td>6</td>
<td>0.19</td>
</tr>
<tr>
<td>anatum</td>
<td>216</td>
<td>6.91</td>
<td>minnesota</td>
<td>57</td>
<td>1.82</td>
</tr>
<tr>
<td>ardwick</td>
<td>1</td>
<td>0.03</td>
<td>molade</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>arkansas</td>
<td>8</td>
<td>0.26</td>
<td>montevideo</td>
<td>296</td>
<td>9.48</td>
</tr>
<tr>
<td>assen</td>
<td>1</td>
<td>0.03</td>
<td>muenchen</td>
<td>10</td>
<td>0.32</td>
</tr>
<tr>
<td>bareilly</td>
<td>43</td>
<td>1.38</td>
<td>muenster</td>
<td>25</td>
<td>0.80</td>
</tr>
<tr>
<td>bareilly var</td>
<td>1</td>
<td>0.03</td>
<td>new-brunswick</td>
<td>9</td>
<td>0.29</td>
</tr>
<tr>
<td>bere</td>
<td>1</td>
<td>0.03</td>
<td>newington</td>
<td>17</td>
<td>0.54</td>
</tr>
<tr>
<td>binza</td>
<td>135</td>
<td>4.32</td>
<td>Newport</td>
<td>13</td>
<td>0.42</td>
</tr>
<tr>
<td>blockley</td>
<td>6</td>
<td>0.19</td>
<td>nienstedten</td>
<td>3</td>
<td>0.10</td>
</tr>
<tr>
<td>bornum</td>
<td>4</td>
<td>0.13</td>
<td>oranienburg</td>
<td>148</td>
<td>4.74</td>
</tr>
<tr>
<td>braenderup</td>
<td>2</td>
<td>0.06</td>
<td>orion</td>
<td>4</td>
<td>0.13</td>
</tr>
<tr>
<td>brandenburg</td>
<td>2</td>
<td>0.06</td>
<td>panama</td>
<td>6</td>
<td>0.19</td>
</tr>
<tr>
<td>bredeney</td>
<td>130</td>
<td>4.16</td>
<td>pensacola</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>california</td>
<td>20</td>
<td>0.64</td>
<td>poona</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>canoga</td>
<td>3</td>
<td>0.10</td>
<td>reading</td>
<td>3</td>
<td>0.10</td>
</tr>
<tr>
<td>cerro</td>
<td>71</td>
<td>2.27</td>
<td>rubislaw</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>colorado</td>
<td>1</td>
<td>0.03</td>
<td>ruiru</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>cubana</td>
<td>57</td>
<td>1.82</td>
<td>saint-paul</td>
<td>19</td>
<td>0.61</td>
</tr>
<tr>
<td>derby</td>
<td>125</td>
<td>4.00</td>
<td>san-diego</td>
<td>3</td>
<td>0.10</td>
</tr>
<tr>
<td>drypool</td>
<td>52</td>
<td>1.66</td>
<td>schwazengrund</td>
<td>97</td>
<td>3.10</td>
</tr>
<tr>
<td>dublin</td>
<td>1</td>
<td>0.03</td>
<td>sonftenberg</td>
<td>326</td>
<td>10.44</td>
</tr>
<tr>
<td>eimsbuettel</td>
<td>233</td>
<td>7.43</td>
<td>siegburg</td>
<td>35</td>
<td>1.12</td>
</tr>
<tr>
<td>enteritidis</td>
<td>10</td>
<td>0.32</td>
<td>simsbury</td>
<td>4</td>
<td>0.13</td>
</tr>
<tr>
<td>eppendorf</td>
<td>2</td>
<td>0.06</td>
<td>taksony</td>
<td>5</td>
<td>0.16</td>
</tr>
<tr>
<td>essen</td>
<td>1</td>
<td>0.03</td>
<td>tennessee</td>
<td>93</td>
<td>2.98</td>
</tr>
<tr>
<td>give</td>
<td>30</td>
<td>0.96</td>
<td>thielallee</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>godesberg</td>
<td>1</td>
<td>0.03</td>
<td>thomasville</td>
<td>98</td>
<td>3.14</td>
</tr>
<tr>
<td>grumensis</td>
<td>7</td>
<td>0.22</td>
<td>thompson</td>
<td>12</td>
<td>0.38</td>
</tr>
<tr>
<td>grundy</td>
<td>1</td>
<td>0.03</td>
<td>typhimurium</td>
<td>43</td>
<td>1.38</td>
</tr>
<tr>
<td>habana</td>
<td>19</td>
<td>0.61</td>
<td>typhimurium</td>
<td>var copenhagen</td>
<td>7</td>
</tr>
<tr>
<td>halmstad</td>
<td>1</td>
<td>0.03</td>
<td>uganda</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>heidelberg</td>
<td>38</td>
<td>1.22</td>
<td>uno</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>illinois</td>
<td>21</td>
<td>0.67</td>
<td>urbana</td>
<td>8</td>
<td>0.26</td>
</tr>
<tr>
<td>indiana</td>
<td>14</td>
<td>0.45</td>
<td>westhampton</td>
<td>7</td>
<td>0.22</td>
</tr>
<tr>
<td>infantis</td>
<td>98</td>
<td>3.14</td>
<td>worthington</td>
<td>75</td>
<td>2.40</td>
</tr>
<tr>
<td>javiana</td>
<td>2</td>
<td>0.06</td>
<td>Untypable</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>johannesburg</td>
<td>28</td>
<td>0.90</td>
<td>Untypable-Group C1</td>
<td>7</td>
<td>0.22</td>
</tr>
<tr>
<td>kentucky</td>
<td>40</td>
<td>1.28</td>
<td>Untypable-Group C2</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>lexington</td>
<td>20</td>
<td>0.64</td>
<td>Untypable-Group E</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>lillie</td>
<td>8</td>
<td>0.26</td>
<td>3, 10: e, h-z48</td>
<td>3</td>
<td>0.10</td>
</tr>
<tr>
<td>litchfield</td>
<td>1</td>
<td>0.03</td>
<td>Total Isolates</td>
<td>3,124</td>
<td>99.87</td>
</tr>
<tr>
<td>livingstone</td>
<td>97</td>
<td>3.10</td>
<td>Serotyped</td>
<td></td>
<td></td>
</tr>
<tr>
<td>london</td>
<td>4</td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>madelia</td>
<td>5</td>
<td>0.16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A RENDERING PLANT MANAGER'S EXPERIENCE IN SALMONELLA CONTROL

Howard R. Norton, II

I wish to thank you for inviting me. I have met and worked with many of you gentlemen, and I realize that many of you have heard me speak at your Salmonella workshops; therefore, I do not plan to give you "warmed over milk". Rather, I am going to be very straight-forward on the very specific subject of the control of Salmonella in the rendering industry. Control as has been accomplished in the past decade — the present accomplishments — the future accomplishments.

In discussing the Salmonella problem as concerns us renderers, we direct our efforts toward the control of Salmonella on a cooperative basis of State-Federal officials with the rendering industry. May I say before we explore the past, present, and future cooperative joint governmental — industry efforts to control Salmonella, that I am proud to be called one of the nation's renderers, we are not "garbage men, nor junk men", but men in an industry converting solid wastes into useful products through manufacturing. The plant of Norton & Company, has been involved for several years in a cooperative effort to control Salmonella. I wish to express my "thank you" to the men of the Departments of Agriculture of the Federal Government and the state of Virginia. Without their dedicated assistance we could not have accomplished what we have. I think more of our industry people should know of the real dedication of the State-Federal people in our project.

Now let us look into the past decade of Salmonella control.

1. The Recommended Sanitation Guidelines for Salmonella Control (ARD 91-47) was printed in 1964, by Dr. John Walker, USDA, and has been in constant use since then.

2. A method of isolation for Salmonella organisms has been agreed upon and is functioning — ARS 91-68.

3. The quality of Salmonella organisms in the 1960 decade of renderers' proteins has improved tremendously — maybe not as significantly on a "positive" — "negative" evaluation, but the number of organisms found in the "positive" samples has been greatly reduced — indicating progress.

4. The industry and State-Federal officials have conducted numerous workshops on a regional basis.

5. Terminal heaters have been installed in plants where their use is practicable, resulting in tons upon tons of "negative" product going into the feed industry. We realize however, that terminal heating has limitations and is not the complete answer to the control of Salmonella.

6. We have been trying to learn how to decontaminate a plant and keep it decontaminated. We may be approaching an answer.

7. We have spent many dollars and hours in an industry research effort to develop an "antagonist". This work still goes on.

8. We have developed a Cooperative Program.

In summary, the past decade has produced real effort and some worthwhile
results in the control of Salmonella, but there are frustrations — the main frustration — “How do we decontaminate a plant and keep it decontaminated as measured by the finished product of that plant”?

We now should look at the present. Where are we? Confused? Yes — we have a right to be confused as renderers. The “cleanest” looking plants are now always the “negative” plants. Have we all “deified” sanitation and neglected concern for other necessary procedures to control Salmonella in a plant? Let us list where we stand in our control of Salmonella at present:

1. We are working into the Phase II (Plant Improvement) Status, and many plants into the Phase III (Approved) Status under the *Summary of Uniform Methods and Rules of 1970*.

2. We know we need “know-how”. Do you realize that in 1970, we were able to decontaminate our first plant? There was no lack of dedication to the job in our plant — *knowledge* was the missing ingredient. By referring to “dedication”, I mean “Dedication” by our plant people as well as “dedication” by State-Federal people. The “dedication” was there. The *knowledge* had to be found.

3. We have many confused renderers today. They do not know which way to “jump”. Can we operate a decontaminated plant — or is it a lost cause? I don’t feel that they can be criticized for their outlook on the basis of performance to date. I am an “Uncle Tomanella” in the minds of a few of our industry people. I can understand fully why I have earned the “distinction”. I have had the good fortune and tremendous assistance from you men in becoming convinced that a plant can be decontaminated and continue operation as essentially decontaminated. Many renderers have not.

4. We have a *Summary of Uniform Methods and Rules* for the control of Salmonella which requires much over-hauling to become a working document for industry as well as for State-Federal officials. I feel that the several sampling procedures utilized are confusing. In those states whose lab facilities only permit doing five samples at a time, please collect the ten-run five samples — run the remaining five samples and keep our program uniform.

5. We have “neglected” renderers in some states — “Neglected” as far as determining their status under the *Summary of Uniform Methods and Rules* new “Phase Program”. Also, “neglected” renderers are reporting they are not being sampled routinely. Please, gentlemen, realize that you must maintain your end of the bargain on sampling routinely or our “cooperative program” fails. I sincerely request officials of those states harrassed by lack of sampling personnel to come to our industry and “deputize” some of us as samplers to help with the routine sampling.

6. The “Evaluation” Phase of the program has been completed on all plants.

In summary I can say sincerely that we are progressing at present, but not without some confusion and frustrations. I can also say the important fact is that
we are closer than we have been in a decade to the real control of Salmonella in rendered animal proteins.

Let me outline some considerations for the future — these are the result of discussing the control of Salmonella with industry and with State-Federal people.

(1) Our industry needs at least the duration of the next three fiscal years to show some significant results in the control of Salmonella.

(2) We must continue to use our presently adopted isolation procedure (ARS 91-68) to determine the presence of the organism. I feel strongly that we must attain our goal of control of Salmonella by using an identical testing procedure throughout the duration of the program.

(3) We must develop a Summary of Uniform Methods and Rules that can be utilized as "workable" and "effective" for industry and State-Federal officials.

(4) We need to clarify our entire thinking on "who does what" in the program. Permit me to draw a vertical line on the blackboard and indicate what may be the practical answer to "who does what"?

State-Federal Officials Do

(1) Sampling for official status performed on a statistically sound method.
(2) Sampling to assist in locating infection in the system if needed.
(3) Act as a consultant to industry on where trouble may be found.
(4) Determine official status.

Industry People Do

(1) Self-monitor its product to a degree not before thought of as necessary.
(2) Make the unilateral decisions of "what" to do to correct positives.

I am more convinced than ever before that State-Federal officials must work with industry members to more clearly define their areas of effort. Our program is a program to control Salmonella in the finished animal protein product, not a program to specify this or that specific plant improvement. Our program is a cooperative program — at this point cooperation is more important than ever if we are to accomplish the control of Salmonella. Who can argue with "negatives"?

Thank you.
SALMONELLA COMMITTEE REPORT

A. A. Erdmann, Madison, Wisc., Chairman


Salmonellosis is of major concern to public health officials, regulatory agencies, and the processors of food items containing livestock and poultry products.

East Coast residents were reminded of its seriousness in July, 1970 when 26 deaths due to Salmonella infection occurred in a Baltimore, Maryland nursing home. Even before the source of the outbreak had been established, news accounts called attention to the fact that poultry products are major reservoirs for the disease.

The basic problems related to Salmonellosis in the United States were adequately defined in the report of the National Research Council, National Academy of Science, "An Evaluation of the Salmonella Problem." This committee feels that this report is an accurate appraisal of the Salmonella situation in the United States and that it offers practical recommendations for ameliorating the Salmonella problem in the United States. We urge the State and Federal regulatory agencies, public health agencies, and the industries involved to implement those recommendations in the report that relate to their activities.

The committee has directed its attention to the following areas:

(1) A review of past recommendations of this committee.
(2) Improvements needed in the program to control Salmonella in rendering plants and industrial fish plants.
(3) Salmonella in environmental pollution.

PAST RECOMMENDATIONS

The committee notes that the following recommendations made in previous reports of the committee have not been implemented:

(1) The committee directs attention to Resolution No. 4 of the 1968 Report of the Committee on Nomination, Resolution, and Internal Affairs which was adopted by the executive committee of this association.

This resolution requested the Animal Health Division of the Agricultural Research Service, U.S. Department of Agriculture to publish on a regular periodic basis a summary of the recovery of Salmonellae by species, by host species or source, and by geographical location of origin as confirmed by the National Animal Disease Laboratory and other approved serotyping laboratories.

This information is essential for an accurate appraisal of changes in the Salmonellae situation and the effectiveness of controlled activity. This information is readily available from the reports of serotyping at the various laboratories.
(2) Standards for vehicles used for handling and transporting rendered animal by-products and fish meal, for storage facilities and for terminal facilities to assure that the products are being handled in such a manner to prevent contamination as mentioned in the 1968 Report of this committee, have not been developed. It was noted in the 1969 Report that the Government-Industry-Carrier Committee was developing recommended standards for rail cars used in handling and transporting these products. But these standards have not been developed to date. We urge the industries involved to take the lead in voluntarily developing all the aforementioned standards; thereby, eliminating the need for State and Federal regulatory agencies to develop such standards.

(3) The committee recommended in its 1969 Report that a concentrated effort be made to encourage blenders to purchase rendered material from plants producing a negative product or to develop a process of terminal treating to insure a negative blended finished product. The need for this effort is even greater, now that the evaluation phase of the program has been completed. It is possible for negative rendered material from several establishments to be mixed with positive material from a single producer at a blending establishment and thereby contaminate the blender's total production. The overall effect is to negate the success that had been achieved by those plants whose product was negative.

The committee recommends not only blender, but all classes of feed processors and feed mixers attempt to acquire protein supplement from plants that are producing a negative product or have met the Uniform Methods and Rules requirements for designation as an approved establishment. If this is done, the purchasing practices of the industry will eliminate the need for additional regulations and enforcement activity.

IMPROVEMENTS NEEDED: SALMONELLA PROGRAM

(1) The objective of the Salmonella Program in rendering plants and industrial fish establishments is to achieve the eventual elimination of Salmonella in feed ingredients of animal and marine origin through the voluntary efforts of individual plant owners.

The program is based upon the cooperative efforts of the representative of the Animal Health Division, the State agency, and the individual plant management. Success in achieving the goals of the program will require each agency to discharge its function in the program. The committee notes that only 20 states made the equivalent of 3 inspections and 30 samples collected from each plant (fish meal plants excluded due to seasonal operation) in the state in FY 1970.

Until the plants have established an approved inplant sanitation program, we believe the minimum level of satisfactory activity for the program should be 3 inspections per year.

We recommend that each state give sufficient priority to the program to permit
the equivalent of 4½ inspections per plant with the collection of 10 samples per inspection (the equivalent of 45 samples per plant) during a 12 month period.

Since the evaluation phase is completed and the status of all plants is known, the allocation of individual inspections and sample collections to specific plants should be based upon the plant needs within a state. The state average, however, would be 4½ inspections with the collection of 45 samples per plant.

To achieve continued progress in the program, we urge that the individual states give recognition to those plants that meet the requirements of the Salmonella Uniform Methods and Rules for designation as approved plants. Approval should be done at the state level.

Program emphasis during the coming year should be placed upon identifying these problems that are preventing plants from moving through the clean-up phase to approved status.

The report from the National Academy of Science recommended that imported foods, feeds and drugs should meet the same standards as those imposed on domestic products.

We concur with this recommendation and recommend that the appropriate Federal agencies, Food and Drug Administration (Department of Health, Education and Welfare), and the U.S. Department of Agriculture take the necessary steps to implement this recommendation by:

1. Requiring certification of sampling and laboratory examinations for Salmonellae by a specified procedure with negative results in the country of origin for imported fish meal and meat and bone meal.
2. Increase in the testing of the lots of fish meal and meat and bone meal imported into the United States.

LABORATORY

The committee recognizes the work of Dr. Ned Robey in calling attention to the apparent emergence of variant, Salmonella that ferment lactose and are not recovered by standard procedures and techniques. This committee directs that the American Association of Veterinary and Laboratory Diagnosticians review ARS 91-68 and develop a protocol for conduct and application of fluorescent antibody tests for tissues and feed ingredients.

ENVIRONMENTAL POLLUTION

The committee notes the increased concern that is being shown for the serious and complex problems of environmental degradation. In the first annual report of the President's Council on Environmental Quality, waste from feed lots was cited as a key source of water pollution. In the 1967 Report of this committee, attention was called to the increased incidence of Salmonellosis in livestock as a result of surface water contamination and the need to acquire more information regarding potability of water supplies for livestock. Waste from beef cattle, poultry, and
swine feeding operations, along with dairy farms, are major sources of actual or potential pollutants and a solid waste problem. Recent studies have shown that Salmonella can be detected with a relative ease in many surface waters. It is reasonable to expect increased effort will be directed toward the control and prevention of the contamination of our rivers and streams with Salmonella.

Recent reports in literature by a number of investigators working independent of each other (Snoeyenbos and Co-Workers, University of Massachusetts; Sadler and Co-Workers, University of California; Pomeroy and Co-Workers, University of Minnesota) have identified the Salmonella contaminated poultry house as a significant source for the perpetuation of Salmonella infection in successive flocks of poultry raised on the same premises. The Agricultural Research Service is urged to acquire, evaluate and disseminate information regarding practical procedures for the elimination of Salmonella from contaminated premises.

It is noted that various programs for control of Salmonella in poultry have been initiated by individual companies. These programs are being tailored to operations in individual premises or flocks. Your committee hopes that these studies will prove to be a successful means to produce animals free of Salmonella.

There is an urgent need for information on the prevalence of Salmonella in the environment where livestock are raised, concentrated and marketed. Without this information, an accurate appraisal of the magnitude of the Salmonella problem is impossible. Today, we do not know how many farms and premises are contaminated with Salmonella. In 1968, a survey by Consumer and Marketing Service, U.S. Department of Agriculture showed the following percent of livestock and poultry to be carrying Salmonella in their intestinal tract at the time of slaughter:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>0.4%</td>
</tr>
<tr>
<td>Swine</td>
<td>10.7%</td>
</tr>
<tr>
<td>Chickens</td>
<td>3.8%</td>
</tr>
<tr>
<td>Turkeys</td>
<td>5.8%</td>
</tr>
<tr>
<td>Sheep</td>
<td>0.0%</td>
</tr>
<tr>
<td>Horses</td>
<td>14.9%</td>
</tr>
</tbody>
</table>

We need to know how many premises or farms are represented in these percentages for proper evaluation of the effectiveness of the various control activities, including the elimination of Salmonella from animal feeds.

A practical procedure using systematic statistical sampling techniques is available to acquire this information.

GOVERNMENT/INDUSTRY/CARRIER COMMITTEE

Your committee calls upon the Government/Industry/Carrier Committee appointed by Dr. E. E. Saulman, ARS, to actively study and report corrective steps that can be taken to improve the sanitary condition of vehicles used for transporting feed and feed ingredients.
The committee considered suggested changes to three paragraphs of the SUMR (Part 1, paragraph C, Part III, paragraph 3a, and 3b (2)). The committee recommended that the three suggestions be adopted into the program administratively and not as changes in the language of the SUMR (Uniform Methods and Rules). The Administrative determination should be based upon a plant's past history pertaining to sampling and test results.

The following resolutions adopted by AAVLD were received, endorsed, and forwarded to the Executive Committee for final action.

RESOLUTION

A genus specific fluorescent antibody conjugate and counterstain have been developed by Diagnostic Bacteriology, Diagnostic Services, Animal Health Division, for the detection of salmonella in animal feeds and by-products. Recent data indicate that the technique has considerable promise as a definitive procedure. Until the evaluation of the technique is completed, the Committee recommends that it be officially approved as a salmonella screening procedure.

The following resolution was adopted by the American Association of Veterinary Laboratory Diagnosticians at the 1970 annual meeting.

Whereas, We believe that diagnostic reagents are an integral part of every laboratory; and

Whereas, We believe that many diagnostic reagents now in use by veterinary diagnostic laboratories do not meet minimum quality standards; and

Whereas, Diagnostic reagents are not tested and licensed; therefore,

RESOLVED, That all diagnostic reagents sold or produced for interstate shipment in the United States and for use in veterinary diagnostic laboratories, be tested to insure minimum standards of accuracy and dependability by the Veterinary Biologics Division with the support of the Animal Health Division.
### FISCAL YEAR 1970 – RENDERING PLANT INSPECTIONS

**PER CENT OF EXPECTED INSPECTIONS**

<table>
<thead>
<tr>
<th>Area</th>
<th>Accomplished At Three Insp./Yr.*</th>
<th>Accomplished At 4½ Insp./Yr.**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eastern Area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connecticut</td>
<td>216</td>
<td>140</td>
</tr>
<tr>
<td>Kentucky</td>
<td>121</td>
<td>81</td>
</tr>
<tr>
<td>Maine (NH)</td>
<td>Main (100)-NH(100)</td>
<td>Maine (69)-NH(100)</td>
</tr>
<tr>
<td>Massachusetts (RI)</td>
<td>Mass. (127)-RI(267)</td>
<td>Mass. (86)-RI(200)</td>
</tr>
<tr>
<td>Michigan</td>
<td>144</td>
<td>96</td>
</tr>
<tr>
<td>New Jersey</td>
<td>88</td>
<td>58</td>
</tr>
<tr>
<td>New York</td>
<td>102</td>
<td>69</td>
</tr>
<tr>
<td>Ohio</td>
<td>44</td>
<td>29</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>53</td>
<td>30</td>
</tr>
<tr>
<td>Vermont</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>West Virginia</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>North Central Area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illinois</td>
<td>134</td>
<td>89</td>
</tr>
<tr>
<td>Indiana</td>
<td>68</td>
<td>46</td>
</tr>
<tr>
<td>Des Moines, Iowa</td>
<td>78</td>
<td>52</td>
</tr>
<tr>
<td>Ames, Iowa</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Kansas</td>
<td>101</td>
<td>68</td>
</tr>
<tr>
<td>Minnesota</td>
<td>416</td>
<td>279</td>
</tr>
<tr>
<td>Missouri</td>
<td>102</td>
<td>68</td>
</tr>
<tr>
<td>Montana</td>
<td>117</td>
<td>78</td>
</tr>
<tr>
<td>Nebraska</td>
<td>104</td>
<td>70</td>
</tr>
<tr>
<td>North Dakota</td>
<td>92</td>
<td>61</td>
</tr>
<tr>
<td>South Dakota</td>
<td>106</td>
<td>71</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>154</td>
<td>102</td>
</tr>
<tr>
<td>Wyoming</td>
<td>142</td>
<td>94</td>
</tr>
<tr>
<td><strong>Southern Area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alabama</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Arkansas</td>
<td>73</td>
<td>49</td>
</tr>
<tr>
<td>Delaware</td>
<td>83</td>
<td>55</td>
</tr>
<tr>
<td>Florida</td>
<td>115</td>
<td>78</td>
</tr>
<tr>
<td>Georgia</td>
<td>95</td>
<td>64</td>
</tr>
<tr>
<td>Louisiana</td>
<td>113</td>
<td>76</td>
</tr>
<tr>
<td>Maryland</td>
<td>155</td>
<td>104</td>
</tr>
<tr>
<td>Mississippi</td>
<td>66</td>
<td>44</td>
</tr>
<tr>
<td>North Carolina</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>South Carolina</td>
<td>73</td>
<td>50</td>
</tr>
<tr>
<td>State</td>
<td>Value</td>
<td>Value</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Tennessee</td>
<td>95</td>
<td>63</td>
</tr>
<tr>
<td>Virginia</td>
<td>150</td>
<td>100</td>
</tr>
<tr>
<td>Western Area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alaska</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>Arizona</td>
<td>147</td>
<td>100</td>
</tr>
<tr>
<td>California</td>
<td>100</td>
<td>67</td>
</tr>
<tr>
<td>Colorado</td>
<td>160</td>
<td>107</td>
</tr>
<tr>
<td>Hawaii</td>
<td>116</td>
<td>78</td>
</tr>
<tr>
<td>Idaho</td>
<td>93</td>
<td>62</td>
</tr>
<tr>
<td>New Mexico</td>
<td>56</td>
<td>37</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>Oregon</td>
<td>183</td>
<td>122</td>
</tr>
<tr>
<td>Texas, Austin</td>
<td>44</td>
<td>29</td>
</tr>
<tr>
<td>Texas, Mission</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Utah</td>
<td>93</td>
<td>63</td>
</tr>
<tr>
<td>Washington</td>
<td>114</td>
<td>76</td>
</tr>
<tr>
<td>Nevada</td>
<td>133</td>
<td>92</td>
</tr>
</tbody>
</table>

* All states with values of less than 100 did not make the equivalent of 3 inspections per plant.

** All states with values of less than 100 did not make the equivalent of 4.5 inspections per plant.
EPIDEMIOLOGIC STUDIES ON SWINE TUBERCULOSIS

Charles O. Thoen, D.V.M. and Alfred G. Karlson, Ph.D
Rochester, Minnesota

Tuberculous adenitis in the United States is in most cases due to *Mycobacterium avium*. In some areas the so-called Battey bacilli, which are similar to avian bacilli, have been isolated from such lesions but their occurrence in this country is not known to be of importance. Little information is available concerning the source of *M. avium* in swine. We do not know if they persist in the soil or if they are carried by birds or animals and excreted into the soil and water.

To obtain a better understanding of some of the sources of mycobacterial infection in swine, we attempted to isolate mycobacteria from swine and to compare them with those from the environment on the premises of origin, including wild birds, domestic fowl, and soil and water.

**METHODS**

Lymph nod lesions from one carcass from each of seven lots of tuberculosis swine were collected at an abattoir, transferred to the laboratory in refrigerated containers, and examined bacteriologically and histologically. From one of the lots, lesions from two additional carcasses were obtained; cultures from these three carcasses from one lot were serologically identical and two of these were not studied further.

Each of the cultures of acid-fast bacteria (representing seven different premises) was subsequently identified by animal pathogenicity tests which included the inoculation of guinea pigs, rabbits, and Japanese quail.\(^1\) In addition the cultures were typed by serologic procedures.\(^2\) Lymph nodes of the swine were examined histologically, including use of appropriate stains for acid-fast bacilli.

Simultaneous tuberculin tests using avian and mammalian tuberculins were conducted on swine and other animals present on each of the seven premises (located in the southern part of Minnesota) from which the infected swine had originated. Free-flying birds, including pigeons, sparrows, starlings, and bluejays, were collected for bacteriologic studies from some of the farms. Soil and water samples were collected for culture from each of the farms. On one farm, innumerable bedbugs (*Cimex lectularius*) were present in the poultry house and a number of these were collected for culture. The investigation also included culture, for mycobacteria, of tissues from some reactor swine and from reactor and nonreactor chickens on the farms.

---

Mayo Graduate School of Medicine (University of Minnesota), Rochester: Fellow in Microbiology (Dr. Thoen). Mayo Clinic and Mayo Foundation: Department of Microbiology and Immunology (Dr. Karlson). Dr. Thoen is on assignment from U.S. Department of Agriculture.
RESULTS

Examination of Original Lesions. — Histologically the lesions varied from caseous abscesses to noncaseating tubercles. Acid-fast bacilli were seen in smears or in histologic sections of lesions from six of the seven carcasses. A positive culture for *M. avium* was eventually obtained from the one lesion which was microscopically negative. Positive cultures for mycobacteria were obtained from six of the seven lesions. The lesion from which no mycobacteria could be isolated was histopathologically typical of tuberculosis and did have many acid-fast bacilli in a smear and in the histologic section.

Five of the six cultures of mycobacteria were pathogenic for Japanese quail and rabbits but not for guinea pigs and were therefore identified as *M. avium*. Serologically these were found to be *M. avium* serotype II. The culture which failed to cause progressive disease in Japanese quail and in rabbits was subsequently identified serologically as a so-called Battey strain, serotype Davis.

The guinea pigs used in the pathogenesis studies were tested for tuberculosis sensitivity with Agricultural Research (ARS) avian O.T. (1:25 dilution) and with two different avian tuberculins, trichloroacetic acid-precipitated proteins (TCAP), 0.01 mg. The latter two were made from different cultures of *M. avium* serotype I, one of which appears to have a combination of serotype I and serotype Watson antigens.* Each of the avian tuberculins elicited significant reactions in guinea pigs infected with each of the isolated including the serotype Davis. The ARS avian O.T. (1:25 dilution) caused reactions equal to or greater than the reactions to TCAP.

Tuberculin Tests on Premises. — Simultaneous tuberculin tests were made on various species on each farm using 0.1 ml. of ARS avian O.T. and 0.1 ml. of ARS mammalian O.T. (Table).

The proportion of swine showing sensitivity reactions to ARS avian O.T. (injected into the vulva) varied from 6 to 71% among the different farms. Although some swine responded to ARS mammalian O.T., the responses to ARS avian O.T. usually were two to five times larger. One cow on each of two farms (no. 1 and 4) developed a 10-mm circumscribed response (caudal fold) to avian tuberculin; however, no significant skin reactions to mammalian tuberculin were observed in these animals or in other cattle tested.

Comparative tuberculin tests were made by injecting different tuberculin in separate wattles of chickens on five of the premises. All flocks showed responses to avian tuberculin and a few birds in some flocks responded to mammalian tuberculin. The responses to avian tuberculin were on the average four times larger than the responses to mammalian tuberculin. It was of interest that, on one farm (no. 1), two dogs over 4 years of age did not react to avian tuberculin whereas two dogs less than 1 year of age did react. Cats on three of the six premises which had them showed suspicious responses to avian tuberculin. No cats of dogs tested showed skin sensitivity to ARS mammalian O.T. Suspicious responses to avian

*Identified by Dr. Werner Schaefer, M.D., Denver, Colo.
tuberculin but not to mammalian tuberculin were observed in a raccoon on premise 1, in a guinea hen on premise 2, and in a duck on premise 6.

**Laboratory Studies on Animals and Materials From Premises.** — Chickens, from each of five premises, sensitive to avian tuberculin were autopsied. Acid-fast vacculi were identified microscopically in liver and spleen lesions in chickens on four of the premises. Isolates from chickens were of the same serotype as isolates from the swine from the corresponding premise. Swine reacting to avian tuberculin on some premises were traced to slaughter, and lymph nodes were collected for bacteriologic and histologic examination. Acid-fast bacilli resembling *M. avian* were found on culture, and serologic identification of these isolates is in progress.

*M. avian* was cultured from a bluejay present on premise 1; this isolate was pathogenic for Japanese quail and rabbits and serologically was *M. avium* serotype II, the same as the *M. avium* isolated from swine and chickens on the same premises. Mycobacteria were not isolated from other wild birds ( sparrows, pigeons, and starlings). *M. avium* was isolated from a suspension of about 50 bedbugs collected from a poultry building housing tuberculous chickens on premise 1; this isolate was identified as *M. avium* serotype II and was identical to that isolated from the bluejay, the 16 reactor chickens, and the swine present on the premise.

Water and soil samples from each farm were treated with equal volumes of 0.3% quaternary ammonium chloride (Zephiran) and with 6% sulfuric acid, respectively, as decontaminants. All were negative for acid-fast microorganisms on culture.

**DISCUSSION**

The findings of *M. avium* in swine on farms where avian tuberculosis occurs in chickens as well as in wild birds provides an explanation for the source of the infection. On five of the seven farms there were unconfined flocks of hens more than 1 year old. However, this does not explain the mode of transmission. Whether or not swine may transmit the infection to other swine may be a matter of speculation. It is known that tuberculous ulcers of the intestinal mucosa may be caused by *M. avium* in swine and thus spread the organism by feces. It is also known that *M. avium* as well as other mycobacteria in soil on these farms were fruitless but perhaps more extensive studies should be made using a variety of techniques.

It may be of interest that wild geese and ducks were frequently observed in large numbers on three of the premises. On one of these, where there was no other known contact with fowl, the swine were in a cornfield where many wild geese stopped to feed during migration.

The incidence of tuberculosis in swine as determined by reports of the Federal Meat Inspection Services. Such data are based on gross examination, and it is possible that the actual rate of infection may be higher. A number of reports indicate that *M. avium* may be isolated from apparently normal animals. The so-called Battey bacilli isolated from lesions in man and animals are of interest because of their distribution in nature and their possible relationship to *M.*
avium. The Davis serotype and the Watson serotype have been isolated from man and from swine.9,10 Perhaps extensive studies on the origin and transmission of these avian-like mycobacteria in swine could provide information of value on the epidemiology of such infections in man. Although M. avium has been isolated from man, no epidemiologic studies have been made to determine the source and mode of transmission.

SUMMARY

Lesions of tuberculous adenitis in a swine carcass from each of seven different Minnesota farms were cultured for mycobacteria. M. avium serotype II was isolated from five and a Davis serotype of mycobacterium was found in one; lesions in one swine had acid-fast bacilli which could not be cultured. Five of the seven farms of origin had unconfined flocks of old chickens. Chickens and swine tested on some farms had reactions generally greater to avian than to mammalian tuberculin. No significant reactions to mammalian tuberculin were found in cattle even when M. avium infection could be demonstrated in chickens and swine on the same farm. M. avium serotype II was isolated from chickens and swine on some farms as well as from a bluejay. M. avium serotype II was isolated from Cimex lectularius present in a poultry house on one farm. No isolates of mycobacteria could be made from soil of water.
Simultaneous Tuberculin Tests on Animals on Farms Where Tuberculous Swine Originated

<table>
<thead>
<tr>
<th>Animal</th>
<th>Tuberculin used*</th>
<th>Promises†</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4±</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine</td>
<td>A</td>
<td></td>
<td>5/44</td>
<td>3/4</td>
<td>9/13</td>
<td>9/12</td>
<td>1/16</td>
<td>1/13</td>
<td>2/22</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>2/44</td>
<td>1/4</td>
<td>5/13</td>
<td>4/12</td>
<td>0/16</td>
<td>0/13</td>
<td>0/22</td>
</tr>
<tr>
<td>Cattle</td>
<td>A</td>
<td></td>
<td>1/6</td>
<td>NT</td>
<td>NP</td>
<td>1/20</td>
<td>0/27</td>
<td>NP</td>
<td>0/19</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>0/6</td>
<td></td>
<td></td>
<td>0/20</td>
<td>0/27</td>
<td></td>
<td>0/19</td>
</tr>
<tr>
<td>Chickens</td>
<td>A</td>
<td></td>
<td>132/206</td>
<td>16/204</td>
<td>NP</td>
<td>3/6</td>
<td>6/35</td>
<td>2/7</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>14/206</td>
<td>2/204</td>
<td>0/6</td>
<td>1/35</td>
<td>0/7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs</td>
<td>A</td>
<td></td>
<td>2/4</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>NT</td>
<td>0/2</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>0/4</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td></td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>Cats</td>
<td>A</td>
<td></td>
<td>1/4</td>
<td>0/1</td>
<td>0/2</td>
<td>0/1</td>
<td>1/2</td>
<td>1/4</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>0/4</td>
<td>0/1</td>
<td>0/2</td>
<td>0/1</td>
<td>0/2</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>Ducks</td>
<td>A</td>
<td></td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>0/3</td>
<td>NP</td>
<td>1/6</td>
<td>NP</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td></td>
<td>0/3</td>
<td></td>
<td></td>
<td></td>
<td>0/6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*A = ARS avian O. T.; M = ARS mammalian O. T.
†Results = number of reactors/total number tested. NT = not tested; NP = none present.
‡No mycobacteria isolated from lesion of swine from this farm; original specimen had acid-fast bacilli and was histologically compatible with tuberculosis.
REFERENCES

THE STATUS OF THE STATE-FEDERAL TUBERCULOSIS ERADICATION PROGRAM

by
A. F. Ranney, D.V.M., M.S., and
J. D. Roswurm, D.V.M., M.P.H.

One year ago, Dr. Frank Wheeler, as president-elect of this organization, stood before us and with sincerity born of clear thinking pointed to the way that we must go to fulfill our respective responsibilities to ourselves, to the livestock industry, to the consumer, and to the taxpayer.

He said, "In the future, animal health officials will no longer be able to ignore coming to grips with potential disease problems until after the disease has become established and the threat a reality. This is the way it was done in the past — the easy way. It turned out to be, however, the most expensive, least professional way, and certainly did not exemplify leadership on the part of animal health officials."

The problem of bovine tuberculosis was allowed to grow as a cancer in this country until the disease brought the livestock industry to the brink of disaster. Only then was a concerted effort begun to control the spread and clean up the population. In spite of the handicap of a late start, and in an atmosphere of distrust and apathy, the leaders in animal health realized that the program they were launching had to be designed to preclude the possibility that bovine tuberculosis would ever again threaten the livestock industry. At that point in history, our predecessors set the goal of total eradication of bovine tuberculosis.

In the year 1917 — the year after the number of cattle and hogs that were consigned to seven midwestern markets and were condemned for tuberculosis would have filled 50 trains of 40 cars each — Dr. J. A. Kiernan, Chief, Tuberculosis Eradication Division, U.S. Bureau of Animal Industry, had the vision and foresight to say "... it is my belief that if this nation succeeds in eradicating tuberculosis in 50 years, it will be one of the greatest heritages our successors will have handed down to them."

We are the successors of whom Dr. Kiernan spoke; the 50 years was up in 1967. Tuberculosis in cattle and hogs has not been eradicated, but eradication of bovine-type tuberculosis in cattle and hogs is in sight. The goal can be achieved in this decade if we are willing to come to grips with the problem.

It has been said that bovine tuberculosis is no longer a serious economic problem in the United States. Dr. Wheeler, in speaking of our approach to problems in the past, said, "Presently we are guilty of this same negligence as there is an urgent need to develop eradication programs for diseases that are already well established and spreading — yet are virtually unchallenged."

1Chief Staff Veterinarian, Tuberculosis Eradication, Animal Health Division, Agricultural Research Service, United States Department of Agriculture.
2Staff Veterinarian, Tuberculosis Eradication, Animal Health Division, Agricultural Research Service, United States Department of Agriculture.
How much of this negligence is due to the drain on our money and manpower by halfhearted efforts to work at old programs that should have been completed years ago? If we allow bovine tuberculosis eradication to drag on another 25 years, the waste in program time and money alone will be a serious economic loss, to say nothing of losses due to diseases that might be halted with the same funds and manpower we waste.

The Uniform Methods and Rules provide a yardstick to measure the tuberculosis program in each State; however, there exist wide variations in the effectiveness of the program in the various States.

Dr. Kiernan said in 1918, "We have too much at stake not to resort to every means that may be put into practice at any time to extirpate this foe. We are compelled to consider this disease from a national standpoint rather than from 48 sectional viewpoints. The livestock industry of each State is but a part of the agricultural resources of the Nation."

And in 1969 Dr. Wheeler said, "In spite of the fact that State-Federal programs are supposed to be the same throughout the United States, there are alarming differences."

The Uniform Methods and Rules are designed not only to provide guidelines to clean up the known infected herds and to protect against the spread of disease from these herds, but they are also designed to insure minimum standards for continual surveillance to detect previously unknown infection. A number of States are actively pursuing a goal of 100 percent effective surveillance by identification through slaughter and selective area tuberculin testing to ferret out the remaining infection while they quickly dispose of the known infected herds. These States have shown remarkable progress in the space of a few years. Many other States, we are sorry to report, fail to meet even minimum standards in some areas of the program and pose a continuing threat to the two million clean herds in the country.

The map (Figure 1) divides the country by States according to the time interval since \( M. \text{bovis} \) was confirmed in a cattle herd in each State. Of the number of States that have not had a confirmed \( M. \text{bovis} \) herd for sometime, only three States and the Virgin Islands are recorded as having Accredited Tuberculosis Free status. In most cases the failure to qualify rests not with the amount of known \( M. \text{bovis} \) in the State, but with deficiencies in surveillance, traceback, or protection against reinfection. The designation of an area as Accredited Tuberculosis Free has little meaning unless we can have a high degree of confidence in the accuracy of that status.

Let us look at the status of the program according to the herds reported with infection in fiscal year 1970 (Figure 2). The breakdown of numbers represent herds with no record of previous infection and herds reported during the year with a history of infection in one or more prior years. A total of 50 infected herds were reported. This is a significant drop from 70 herds reported in fiscal year 1969. Even more significant is the drop from 50 new herds in fiscal year 1969 to 25 new herds this past year (fiscal year 1970). These drops are significant but in what way?

Two possibilities are most likely. The obvious explanation is that increased
herd depopulation and increased attention to known infected herds has reduced the spread of the disease and lowered the prevalence. We must, however, examine the other side of the coin and ask whether our surveillance efficiency has decreased to the point where we are finding a smaller proportion of the newly infected herds. Is the number of herds with undiscovered infection remaining steady or even increasing? We must not overlook the possibility of there being five to ten tuberculosis herds unknown to us for every one that is known.

During the early sixties, concentration of Red Flag herds produced dramatic results. The graph (Figure 3) shows that we have reached a plateau and that the number of Red Flag herds has shown little change since the middle sixties. The map (Figure 4) shows the location of the current Red Flag herds and the relative number of years they have been infected. It should be noted that six of the 23 Red Flag herds have had infection for 11 or more years.

Turning to our progress in herd depopulation (Figure 5), the percent of infected herds destroyed during the past year rose slightly from 30 to 32 percent, but the number of herds destroyed dropped from 21 to 16. There appears to be danger of stagnation here also.

We mentioned the other side of the coin — surveillance efficiency (Figure 6). The number of suspicious granulomas reported during the year declined this year and is continuing to decline. Of the 1,025 6-35 cases (reports of non-reactors showing tuberculous lesions or thoracic granulomas) completed during fiscal year 1970, 240 cases were suspicious of or confirmed M. bovis. Fifty percent were reported as successfully traced to a herd of origin. The graph shows that this is the second year in a row with a reported decline in tracing success.

On the other hand, the confirmation rate in suspicious cases remains remarkably stable (Figure 7). As we approach eradication and the reduction of M. bovis, the percent of suspicious lesions due to other Mycobacteria will increase. We must expect that when M. bovis is eradicated some cattle will continue to be sensitive to tuberculin and suspicious lesions will be found from which it will be impossible to isolate M. bovis.

Looking at only the confirmed 6-35 cases for fiscal year 1970 (Figure 8), we see that further infection was located as a result of tracing 27 percent of the cases, while no further infection was found in the remaining 73 percent.

We know that cattle are not marketed in a random manner. Herd owners tend to sell in the same channels of trade year after year. Each year we succeed in tracebacks leading to the infected herds that are the easiest to find. The undiscovered infected herds that remain are more difficult more difficult to locate with each succeeding year. Thus, we must constantly expand and improve our identification and traceback system just to maintain our current level of progress.

Now let us turn from our statistics and their questionable interpretation to some projections and possibilities for the future.

In connection with our Program Planning and Budget System, a study was made to project our current progress and the expected progress of several alternate approaches to the program.
The graph (Figure 9) depicts the estimated number of undiscovered tuberculosis cattle herds in the country that we can expect each year in the future under several different conditions. Eradication, at the bottom of the graph, is defined as the absence of undiscovered infected herds in our cattle population. The model for this graph ignores the effect of infection entering from outside the country.

The first line projects our current rate of progress and estimates the year of eradication to be 1995.

The second line is a projection of the current program with destruction of 95 percent of the infected herds beginning in fiscal year 1972. This projection estimates that increased depopulation could speed eradication by 9 years and save an estimated $130 million in total program costs.

The third line projects the present program changed to increase the discovery rate of the new infected herds by 50 percent. This could be accomplished through improvement in cattle identification and a tenfold increase in the number of granulomas submitted for laboratory examination from regular kill. It is estimated that this improvement in the program alone would bring eradication in the year 1983 with a saving of $100 million.

The fourth line projects the results with both increased herd depopulation and an increased herd discovery rate beginning with fiscal year 1972. These two factors together would have the effect of drastically reducing disease spread and accelerating the discovery of unknown infected herds. From this projection it is estimated that eradication would occur in fiscal year 1979 at a program savings of $168 million as compared to our present rate of progress.

These projections, of course, are fallible; and we do not know that they truly predict the future. However, several things are made very clear by this study. Two important factors are shown to have a great effect on the time required to finish the job:

1. Curtailing the spread of tuberculosis through herd depopulation and tight quarantine measures is essential.
2. Effective surveillance and traceback will increase our ability to find infected herds and also decrease spread.

The model also shows that all measures to speed eradication, even at increased costs now, will save many millions of dollars.

The third and most important point is that it requires time to harvest benefits from program improvements so that we must act now if we are to prevent a long and wasteful conclusion to the bovine tuberculosis eradication program.

We have the prospect of seeing the eradication of this disease in this decade if we are willing to make the necessary commitments and the detailed follow through to get the job done. But we must start now! We cannot delay, we must charge with full speed ahead!

Uniformity must be returned to the program. Each State must evaluate its own practices. All States need to look at what has been done and is being done in States that are rapidly eliminating the disease from their cattle. All States should examine their animal identification, lesion collection, and traceback systems and make every
possible improvement at the earliest possible moment. This must be done in States with little or no known infection as well as States where infection is known to exist. As long as one State has a problem, all are threatened.

It is encouraging to note (Figure 10) that more than half of the States now require reactors to be shipped direct to slaughter to alleviate the spread of infection in marketing channels.

Quarantine practices in all States should be examined to insure that spread cannot occur from known infected herds and that feeding and grazing animals of questionable status are not permitted to infect our breeding herds. The map (Figure 11) shows that most of the States have the legal means to depopulate infected herds.

False positive and false negative test results are often blamed as major deterrents to tuberculosis eradication. Careful follow up of infected herds by trained veterinarians and herd depopulation will adequately handle the false negative problem and eliminate most, if not all, repeat infection in Red Flag herds.

We are conducting and sponsoring studies on tuberculins. A field trial to study the specificity of two *M. bovis* PPD's (Purified Protein Derivative) will begin soon. Field testing of a comparative test, using bovine and avian PPD's that are biologically balanced, will also begin within the next few months. Plans are being made for field testing of a new type of tuberculin developed at Michigan State University. This product holds promise of being highly specific. If controlled studies on this tuberculin remain favorable, field trials should get underway within a year.

We have reason to hope that improved tuberculins will become available. Continued insistence, however, on complete reporting of tuberculin responses and careful technical supervision by trained epidemiologists, plus judicious branding of animals, will clarify the status of most problem herds.

We have received complaints that if we enforce our regulations, owners will not cooperate. It has been our experience that livestock owners in trouble because of diseased stock will sometimes complain bitterly at the measures necessary to restore their herd to a healthy status. However, they will usually forgive us or thank us later for everything except leaving the disease with them. Half measures can leave an owner worse off in the long run and endanger other herds that are now free of tuberculosis.

Last year Dr. Wheeler stated, "...the 1970's will present the United States Animal Health Association its greatest challenge and its greatest opportunity. It will be a very crucial and trying decade for us. Our success or failure will depend on what we do or fail to do today." The long and trying campaign against bovine tuberculosis has come to the point where victory is within our grasp is we will just come to grips with the problems now.

Like Dr. Wheeler, we are confident that this organization along with our allied organizations are still capable of the dedication and leadership to see a tough job through to the finish. If we fulfill our moral obligation and do what has to be done now, bovine tuberculosis can be eradicated from our cattle population before 1980.
Success requires constant vigilance — the cost of failure is too high.

FIGURE 1

Tuberculosis Eradication
TIME INTERVAL SINCE LAST REACTOR CONFIRMED M. BOVIS
July 1, 1970

Less than 1 Year
1-3 Years
4-6 Years
7 Years or More

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH DIVISION
FIGURE 3

Tuberculosis Eradication

239

"RED FLAG"

HERDS*

50


*as of July 1, Yearly

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH DIVISION
FIGURE 4

CURRENT "RED FLAG" HERDS

July 1, 1970
FIGURE 5

Tuberculosis Eradication

TUBERCULOSIS INFECTED HERDS

<table>
<thead>
<tr>
<th>Year</th>
<th>% of Herds Destroyed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1966</td>
<td>9</td>
</tr>
<tr>
<td>1967</td>
<td>16</td>
</tr>
<tr>
<td>1968</td>
<td>22</td>
</tr>
<tr>
<td>1969</td>
<td>30</td>
</tr>
<tr>
<td>1970</td>
<td>32</td>
</tr>
</tbody>
</table>

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH DIVISION
FIGURE 6

TRACEBACK RESULTS - LESION CASES
REGULAR KILL

PERCENT
100
80
60
40
20
0


UNSUCCESSFUL AND INCONCLUSIVE
SUCCESSFUL

(Completed Cases Suspicious & Confirmed M. bovis)

U.S. DEPARTMENT OF AGRICULTURE ANIMAL HEALTH DIVISION AGRICULTURAL RESEARCH DIVISION
FIGURE 7

Tuberculosis Eradication

SUSPICIOUS AND CONFIRMED LESIONS CASES
REGULAR KILL

TOTAL CASES: 194 232 295 267 240


% CONFIRMED M. BOVIS

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH DIVISION
FIGURE 8

TRACING CONFIRMED M. BOVIS CASES

REGULAR KILL

27% TRACED TO INFECTED HERDS

73% NO FURTHER INFECTION FOUND

FY 1970-55 CASES
FIGURE 9

BOVINE TUBERCULOSIS ERADICATION PROGRAM
PROJECTION AND THREE ALTERNATE APPROACHES

ERADICATION IN F.Y.
PRESENT TB PROGRAM 1995
1. DESTRUCTION OF 95% OF INFECTED HERDS 1986
2. 50% INCREASE IN INFECTED HERD DISCOVERY RATE 1983
3. COMBINATION OF INCREASED HERD DESTRUCTION & INCREASED HERD DISCOVERY 1979

UNCOVERED TUBERCULOSIS INFECTED HEIFERS OF CATTLE

FISCAL YEAR

US DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
FIGURE 10

Tuberculosis Eradication

 STATES REQUIRING SHIPMENT OF REACTORS
DIRECT TO SLAUGHTER

July 1, 1970

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH DIVISION
FIGURE 11

Tuberculosis Eradication

AUTHORITY FOR HERD DEPOPULATION
July 1, 1970

- Force Depopulation and Pay Indemnity for Exposed Cattle
- Pay Indemnity for Exposed Cattle Only
- Neither

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH DIVISION
COLORADO'S BOVINE TUBERCULOSIS TRIAL

Mitchell A. Essey

This past April Dr. W. C. Tobin, Colorado State Veterinarian, stood trial by jury in the District Court of Denver. The charge — negligence. Three Colorado dairymen who had incurred extensive losses under Colorado's Bovine Tuberculosis Program charged "the defendant, Dr. William C. Tobin, negligently performed his duties as a veterinarian and employee of the State of Colorado, and on insufficient and faulty evidence declared certain of the plaintiffs cattle to be disease and caused said cattle to be destroyed." "The plaintiffs pray damages against the defendant . . . that total $740,000 . . . and further pray for their costs, interest and for such other relief that court may deem appropriate."

The plaintiffs charged personal negligence. What was challenged and tried, however, was Colorado's Tuberculosis Eradication Program and, in turn, the national program. Their attacks were against the tuberculin test itself and against our criteria for establishing the existence of bovine tuberculosis. Central to their case was the existence of non-specific tuberculin sensitivity, and the incidence of NGL Reactors.

The trial lasted three days. Only the plaintiffs case was stated, after which the defense moved for dismissal. This was granted. The court ruled that "the case be dismissed because the plaintiffs failed to show any evidence of negligence on the part of the defendant. In all cases Dr. Tobin upheld the state law. That, no man had a right to modify, neither to add to, nor subtract from. This is his prime concern, all other considerations are little relevant and are but opinions and conjectures by contrast to the statutes under which the program is administered. Case dismissed."

It was not appealed.

This trial demonstrated that individuals in highly responsible governmental positions can be held personally liable for losses incurred under programs they are duty bound to administer. Pre-trial decisions in this case denied Dr. Tobin sovereign immunity as an agent of the state; and, paradoxically, denied him a 2 year statute of limitations that applied in individual liability cases. He was caused to incur the 6 year statute of limitation that applied in sovereign matters!

Legal assistance for this case was furnished by Colorado's Attorney General's office. We had excellent legal talent in two young attorneys but it took a great deal of research on their part, and tutoring on our part to familiarize them with the medical and disease eradication principles involved.

Case histories. For the most part, the claimed losses occurred under Colorado's original Bovine Tuberculosis Statute, written in 1923, that made no distinction between tuberculin responses and tuberculous animals.

Herd I problems dated from 1962, when early tests revealed reactors, of which one was condemned for generalized tuberculosis. Several demonstrated thoracic and other lesions. Periodic testing continued to produce reactors, mostly NGL, until by Dec. 1964, 142 of this 450 cow herd had been sent to slaughter because of the tuberculin test.
Herd II entered the picture July 1964 when TB Reactors were disclosed on a test conducted for change of ownership. By Dec. 1964, 111 reactors, all NGL, had left this herd of 600 initial count. This herd was considered tuberculous, however, during 1962 and 1963 when of eighty reactors several were reported with thoracic and other lesions indicative of Bovine Tuberculosis. It was released after two negative tests and was not retested until 2½ years later when the test for transfer of ownership initiated a long string of lesionless cases.

Herd III made an abrupt entry in Dec. 1964 when a retest, to meet Colorado import requirements, revealed reactors and led to a herd test. Result, 41 TB Reactors in 150 head tested. Lesions submitted to NADL from one with hepatic portal and thoracic lesions were “compatible” with a diagnosis of tuberculosis. Two others displayed lesions that were not submitted for confirmation. A confirmed lesion (hepatic) was submitted from a non-reactor yearling steer a month later.

Beginning March 1965 all three herds became participants in Special Tuberculosis Study No. 16 (known as the Colorado Project), a well founded 3 year study to determine the cause of causes of Tuberculin Sensitivity in Colorado Dairy Cattle. These herds were given a special dispensation to permit the use of special tests without the necessity of classifying tuberculin responding animals as TB Reactors. This study revealed, for purposes of this report, herd sensitivity levels ranging from 74-96% of animals tested by the cervical route, and no other evidence of tuberculosis.

Non-official caudal fold testing showed conclusively that continued test and slaughter would have quickly depopulated herd III, and would have ultimately done the same with herd I. For example, the first caudal fold retest of herd III yielded responses in 41% of the animals tested. As it turned out, however, Herds I and III that followed this project to completion experienced two successive drops of 84% each in caudal fold sensitivity, and were released from quarantine.

The owner of Herd II, on the other hand, rejected its “Project Herd” status after an initial cervical test (94% herd response level), and demanded resumption of the test and slaughter program. To the credit of the defendant, it was documented that Dr. Tobin had appealed to the Colorado Agricultural Commission, and to the owner, his conviction that tuberculosis no longer existed in this herd and to resume official testing would result only in needless losses with no assurance that the real problems would be resolved in any way. Only the advent of Colorado’s Bovine Tuberculosis Statute of 1967 that permitted the use of TB Suspect classification, prevented the complete depopulation of this herd as a result of sensitivity to tuberculin. Resumption of testing produced an additional 56 Reactors. Of the remainder of the herd, most were classified as Suspects on one test or another.* Significantly this herd that yielded 11 reactors at the onset, all NGL, provided us with our first clear picture of extensive non-specific sensitivity in the absence of

*On the final test this herd revealed 9 responses in 324 animals tested (only 4 were 2X, P1 or greater). The only change in herd management was the adoption of phosphorus supplementation in line with the principal finding of Special Tuberculosis Project No. 16.
tuberculosis, and was the herd most instrumental in the updating of Colorado’s tuberculosis statute.

This brief resume of specific herd histories, rendered as uncomplicatedly as possible, was necessary to make the following points. (1) By criteria still valid today, Bovine Tuberculosis existed at one time in all three herds. Standard eradication procedures successfully eliminated the infection from all three (2) Non-specific sensitivity was a distinct entity that obscured the end point of specific sensitivity. This problem was resolved under prevailing circumstance in the best possible way, albeit novel, by a Special Project, and (3) Upon clear demonstration for the need, there occurred an up-dated Bovine Tuberculosis Statute in complete accord with the Uniform Methods and Rules.

I would like to close on a thoughtful note. This trial closed as it should have. The outcome reaffirms that good laws and good programs, when firmly adhered to, will withstand the most serious of challenges. The very nature of our work, however, lends itself to such challenges. If a state official is to function in the best interests of such programs, he can expect extreme situations such as this to arise; but then, he should expect the best help possible. Dr. Tobin received the best assistance available under our present structure. But, was this enough? Should we not begin to consider sharpening our legal defenses? Perhaps the answer lays in the creation of a medically oriented legal team that has a total grasp of even the most salient aspects of disease eradication principles. Perhaps we need only provide that the transcripts of such trials be gathered at a central location, analyzed, and such information made available to states or individuals finding themselves in need for a defense. Perhaps certain aids exist that all are not presently aware of. I submit that we should ferret out whatever aids we have in order to determine what we need. Only after we recognize our weaknesses can we take the steps necessary to eliminate them. Thank you.
REPORT OF THE COMMITTEE ON TUBERCULOSIS AND PARATUBERCULOSIS

D. S. Ingraham, Harrisburg, Pa., Chairman

This past year has been a very active one for members of the committee. A coordinated plan to achieve the goal of eradication of bovine tuberculosis from cattle by 1979, was developed by Animal Health Division, State-Federal Meat Inspection Programs, and the Tuberculosis Committee of this organization. The proposed plans were presented by these three groups at the four regional meetings of this Association not only to stimulate interest in renewed effort toward eradication but also to provide the committee with information about special problems that affect eradication in each region. We are encouraged with the general attitude toward tuberculosis eradication which has been expressed to us. The concern to accomplish eradication and not tolerate \textit{M. bovis} infection anywhere is significant. The committee's responsibilities to improve the Uniform Methods and Rules based on the results of these meetings has been discussed in detail. The committee has decided that a major revision of the Uniform Methods and Rules is necessary to provide each state with adequate minimum standards for tuberculosis eradication recognizing the varied disease and livestock management conditions in all states. The committee plans to meet during the coming year to complete the revision.

The committee has prepared a comprehensive questionnaire to provide the Committee on Evaluation and Development of State-Federal Programs with facts concerning all phases of the tuberculosis eradication program in each state. It is the intent of the committee to provide minimum standards to achieve eradication in all states. It is again recommended by this committee that the eradication procedures used in each state be evaluated periodically to assure full compliance with the Uniform Methods and Rules. Many states have developed their programs beyond these minimum standards and enforce more stringent requirements. The leadership of these states is commended.

Dr. W. C. Tobin, State Veterinarian, Colorado and Dr. M. A. Essey, Tuberculosis Epidemiologist, ANH, Colorado, presented a summary of the legal action brought by three herd owners against Dr. Tobin as state veterinarian. This suit challenged the tuberculosis eradication program in Colorado. Even though the suit was dismissed, your committee believes certain details should be made available to state and federal regulatory veterinarians. It requests that this special report be published in the proceedings of this organization. Brief reports concerning special
tuberculosis problems in several states were presented to the committee. It was pleased with the progress that has been made by State-Federal cooperating officials in attacking and solving problems that have plagued those states for years.

A progress report was presented by Dr. W. L. Mallman of results obtained with the sensitin, Band 24, applied as a skin test to cattle infected with *M. bovis*, *M. avium* and Group III mycobacteria. Preliminary studies are very encouraging and we hope they will lead to an improved tuberculin. Additional research in the development of specific blood tests were described and demonstrated. Your committee is hopeful that the eradication program will benefit soon from this research.

It has been called to the attention of the committee that there are no universally accepted standards for tuberculin testing nonhuman primates. Your committee recognizes the need for the standardization of such procedures. It, therefore, offers its cooperation with interested groups to assist in the preparation of appropriate standards based on the recommendations of specialists in this field.

As the incidence of bovine tuberculosis declines and we approach eradication, our concern for the introduction of tuberculosis from other countries increases. We are aware that tuberculosis lesions are being found in cattle that only a few months prior to slaughter originated from outside of the United States. The committee recommends that the import regulations of the United States Department of Agriculture be amended as soon as possible to require that all cattle, including steers, except cattle for immediate slaughter, shall pass a tuberculin test within 60 days prior to entry and shall be individually identified by ear tag or other satisfactory means. This committee further recommends that immediate attention be directed to this problem and that import regulations of the United States Department of Agriculture be amended, no later than January 1, 1973, to require that all cattle entering this country, except those for immediate slaughter, meet the following conditions:

1. Cattle to be accepted only from countries that have an official progressive bovine tuberculosis eradication program comparable to our Uniform Methods and Rules.
2. Cattle to come directly from:
   (a) herds that meet standards in the country of origin equal to those from accredited herds as outlined in our Uniform Methods and Rules, or
   (b) areas that meet the standards in the country of origin equal to those for bovine tuberculosis free areas as outlined in the Uniform Methods and Rules, or
   (c) herds with no evidence of bovine tuberculosis for at least two years prior to being offered for entry in areas that meet standards in the country of origin equal to those for modified accredited status as outlined in our Uniform Methods and Rules. The individual animals must pass a tuberculin test within 60 days prior to entry, or
   (d) herds with no evidence of bovine tuberculosis for at least two years prior to being offered for entry which have passed a complete herd...
test within twelve months and the individual animals must pass a tuberculin test within 60 days prior to entry and be retested in not less than 60 days after arrival in this country and remain under quarantine for an additional 60 day retest.

The subcommittee on Johne's disease, consisting of Drs. A. B. Larsen, W. D. Yoder, and A. R. McLaughlin, submitted suggested uniform guidelines for handling infected herds and the standardization of procedures to establish Johne's disease-free herds, known as the "Individual Qualified Herd Plan." These suggestions were endorsed by the committee. A copy of these guidelines has been made part of this report.

Mycobacterium paratuberculosis can be found in the semen of infected bulls. The organism will survive antibiotic treatment used in processing. This committee recommends that bulls used for artificial insemination should be fecal-cultured and found free of Johne's disease.

REPORT OF THE SUBCOMMITTEE ON JOHNE'S DISEASE
GUIDELINES FOR CONTROL OF JOHNE'S DISEASE (PARATUBERCULOSIS)

The following procedures are recommended for the control of Johne's disease in cattle.

Definitions:

Infected Herd — A herd which contains animals with laboratory confirmed Johne's disease confirmed by histopathological examination or culture.

Infected Animal — An animal with confirmed clinical disease or shedding Mycobacterium paratuberculosis in the feces. (Probability is high that calves of infected dams are also infected even though the causative organism cannot be isolated from their feces.)

Negative Animal — An animal that is negative to a test and culture for M. paratuberculosis.

Exposed Animals — Other animals in an infected herd.

Suspect Animal — An animal with symptoms resembling those of Johne's disease which has not yet been confirmed by laboratory examination.

Cleaning and Disinfecting — Those procedures employed in the tuberculosis eradication program. Manure should not be spread on land which will be used for grazing or forage within two years.

Positive Fecal Culture — Isolation and identification of M. paratuberculosis by an individual who has received special training in the Mycobacteriology Section, Diagnostic Services, ANH Division, NADL, Ames Iowa.

Control Procedures:

1. Cattle may be sold only to quarantined feedlots, as defined in CFR part 78 (1) (v), or directly to slaughter except calves of negative dams. These calves shall be at least six months of age, separated from the herd at birth
and raised apart, tested and found negative to johnin not more than 30 days prior to sale.

2. Animals with persistent diarrhea or other clinical signs of Johne’s disease shall be isolated until the cause is determined.

3. Fecal culture of all animals, two years of age and older, semi-annually with removal of positive animals and their offspring.

4. Cleaning and disinfecting of premises after removal of infected animals.

5. Purchases limited to johnin-negative animals from herds with no history of disease.

6. Continued surveillance (at intervals of not less than five nor more than seven months) until there have been four consecutive negative fecal cultures of all animals two years of age and older.

7. Every effort should be made to assure a nutritionally adequate, balanced ration. The Ca:P ratio should be within normal limits.

8. Herd additions should be limited to:
   a. Cattle from qualified herds.
   b. Cattle from herds with no clinical history of Johne’s disease which have been tested and found negative to johnin not more than 30 days prior to purchase.

9. If artificial insemination is used, semen should come from negative bulls.

**Individual Qualified Herd Plan**

1. There shall have been no clinical Johne’s disease in the herd within the past three years.

2. On two tests at least five and not more than seven months apart, all cattle six months of age and older shall have no increased skin thickness greater than three mm. to an intradermal test applied in the cervacal region using 0.2 ml. ARS johnin, read at 48 hours post-injection. These tests shall be made by or under the supervision of a full-time employed State or Federal veterinarian.

3. At the same time as the johnin test, fecal samples shall be collected from each animal over two years of age in accordance with ANH Division Memorandum 587.24, dated July 16, 1969, and submitted for culture of *M. paratuberculosis*. This examination shall be conducted by a qualified individual as stated in the definitions.

4. In the event that skin thickness increases greater than 3 mm. are found in one or more animals although no fecal positive animals are found, at least two additional negative fecal samplings shall be required. Samplings shall be at five to seven months intervals and shall include all animals two years of age and older in the herd. (Since a minimum of 200 negative samples are required, smaller herds will require additional samplings.)

5. Herds which have met the above requirements shall be designated as “Johne’s Qualified” for a period of one year following the report of the last fecal culture.
6. To maintain "Qualified" Status, negative fecal cultures shall be required of all animals two years of age and older at 10 and 14 month intervals.

7. There shall be no co-mingling of animals with goats, sheep, or swine.
# AMERICAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS

## CONTENTS

13th Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians
(formerly Conference of Veterinary Laboratory Diagnosticians)

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>502</td>
</tr>
<tr>
<td>515</td>
</tr>
<tr>
<td>522</td>
</tr>
<tr>
<td>527</td>
</tr>
<tr>
<td>537</td>
</tr>
<tr>
<td>549</td>
</tr>
<tr>
<td>561</td>
</tr>
<tr>
<td>568</td>
</tr>
<tr>
<td>572</td>
</tr>
<tr>
<td>577</td>
</tr>
<tr>
<td>584</td>
</tr>
<tr>
<td>589</td>
</tr>
<tr>
<td>601</td>
</tr>
<tr>
<td>607</td>
</tr>
<tr>
<td>620</td>
</tr>
<tr>
<td>624</td>
</tr>
</tbody>
</table>

Comparison of Tissue Section and Cell Culture Immunofluorescent Techniques for the Detection of Hog Cholera Infection in Experimentally Infected Pigs — by E. A. Carbrey, H. A. McDaniel, W. C. Stewart, E. J. Henry, and J. I. Kresse

Use of the Microtiter Serum Neutralization Test for the Diagnosis of IBR, BVD, and Other Bovine and Porcine Viral Diseases — by J. W. Black

Investigation of Feed Related Porcine Hemorrhagic Disease — by G. D. Osweiler

Salmonella Infection in Ontario Market Swine — by B. I. Groves, N. A. Fish, and D. A. Barnum


Fat Soluble Toxicants - Intoxication and Detoxication — by D. J. Wagstaff, and J. C. Street

Isolation and Identification of Obligate Anaerobic Bacteria — by A. R. Dommert

Immunodiffusion Test for Equine Infectious Anemia — by L. Coggins, and V. Patten


Preliminary Studies in Interferon Induction on the Respiratory Tract of Cattle — by A. B. Angulo, and M. Savan

The Fluorescent Antibody Technique in the Diagnosis of Bovine Respiratory Virus Disease — by L. van der Heide

Observations on Thromboembolic Meningoencephalitis (TEM) in Cattle in Indiana Feedlots — by H. J. Olander, A. M. Gallina, D. Beckwith, and M. Morrow

Comparison of Techniques for the Diagnosis of IBR Virus Abortion — by E. J. Bicknell, and D. E. Reed

Parainfluenza 3 Virus Infection of Tracheal Organ Cultures — A. B. Angulo and M. Savan

Diagnostic Methods for Detection of Paratuberculosis (Johne’s Disease) — by R. S. Merkal

Histological Technique for Diagnostic Laboratories — by D. C. Tudor
The Diagnosis and Control of Anaplasmosis — by H. D. Anthony ............ 633
The Passive Hemagglutination Test in Avian Virology — by E. G. Trewick and
G. Lang ............................................................... 637
Techniques for Detection of Infection of Chickens with the Herpesvirus of
Marek's Disease — by S. H. Kleven, C. S. Eidson, and D. P. Anderson ... 650
The Operation of a Mastitis Diagnostic Laboratory — by H. D. Emmette, and
D. S. Postle ............................................................ 657
Examination of Specimens from Suspected Hog Cholera Cases by the
Fluorescent Antibody Tissue Section and Cell Culture Techniques — by E.
J. Henry, and H. A. McDaniel .................................... 664
Neonatal Immunohemolytic Disease in a Georgia Beef Herd — by F. E.
Mitchell and H. C. Morgan ....................................... 668

Constitution and Bylaws may be found in the Seventy Third Proceedings of
the USAHA — 1969
A.A.V.L.D. COMMITTEES FOR 1970-71

OFFICERS OF 1971
Dr. H. L. Chute, Chairman
Dr. N. W. Rokey, Chairman-elect
Dr. E. P. Pope, Secretary-Treasurer

Committee on Program Credentials, and Ways & Means
N. W. Rokey, Chairman
J. F. Frank
T. B. Ryan
W. C. Bolton
M. J. Twiehaus
N. W. McIntyre
W. W. Worcester

Committee on Continuing Education
W. L. Sippel, Chairman
M. E. Bergland
V. A. Seaton
E. S. Bryant

Committee for Hog Cholera Standard Techniques
L. W. Turner, Chairman
H. A. McDaniel, Secretary
L. G. Morehouse
H. L. Rubin
T. B. Ryan
V. A. Seaton
M. K. Twiehaus

Committee on International Symposium
J. C. Miller, Chairman
V. A. Seaton
E. P. Pope
E. E. Wedman

Committee on Wildlife and Zoo Diseases
L. A. Griner, Chairman
L. H. Karstad
D. O. Trainer
M. E. Macheak
R. P. Houk
T. P. Kistner
L. A. Page
T. P. Kistner
L. A. Page

Committee on Recognition
D. M. Bedell

Committee on Biological Security
D. H. Smith, Chairman
D. Johnson
E. J. Bicknell
J. C. Jefferies
E. Broughton
D. R. Cassidy
C. John Mare

Committee on Accreditation
J. G. Miller, Chairman
S. R. Nusbaum
L. G. Morehouse
W. W. Worcester
D. E. Cooperrider
W. E. Ketter

Committee on Animal Disease Reporting and Surveys
N. E. Hutton, Chairman
D. E. Cooperrider
H. D. Anthony
C. D. Murphy

Committee on Salmonella
E. M. Ellis, Chairman
J. E. Williams
B. S. Pomeroy
J. C. Olson, Jr.
E. T. Mallinson
W. J. Martin
P. Middaugh
N. Insulata
G. H. Snoeyenbos

Representatives to the National Academy of Sciences on Veterinary Microbiological Standard Methods
H. W. Dunne
W. L. Sippel

Committee for Recommended Standard Techniques for Diagnosing Bovine Respiratory Diseases
E. A. Carbrey, Chairman
L. N. Brown
T. L. Chow
R. F. Kahrs
D. G. McKercher
L. K. Smithies
T. W. Tomoglia

Committee on Equine Infectious Anemia
E. A. Carbrey, Chairman
J. E. Pearson
W. W. Kirkham
W. L. Kadel
L. Coggins
E. E. Roth
J. R. Gorham
D. Barnett

Committee on Reference Assistance
L. W. Turner, Chairman
W. E. Lyle
H. D. Anthony
L. G. Morehouse
D. E. Cooperrider

Committee on Resolutions
W. E. Lyle, Chairman
C. S. Roberts
F. E. Mitchell

Committee on Resolution Assistance
Southern Region 1973
Northesatern Region 1974
North Central Region 1972
Western Region 1971
Secretary 1971
Assistant Secretary 1971
1970-71 OFFICERS OF THE A.A.V.L.D.

H. L. Chute
*Chairman*

Dr. Ned Rokey
*Chairman-Elect*

Dr. E. E. Pope
*Secretary-Treasurer*
COMPARISON OF TISSUE SECTION AND CELL CULTURE IMMUNOFLUORESCENT TECHNIQUES FOR THE DETECTION OF HOG CHOLERA INFECTION IN EXPERIMENTALLY INFECTED PIGS

by
E. A. Carbrey, VMD, MS; H. A. McDaniel, DVM, PHD; W. C. Stewart, DVM, MS; E. J. Henry, BA, MS; and J. I. Kresse, BS

SUMMARY
Appropriate sets of tissues for hog cholera (HC) examination by the fluorescent antibody tissue section technique (FATST) and the fluorescent antibody cell culture technique (FACCT) were collected from 122 pigs and their identities were concealed by code numbers. The pigs were arbitrarily divided into two categories according to the experimental treatment received; 48 were classified as "Hog Cholera Exposed" and 74 as "Hog Cholera Free or Immune."

In the first category 32 sets of tissues were found positive and 3 negative by agreement in the findings of both techniques. Of the remaining 13 sets, 8 were negative by the FATST and positive by the FACCT; and 5 were positive by the FATST and negative by the FACCT.

Of the 74 pigs in the "Hog Cholera Free or Immune" category, all were found negative by the FATST and 72 were negative by the FACCT. The 2 sets of tissues found positive by the FACCT and negative by the FATST were from pigs exposed to bovine virus diarrhea (BVD) virus.

The differences in the results of the two fluorescent techniques were not statistically significant.

The effect of serum therapy on the diagnostic procedures was studied in detail by inoculating 10 out of 20 HC sick pigs with 1.0 ml of serum per lb. body weight and killing one pig a day from each group sequentially.

INTRODUCTION

The laboratory confirmation of hog cholera (HC) infection by means of a fluorescent antibody conjugate prepared from the serum of a pig hyperimmunized against HC virus has been of great assistance to the HC eradication program. Two different techniques employing this conjugate have been developed and applied extensively in veterinary diagnostic laboratories.

From the Virology Section (Carbrey, Stewart, and Kresse) and the Pathology Section (McDaniel, and Henry) Diagnostic Services, Animal Health Division, National Animal Disease Laboratory, P.O. Box 70, Ames, Iowa.

Appreciation is expressed to Mr. G. D. Booth for the statistical analysis and to personnel of Diagnostic Services, Animal Health Division for assistance and consultation.
The fluorescent antibody cell culture technique (FACCT) was first described by Mengeling et al. and involved the staining of HC infected PK-15 cell cultures after overnight incubation. The cell cultures were exposed to blood or suspension of spleen tissue (33-1/3%) from the affected pig.

During the same year Stair et al. described a fluorescent antibody tissue section technique (FATST) in which thin sections were cut from tissues of infected pigs on a cryostat, fixed, and stained with the HC conjugate. This technique was later modified by the use of a blocking or inhibition procedure by Henry and McDaniel. The FACCT has been evaluated on both experimentally infected pigs and specimens from field outbreaks and found to be a sensitive method for the isolation and identification of hog cholera virus. Studies have also been conducted on the FATST and the technique has been found to be a rapid and accurate procedure for detecting HC viral antigen in tissues. However, several laboratories have reported studies in which the two techniques were compared.

Ressang and den Boer reported both procedures to be of relatively equal merit when all features were considered. However, the cell culture method confirmed 4 field cases of HC that were missed by the tissue section technique. The cell culture technique was considered more complicated but had the advantage of being able to replicate the small amounts of virus present in some tissues.

Torlone et al. infected 40 pigs with HC virus, 20 orally and 20 i.m. and killed the pigs at various stages of the infection. Tissues from the pigs were examined by the FATST and the FACCT. The PK-15 cell culture method was found to give the best results for "constancy and accuracy." However, the tissue section method was considered to be rapid and efficient and both techniques were employed routinely in their diagnostic work.

Meyling and Schjerning-Thiesen compared the techniques on 20 experimentally infected pigs, 10 with a virulent strain of HC virus and 10 with a weakly virulent strain. Tissues from the pigs infected with the virulent strain were positive by both the FATST and the FACCT. Although tissues from 8 out of the 10 pigs given the weakly virulent strain were positive by the FATST, HC virus was not isolated from any of the 10 pigs by cell cultures. However, primary pig kidney cell cultures derived from 6 month old pigs rather than PK-15 cell line cultures were employed in the FACCT.

Kubin and Kolbl found that the two techniques were accurate in confirming HC infection in experimentally infected pigs. However, with tissues submitted from the field the FATST was positive on only 75% of the cases from which HC virus was isolated by the FACCT. This difference was attributed to tissue deterioration during shipment that affected the quality of the frozen sections but did not inactivate the virus.

Unfortunately the designs of these comparison studies did not include some means of coding the tissues so that the workers would not be biased by previous knowledge of the history, clinical signs, and necropsy findings. A blind study was needed to make a valid comparison between the FATST and the FACCT and to acquire specific knowledge concerning their true limitations. This experiment was
performed for this purpose.

In addition to healthy pigs and pigs exposed to HC virus, other pigs were given HC antiserum after the onset of clinical signs, some pigs were immunized and inoculated with virulent virus, and a large number of pigs were exposed to a strain of bovine virus diarrhea (BVD) virus that was virulent for cattle. The knowledge that the HC fluorescent antibody conjugate will stain BVD infected cells was the reason for the inclusion of BVD infected pigs in the experiment.

The appropriate tissues for each technique were placed in separate bags and identified only by number. Great care was taken to conceal the experimental treatment of all of the pigs until the completion of the study.

MATERIALS AND METHODS

Viruses. — The HC virus strains employed included the Ames strain, a highly virulent strain administered as 1.0 ml of blood collected from a febrile pig with acute signs of HC, and a variety of field strains isolated from specimens submitted to the laboratory. The virus strain employed in the serum therapy trial was first inoculated in a test pig and classified as of low virulence since the pig developed a chronic course of the disease and died 32 days postinoculation (DPI). The spleen suspension obtained from this pig produced 175 virus plaques per ml of inoculum with the FACCT.

The BVD virus employed was the NADL-Md strain*, 4th passage in embryonic bovine kidney (EBK) cell cultures, obtained from a specific pathogen free (SPF) calf inoculated in the course of another experiment. The virus suspension contained $10^5$ TCID$_{50}$ per ml as determined by titration in EBK cell cultures.

Pigs. — The pigs employed were first or second generation SPF pigs weighing 30 to 50 lbs.

Fluorescent Antibody Cell Culture Technique (FACCT). — The HC virus isolation technique was performed with PK-15 cell cultures as previously described and standardized. The organs selected for the FACCT were spleen, tonsil, and mandibular lymph node. Two sets of PK-15 cell cultures were inoculated, one with spleen suspension and the other with a pooled suspension of tonsil and mandibular lymph node.

Fluorescent Antibody Tissue Section Technique (FATST). — The detection of HC viral antigen was performed on frozen sections of tonsil as previously described and standardized.

Buffy cell cultures for Hog Cholera Virus. — Pig blood was collected in a sterile manner into a flask containing sufficient heparin to make a final concentration of 0.3 mg percent. The buffy cell cultures were prepared according to the method of Hess and DeTray. Spleen suspension as prepared for the FACCT was diluted 1-10 and 1-100 in Earle's balanced salt solution and 0.1 ml of each dilution was

---

* NADL-Md strain of BVD virus was kindly supplied by Dr. A. L. Fernelius, Veterinary Sciences Research Division, NADL, 90 Dayton Road, Ames, Iowa 50010.
inoculated in 3 Leighton tube cultures of buffy cells. The cultures were incubated at 37°C for 6 days and then frozen and thawed rapidly. The fluid from the culture tubes was subcultured for HC virus on PK-15 cell cultures.

**Tissue Collection.** — Tissue specimens were collected from 122 pigs as described in the next section, “Experiment and Results.” Sections of tonsil, spleen, and mandibular lymph node were placed in a plastic bag for the FACCT and a section of tonsil and, in some cases, spleen, mandibular lymph node, and ileum, were placed in a separate bag for the FATST. The bags of tissue were each placed in second plastic bags containing a strip of cardboard marked with the same code number. This number was recorded with the ear tag number of the pig and this record was concealed until the examinations by both techniques were completed. The coded bags of tissue were stored at -80°C. The FATST and FACCT were performed and reported by the code number in the bag. After all the tissues were examined the code was broken and the findings were evaluated.

**Fluorescent Antibody Serum Neutralization (FASN) Test.** — The neutralization test for antibody titers against HC virus was performed on sera samples as previously described.4

**Antiserum** — The Hc antiserum given to the pigs was obtained commercially.**

**EXPERIMENT AND RESULTS**

Although the 122 pigs in the experiment received a variety of treatments it was considered of interest to tabulate all of the findings for evaluation. Some pigs were immune when exposed to virulent virus and others received HC antiserum after developing clinical signs of acute infection. The pigs were arbitrarily classified according to the treatment received as “Hog Cholera Exposed” or “Hog Cholera Free or Immune.”

Of the 48 pigs placed in the first category 25 were inoculated with strains of HC virus isolated from specimens submitted from the field. These pigs were killed at from 2 to 15 DPI or permitted to die of the infection. A group of 12 pigs was inoculated with specimen tissues that did not contain HC virus and was later inoculated with the Ames strain to challenge their immunity. These pigs died or were killed in a moribund state at from 7 to 12 DPI. Another group of 10 pigs was inoculated with 1.0 ml of HC antiserum per lb. body weight 3 days after inoculation with a field strain of low virulence. The tissues were collected from these pigs by killing one a day starting the day after serum therapy. The remaining pig in this category was vaccinated with inactivated HC vaccine and inoculated with Ames strain 23 days later. This pig developed clinical signs of HC and was still sick when killed 14 days after inoculation with the challenge virus. The findings obtained on these 48 pigs were summarized (Table 1).

**Hog Cholera Antiserum, Serial No. B-781, Diamond Laboratories, Inc., Des Moines, Iowa.**
The two techniques were in agreement on 32 sets of tissues found positive and 3 sets found negative. There was disagreement in the results on 5 sets for which the FATST was positive and the FACCT was negative and on 8 sets for which the FACCT was positive and the FATST was negative.

The 3 sets found negative by both techniques were taken from pigs that were killed 8, 9, and 10 days after antiserum therapy (1.0 ml per lb. body weight). These pigs were clinically normal when killed although they had been quite sick at 3 DPI when the serum was administered.

Three of the 5 sets of coded tissues found positive by the FATST and negative for virus isolation by the FACCT were from pigs that were killed 5, 6, and 7 days after the serum therapy. Another set was obtained from the pig that was vaccinated with inactivated HC vaccine and inoculated with the Ames strain, 23 days later. This pig reacted severely to the challenge virus and was still sick when killed 14 days later. The remaining set of tissues was obtained from a pig with acute signs of HC killed 5 days after inoculation with a field strain of low virulence. After the code was broken a second attempt at virus isolation on this set of tissues was positive with a high count of fluorescent virus plaques per ml of spleen suspension.

Of the 8 sets of coded tissues found negative by the tissue section technique and positive by the cell culture technique, 7 were from pigs killed early in the course of HC infection. Five of the pigs were killed 2 DPI and 2 pigs, 3 DPI. The pigs were inoculated with HC virus strains isolated from field outbreaks. Six of these pigs had pyrexia and inappetence and the remaining pig had a subnormal temperature and was depressed. The remaining set of tissues was from a pig with acute HC infection that died 6 days after inoculation with a field virus isolate. Slight lesions of HC were found on necropsy but high counts of virus infectious units were recovered in cell culture from the spleen and tonsil lymph node pool, 4000 and 4900 units per ml of tissue suspension, respectively.

Of the 122 pigs from which coded specimens were collected 74 were placed in the category "Hog Cholera Free or Immune." Six of these were healthy pigs randomly selected for negative controls. Another group of 17 pigs was killed at the termination of an erysipelas vaccine trial. All were healthy with the exception of one unvaccinated, control pig that developed clinical signs of erysipelas infection and was sick when killed 9 days after inoculation with the challenge strain of *Erysipelas rhusiopathiae*.

Another group of 40 pigs was exposed to BVD virus, 16 by the nasal route, 14 intramuscularly, and 10 were placed in close contact with 3 calves that were infected with BVD virus and developed clinical signs of the disease. The pigs from each of these 3 trials were killed sequentially at the rate of 2 pigs per day starting with the 3rd day after inoculation or, in the case of the calf trial, 3 days after the initial viremia in the calves. The intention was to obtain tissues from pigs while the BVD virus was actively replicating in the cells and before the antibody response had taken place. Additional data was recorded in the nature of clinical signs, total white blood cell counts, and virus culture in a bovine cell system.\(^3\)

The remaining 11 pigs placed in this category were actively immunized by
vaccination or by inoculation with avirulent field strains of HC virus. Eight of these pigs were vaccinated with an inactivated vaccine and inoculated with the Ames strain 23 days later. The pigs became sick 4 days after receiving the challenge virus and had temperatures ranging from 105.0 to 107.6 F. The pigs remained sick through the eighth day and then recovered. Fourteen days after inoculation with the challenge virus the pigs were killed and coded sets of tissues were collected.

Two of the pigs in this group were given field strains of HC virus and were immune when inoculated with Ames virus, 14 and 26 days later. The pigs remained healthy after the challenge and were killed 13 and 8 days later.

The remaining pig was inoculated with 136 ml of tissue suspension which was a pool of suspensions from 3 different submissions all from the same herd. The pig was immunized by HC virus in the specimen and remained healthy following inoculation with the challenge virus. There was some delay in disposing of this pig and before any action was taken the pig died. On necropsy, lesions of acute anemia and blood destruction were found. Death was attributed to acute isoemolytic anemia caused by the large inoculation of tissue suspension.

Of the 74 coded sets of tissues from pigs in the category “Hog Cholera Free or Immune”, 74 were negative by the FATST and 72 were negative by the FACCT (Table 2).

The 2 pigs found positive by the FACCT and negative to the FATST were inoculated with BVD virus. One of the pigs was given 4.0 ml of BVD virus suspension via the nasal route and killed 3 DPI. Fluorescent plaques of PK-15 were detected after overnight incubation, 36 plaques per ml of spleen suspension. The presence of BVD virus in the spleen of this pig was confirmed by calf inoculation. The other pig was given 1.0 ml of BVD virus suspension intramuscularly and killed 7 DPI. No fluorescent plaques were found after overnight incubation but 4 plaques per ml of suspension were detected after 48 hours of incubation. Inoculation of a susceptible calf did not confirm BVD virus in the spleen suspension of this pig. The fluorescing cells stained with the HC conjugate were considered to be infected with BVD virus.

All of the BVD exposed pigs were negative by the tissue section technique including the 2 found positive by the FACCT.

None of the 11 HC immune pigs were positive with either technique even though one of the pigs had received virulent Ames strain as little as 8 days previously. This included the 8 pigs given killed vaccine that were sick following the challenge and had been normal for only 5 days before being killed.

The comparison of the two techniques on the 122 pigs was evaluated statistically. Assuming the arbitrary classification of the pigs into the two categories was valid, the percent of correct classifications for each procedure was calculated. In the category “Hog Cholera Exposed” the FACCT had 83.3 percent correct and the FATST 77.1. In the category “Hog Cholera Free or Immune” the FATST had 100 percent correct and the FACCT 97.3. Neither of the differences in the percent of correct findings between the two methods was statistically significant.

Additional information was obtained from the trial in which 20 pigs were
inoculated with 1.0 ml of a filtered spleen suspension containing 175 VPU per ml obtained from a pig inoculated with a field strain of HC virus. By 3 DPI all of the had fever and anorexia and 10 of the pigs were given 1.0 ml of HC antiserum per lb. of body weight. Starting on the next day, 4 DPI, one pig from each group was killed each day. In addition to performing the FATST and FACCT on the appropriate tissues, blood specimens were cultured for HC virus,uffy cell cultures were inoculated with spleen suspensions, and neutralization tests were performed on the sera.

The 10 pigs that were not given serum developed signs of acute HC and 3 were found dead at 9, 10 and 11 DPI. Tonsil and spleen were found positive from all of the pigs by both techniques and high titers of virus (> 10,000 plaques/ml) were obtained from the blood specimens collected on the day each pig was killed or from the heart after death. The secondary culture for HC virus onuffy cell cultures was positive on spleen suspensions from 9 of the 10 pigs and the tenth was toxic to the cell cultures.

In contrast the 10 pigs receiving the serum began to recover and toward the end of the period were normal and alert. Normal rectal temperatures were found in all of the pigs by the sixth day after serum therapy and lesions of HC virus were not detected after the sixth day (Table 3). To enhance the chances of confirmation of HC infection the most severely affected pig was killed each day. As noted previously the FATST was positive on the pigs killed through the seventh day after serum therapy while the FACCT was positive only through the fourth day. However, virus was isolated fromuffy cell cultures inoculated with tissues from the pig killed on the sixth day. The HC antibody titer of the pigs expressed as $\log_{10}$ was 1.8 (1-64) on the day following serum therapy and remained at this level in all but 2 of the pigs throughout the 10 days (Table 4).

It was of interest to note that the only virus isolations from the blood were made in the pigs killed on the third and fourth days, and that these two pigs had lower antibody titers, 0.6 (1-4), than the others. Since virus isolation was performed on a heparinized whole blood sample and the neutralization test on serum from a clotted sample, it was demonstrated that these pigs had HC virus and circulating antibodies in their blood at the same time. The virus plaque counts obtained from the serum pigs were lower by a factor of 10 than those obtained from the pigs that did not receive serum. The 3 pigs killed 8, 9, and 10 days after serum therapy were negative by both FATST and FACCT and appeared to be recovering from the infection.

**DISCUSSION**

There was no significant evidence that either of the two fluorescent antibody techniques was of greater merit than the other when performed on the coded specimens from 122 pigs. Confirmation of HC infection was obtained by the FATST with 77.1 percent of the 48 specimens classified as “Hog Cholera Exposed” and 100 percent of the 74 specimens designated “Hog Cholera Free or Immune.”
The FACCT confirmed 83.3 percent of the “Hog Cholera Exposed” group and 97.3 percent of the second group. Since the specimens were identified by code numbers the results were obtained without the assistance of history, lesions, or the opinions of the field diagnostician.

The failures of the two techniques on the positive specimens were attributed to different factors. The FACCT was negative on tissues from 4 pigs receiving HC antiserum or recovering from exposure to virulent virus following inadequate immunization with an inactivated HC vaccine. The failure to isolate virus was attributed to the presence of serum antibodies that neutralized the virus as it was released from the cells during the trituration of the tissues.

The FATST was negative on 7 pigs killed early in the course of the infection and it was considered that insufficient viral antigen was present at this time for these sections to be diagnosed as positive.

The two techniques failed on 3 sets of tissues from HC infected pigs killed 8, 9 and 10 days after inoculation with antiserum. These pigs had apparently recovered from the disease. In a HC infected herd in the field these pigs would not have been selected for necropsy and specimen collection.

The two techniques each had one negative finding on HC infected pigs that should be attributed to inaccuracies in manipulation and record keeping rather than failures of the methods.

The FATST had a score of 100 percent negative on the 74 pigs in the category “Hog Cholera Free or Immune,” while the FACCT had a score of 97.3 percent because of 2 positive findings. These were obtained from pigs exposed to BVD virus and were attributed to the propagation of BVD virus in the PK-15 cell cultures. The HC conjugate will stain either bovine or porcine cells infected with BVD virus. Since the FACCT was positive in only 2 of the 40 pigs inoculated or exposed to BVD virus and killed at the optimum time, the isolation of BVD virus on PK-15 cells was considered a difficult and unusual event that would be an infrequent occurrence in the processing of field specimens. The reason for the negative calf inoculation for BVD virus with the tissues from one of these pigs was not clear. However, the virus plaque count, 4 plaques per ml of spleen suspension, was low and the virus had been passaged in a pig.

The effectiveness of adequate serum therapy was obvious from the response of the pigs in the HC antiserum trial. Also noted was the interference with isolation of the virus associated with passive antibody titers. However, an isolation was made 6 days after serum therapy by initial culture on pig buffy cells and subculture to PK-15 cells. The phagocytic nature of the buffy cells may account for this isolation in the presence of antibody. Two of the pigs were of interest in that virus and antibody were coexistent in their blood at the same time. The 3 pigs killed 8, 9, and 10 days after serum therapy whose tissues were negative by both techniques might have yielded HC virus by pig inoculation.
### TABLE 1

**FINDINGS OF TISSUE SECTION (FATST) AND CELL CULTURE (FACCT) IMMUNOFLOUORESCENT TECHNIQUES ON SPECIMENS FROM PIGS EXPOSED TO HOG CHOLERA VIRUS**

<table>
<thead>
<tr>
<th>FLUORESCENT ANTIBODY</th>
<th>FLUORESCENT ANTIBODY</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>TISSUE SECTION</td>
<td>CELL CULTURE</td>
<td></td>
</tr>
<tr>
<td>TECHNIQUE</td>
<td>TECHNIQUE</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>32</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>8</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>3</td>
</tr>
</tbody>
</table>

### TABLE 2

**FINDINGS OF TISSUE SECTION (FATST) AND CELL CULTURE (FACCT) IMMUNOFLOUORESCENT TECHNIQUES ON SPECIMENS FROM PIGS THAT WERE HOG CHOLERA FREE OR IMMUNE**

<table>
<thead>
<tr>
<th>FLUORESCENT ANTIBODY</th>
<th>FLUORESCENT ANTIBODY</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>TISSUE SECTION</td>
<td>CELL CULTURE</td>
<td></td>
</tr>
<tr>
<td>TECHNIQUE</td>
<td>TECHNIQUE</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>2</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>72</td>
</tr>
</tbody>
</table>
### TABLE 3

**CLINICAL SIGNS AND LESIONS OF 10 PIGS KILLED SEQUENTIALLY STARTING 4 DAYS AFTER HC INFECTION AND 1 DAY AFTER INOCULATION OF 1.0 ML OF HC ANTISERUM PER LB. BODY WT.**

<table>
<thead>
<tr>
<th>KILLED DAYS POST-INOCULATION</th>
<th>DAYS AFTER SERUM THERAPY</th>
<th>RECTAL TEMPERATURE</th>
<th>CLINICAL SIGNS</th>
<th>NECROPSY LESIONS OF HOG CHOLERA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1</td>
<td>103.8</td>
<td>anorexia, depression</td>
<td>moderate</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>106.2</td>
<td>anorexia, depression</td>
<td>moderate</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>108.0</td>
<td>anorexia, depression</td>
<td>moderate</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>107.8</td>
<td>depression</td>
<td>moderate</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>103.8</td>
<td>depression</td>
<td>slight</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>102.3</td>
<td>normal</td>
<td>slight</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>103.2</td>
<td>normal</td>
<td>negative</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>101.0</td>
<td>slight anorexia</td>
<td>negative</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>102.6</td>
<td>normal</td>
<td>negative</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>101.2</td>
<td>normal</td>
<td>negative</td>
</tr>
</tbody>
</table>
**Table 4**

Diagnostic findings on 10 pigs killed sequentially starting 4 days after HC infection and 1 day after inoculation of 1.0 ml of HC antiserum per lb. body wt.

<table>
<thead>
<tr>
<th>Killed</th>
<th>Days</th>
<th>FACCT** (Plaques/ml)</th>
<th>Buffy Cell</th>
<th>Serum Titer (Log&lt;sub&gt;10&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days Post-</td>
<td>After Serum</td>
<td>Therapy</td>
<td>Blood</td>
<td>Spleen</td>
</tr>
<tr>
<td>Inoculation</td>
<td>Therapy</td>
<td>FATST*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>Pos</td>
<td>Neg</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>Pos</td>
<td>Neg</td>
<td>110</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>Pos</td>
<td>3400</td>
<td>600</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>Pos</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

* Fluorescent Antibody Tissue Section Technique

** Fluorescent Antibody Cell Culture Technique
COMPARISON OF IMMUNOFLUORESCENT TECHNIQUES

REFERENCES


20. Turner, L. W., Chairman: Recommended Minimum Standards for the Isolation and


USE OF THE MICROTITER SERUM NEUTRALIZATION TEST FOR THE DIAGNOSIS OF IBR, BVD, AND OTHER BOVINE AND PORCINE VIRAL DISEASES

by

John W. Black
Chief Virologist, Diagnostic Laboratory
Kentucky Department of Agriculture
North Drive, Hopkinsville, Kentucky 42240

Since the introduction of the microtiter (MTC) technique, many adaptations to tissue culture and serum neutralization (SN) of viruses using this method have been published. Most of these procedures, however, have been developed for diagnosing virus diseases of man. Serologic detection of virus diseases of large animals has been confined to the classic tube dilution (MACRO) method. While accurate and usually reliable, the MACRO method of viral SN is laborious and time consuming when done in quantity. The MTC technique, however, is far less costly and cumbersome while allowing numerous antibody or virus titrations to be performed simultaneously.

Most veterinary diagnostic facilities now perform some type of bovine and porcine virus serology. Reluctance to accept MTC procedures for these tests, however, usually stems from uncertainty as to its reliability and reproducibility. This report will attempt to demonstrate the ease and accuracy with which infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD), transmissible gastroenteritis (TGE) of swine and many other bovine and porcine virus diseases may be diagnosed by the MTC method.

MATERIALS AND METHODS

Media and Diluent — Minimal Eagle’s Medium (MEM) with Earle’s salts, 0.5% Lactalbumin hydrolysate (LAH) and 15% heat-inactivated fetal bovine serum was used as diluent and media. Penicillin and Streptomycin were added to the above to make a final concentration of 200 units and 200 mg per ml of medium, respectively.

Cell Cultures — For this study, the bovine tubinate cell line (diagnostic serv. NADL) was used for IBR and BVD. For TGE and other cytopathogenic porcine viruses, an established line of porcine testicle (PT-1) was used (Dr. A. W. McKlurkin, NADL).

Viruses — All virus strains used in this study were isolated and characterized at this laboratory. The Kentucky isolates of IBR (KBR-3), BVD (KMD-2) and TGE (KEG-1) were all of greater than 25 passages in tissue culture and were plaque or terminal dilution purified at least once. Virus titrations were carried out in MTC by preparing ten-fold serial dilutions of the virus in tubes before delivery to the MTC plate. Four wells were inoculated per dilution.

Antisera used in this study were clinical specimens submitted to this laboratory.
for diagnosis. The sera were centrifuged to eliminate red cells and stored at -20°C. Just prior to use, the specimens were heat-inactivated at 56°C for 30 minutes.

MTC plates — Numerous types of plates were tested but only one was adopted for routine use. The plates used were flexible vinyl (Cooke Engineering Co.) with 96 flat-bottomed wells (12 rows of 8). These plates required pre-treatment for tissue culture before use. They were immersed in either 95% ethanol, concentrated H2SO4 or warm detergent for 30-60 minutes and then rinsed thoroughly 7 times each in deionized and distilled water. The plates were allowed to air dry and then dipped in warm (40°-50°C) 0.5% gelatin solution. After immersion in gelatin, the plates were drained and allowed to air dry. They could then be sterilized with 60 minutes exposure to ultraviolet light, but this was not found to be absolutely necessary. The gelatin coating appeared to be stable and capable of withstanding long-term storage, providing dryness was maintained. Commercially treated and sterilized plates of rigid styrene (Cooke, Linbro, Falcon) were also used and evaluated.

MTC equipment — Disposable 25 ml pipettes (Cooke) were used throughout this study. Serial twofold serum dilutions were performed in MTC plates with 25 ml, slotted-heal microdiluters (Cooke). Calibrated 25 ml blotters were used to check microdiluter delivery. No attempt was made to sterilize the disposable pipettes.

MTC procedures — Serum for testing was initially diluted 1:2 in one-dram vials. Microdiluters were flame sterilized and pre-wetted before priming with the serum dilution. One primed microdiluter (25 μL) of serum dilution was transferred to the first well of a row, each of which contained one drop (25 μL) of diluent. The microdiluter was then spun 3-5 times and transferred to the nest well. This procedure was repeated until the end well of the row was reached. The last 25 μL of serum dilution was then discarded by rinsing the diluter in distilled water. The serum had, by the preceding technique, been carried from 1:4 in the first well, through a two-fold serial dilution scheme to 1:512 in the eighth well. One drop (25 μL) of virus suspension containing 100-1,000 50% tissue culture infective doses (TCID50) (MTC titrated) was added to each well, and the plate was covered and incubated at 37°C and 5% CO2 for 60 minutes. Turbinate cells were trypsinized by standard methods and resuspended in a volume of medium equal to one-half the volume of media in the culture vessel. The PT-1 cells were resuspended in the same amount of media as originally in the culture flask. At the end of the incubation period, two drops (50 μL) of cell suspension was added to each well and overlayed with 0.2 ml of heavy mineral oil (Rexall). The plates were then placed at 37°C under a 5% CO2 atmosphere.

MACRO methods — Cells were grown in 16x125 mm disposable plastic roller-tubes (Falcon). Sera were heated to 56°C for 30 minutes prior to use. Serial dilution os the serum was carried out in glass tubes using pipettes. Virus suspensions containing 100-1,000 TCID50 (as determined in roller tubes) per 0.1 ml were added to the serum dilutions and incubated for 60 minutes at 37°C. At the end of this time, 0.2 ml of virus-serum mixture was added to each roller tube containing cells
and 1.0 ml medium. The tubes were then returned to the 37°C incubator.

Reading and interpretation — Virus titrations were read and recorded according to Reed-Munch method of determining 50% end points, and the resultant titers expressed as TCID$_{50}$. Antiserum titrations were expressed as the reciprocal of the highest two-fold serial dilution of serum completely inhibiting cytopathic effect (CPE). Split dilutions (titers half way between dilutions) were recorded when the next highest serum dilution contained only one observable infectious unit.

RESULTS

When the same serum specimen was titrated numerous times with different batches of cells, different plates, and new media, the reproducibility of the MTC method was demonstrated. For both IBR and BVD, the maximum deviation in repeated titration of the same specimen was one half of one dilution to either side of the mean titer (Table 1). The virus titer was found to be a critical factor in reproducibility. With deviations of one log in virus titer, 2-3 fold deviations in antiserum titer were seen. If the virus quantity was held to the 100-1,000 TCID$_{50}$ range, slight variations in titer did not occur.

When grown in turbinate cells (fig. 1), IBR CPE was usually observable in 24 hours, and the titrations were readable in 48 hours (fig. 2). The BVD virus CPE in turbinates was observable at 48 hours and readable at 72 hours. TGE CPE in PT-1 cells was readable at 48 hours. Vesicular stomatitis (VS) and Aujeszky’s virus CPE were readable at 24 hours and 48 hours, respectively. Various other cytopathic bovine and porcine viruses were grown in MTC and none took more than 72 hours for readable cell destruction to occur.

The reliability of the MTC as compared to the MACRO or tube test appears good. A number of serum specimens were titrated for IBR and BVD simultaneously by both methods and found to correlate closely (Table II). The few discrepancies that occurred in this study and subsequent work have shown the MTC method to be somewhat more sensitive than the MACRO.

The source and treatment of the MTC plates were critical. Commercially prepared and sterilized plates worked well, but were brittle and scarred rather badly when microdiluters were spun in the wells. The circular scars produced by the “slot-type” diluter in some cases prevented microscopic observation of the well bottoms. The use of “spiral-loop” type diluters in these rigid plates alleviated this problem. Cells of a fibroblast type grew best in plates coated with gelatin, while epithelial cell types formed confluent monolayers best in acid treated plates with no coating. The type of plate treatment seemed to have no effect on virus and antiserum titers.

Even though materials and methods at times were not kept sterile, the contamination rate was very low, with most occurring due to contaminated serum specimens.

Mineral oil was found to be the most acceptable method of sealing the wells. Clear adhesive tape and rigid plastic covers were evaluated but found less...
satisfactory. Humidity control was not necessary when mineral oil was used and CO₂ requirement was low. The oil also acts as a lens to fully illuminate the well for ease of observation.

**DISCUSSION**

The obvious advantage of MTC SN for the large animal diagnostic facility is in the saving of time, labor and funds. In this laboratory complete titrations of six serum specimens for IBR and BVD antibodies by the MACRO method consumed over a hundred roller tubes of cells, 500 ml of media and diluent, six cans of pipettes and one-hundred and fifty sterile, screw capped tubes. Most of one work day was required for one technician to complete titrations. When the tubes were ready to read, 30–60 minutes was required to read them. The same six sera could be assayed by one technician in two hours using one MTC plate, 2 ml diluent, two microdiluters, three disposable micropipettes, one 25 cm² flask of cells and 5 ml mineral oil. After attaining proficiency at reading the plates, all six sera for both viruses could be quantitatively read in 5–10 minutes.

Perhaps the most difficult problem to overcome with MTC was the training and manual dexterity needed to become proficient in its use. This may best be obtained in a laboratory where the MTC technique is in use. Proficiency and speed are then developed with practice.

The minor differences in titers seen occasionally between MTC and MACRO tube test should not serve as a deterrent to acceptance of the former. The diagnostic difference between a low titer and a high titer is still valid in MTC. In this laboratory, we have not had the opportunity to evaluate enough paired specimens to make a statement on the validity of the classic “4-fold increase” in titer on MTC. Other literature, however, rate the MTC SN as somewhat more sensitive than the tube test at detecting diagnostic rises in titer.

MTC is also being used in this laboratory to perform porcine enterovirus (SMEDI) serology for swine reproductive disorders. Four paired specimens can be titrated against ten enteroviruses on one plate, and unknown porcine virus isolates can be identified in a MTC plate without undue waste of typing antiserum.

The MTC plates were the most important part of the test. In this laboratory, the flexible vinyl plates were used solely because the “slot-type” diluters did not damage them a great deal. The rigid, commercially prepared plates supported all cell growth quite well, but should be used with the “spiral-loop” type microdiluters only. A drawback to the flexible vinyl plates was that approximately half were not suitable for use due to roughness in the well bottoms. These rough plates grew cells well, but viral CPE was quite difficult to see in them. The rigid plates, however, had excellent optical qualities.

It was found that a high humidity was required for plates not sealed with oil, and if incubator conditions were too dry, media in some of the wells would evaporate. CO₂ was required even with mineral oil because some gas transfer apparently occurred through the oil or plastic.
In conclusion, the primary purpose of this presentation has been to offer the MTC techniques and procedures as a great savings in time, labor and funds over the MACRO tube test, with no apparent loss in accuracy and reliability. It is hoped that the proceeding experiences with the MTC SN for large animal viruses may be of some assistance to other laboratories wishing to adopt this technique.

### TABLE I

Repeated Separate Titration of the same Serum Specimen for IBR and BVD Antibodies at Three Day Intervals by the MTC Technique

<table>
<thead>
<tr>
<th>Separate Titrations (three day intervals)</th>
<th>Mean</th>
<th>Deviation**</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVD Titors*..... 32 32 32 48 32 32 24 48 32 32</td>
<td>32...32...0.50</td>
<td></td>
</tr>
<tr>
<td>IBR Titors..... 128 128 100 100 128 200 128 128 128...128...0.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Titer expressed as reciprocal.

**Deviation expressed as a decimal fraction of a whole dilution.

### TABLE II

Comparison of Antibody Titrations for IBR and BVD Performed by the MACRO (Tube) and the MTC Method

<table>
<thead>
<tr>
<th>Separate Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVD Titors</td>
</tr>
<tr>
<td>MTC..... 32 128 64 100 16 &gt;512 256 &lt;4 12</td>
</tr>
<tr>
<td>Deviation</td>
</tr>
<tr>
<td>IBR Titors</td>
</tr>
<tr>
<td>MTC..... &lt;4 16 200 8 382 6 16 128 100</td>
</tr>
<tr>
<td>Deviation</td>
</tr>
</tbody>
</table>
Figure 1 — Uninfected bovine turbinate cells growing in a MTC well.

Figure 2 — IBR CPE in bovine turbinate cells grown in a MTC well; 48 hours PI.
REFERENCES


INVESTIGATION OF FEED RELATED PORCINE HEMORRHAGIC DISEASE

by
Gary D. Osweiler, D.V.M.

A hemorrhagic condition in Iowa swine has been reported with increasing frequency by veterinary practitioners in the midwest. During the winter of 1969 and 1970, the condition was often observed; and a number of cases were referred to the Iowa Veterinary Diagnostic Laboratory.

The epidemiology and pathology of these cases have been reported previously. The condition is characterized by sudden onset of lameness, anorexia, and lassitude. Dyspnea and hemorrhagic feces have also been observed. Frequently lameness is accompanied by soft fluctuating joint swelling. Soft subcutaneous swellings are seen or palpated in the inguinal area, underline, submandibular region, and encompassing the hamstring muscles of the pelvic limbs. Epistaxis may be an early sign. Affected swine may live from 4 to 72 hours after clinical signs appear. In other cases apparently normal, intact males die from hemorrhage as a result of surgical castration. In such animals blood loss and death occur within 2 to 24 hours after the castration procedure.

Gross necropsy lesions in swine dying of porcine hemorrhagic disease are anemia, hemoarthrosis, hemorrhagic lymph nodes, subcutaneous hematomata and edema, intramuscular hemorrhage following fascial planes, hemorrhage in the intestinal lumen, and hemoperitoneum. Characteristically, the blood is unclotted and remains so for 12 to 72 hours. Gross pathologic changes in the liver are not seen.

Microscopic changes appear limited to hemorrhage in the affected tissues. Microscopic hepatic damage is not observed.

Clinical pathologic alterations in affected swine include depressed hemoglobin and hematocrit and markedly elevated prothrombin times. Platelet numbers are normal. Serum glutamic-oxalacetic transaminase levels are not elevated in affected swine.

Bacteriologic examinations of appropriate tissue (intestine, liver, kidney, lymph nodes) are consistently negative for pathogenic microorganisms.

The prominent epidemiologic features of the disease are that it occurs in winter months almost exclusively in recently weaned swine which are consuming complete pelleted feeds. Signs usually occur within 5 to 10 days after weaning or a feed change. A majority of rations have been found to contain antibiotics, primarily sulfonamides and tylosin. Most rations involved used soybean meal and a premix as the source of supplemental protein and dietary factors.

Experimental Production of Porcine Hemorrhagic Disease

Because of the frequency and similarity of cases of porcine hemorrhagic disease observed at this laboratory and because of circumstantial incrimination of swine
feed as the source of the problem, experimental production of the disease was attempted.

MATERIALS AND METHODS

For the trial described, ten healthy swine weighing approximately 50 pounds (22.5 kg.) were utilized. Four of these were kept as controls and six were utilized in the experimental group. The control group was fed a locally prepared ration consisting of corn, soybean meal, and premix ingredients adequate for swine of that age. The control group feed was not pelleted. The experimental group was fed a pelleted weaner ration of the same lot number associated with a field case of porcine hemorrhagic disease. All swine were fed ad. lib. of the respective feeds.

All swine were bled prior to initiation of the trial and at two day intervals thereafter. Additional samples were taken when clinical signs appeared or when death appeared imminent. Blood samples were taken by the ocular technique.

Hematologic and clinical chemistry studies done included packed cell volume, hemoglobin determination, leucocyte count, differential leucocyte count, platelet count, prothrombin time (PT), and serum glutamic-oxalacetic transaminase (SGOT). Coagulation factor determination was done on three experimental pigs and one control pig.

Portions of liver, kidney, lung, heart muscle, skeletal muscle, lymph nodes, stomach, duodenum, ileum, spleen, brain, adrenals and thyroid were collected for histopathologic examination. Sections were imbedded in paraffin, cut at six u, and stained with hematoxylin and eosin.

Portions of liver, kidney, blood, and urine were taken at necropsy and frozen for chemical analysis.

RESULTS

No clinical signs were observed in any swine for the first five days of the experiment. The control group remained clinically normal for the 28-day period of the experiment. On day 6 of the trial, one experimental pig (no. 10) developed epistaxis which continued through the seventh day. By day 8 of the trial, all experimental pigs were mildly depressed, and feed consumption was down slightly. On day 9, pig no. 6 and pig no. 10 had epistaxis. Two other experimental swine developed lameness. Pig no. 8 was severely lame in the right front leg, and no. 9 had a swollen tibiotarsal joint with lameness in that limb. By day 10 of the trail, three swine were visibly lame (no. 8, 9 and 10) and no. 9 had visible enlargement of the left tibiotarsal joint. Pigs 6 and 7 were depressed and showed moderate dyspnea when handled. Pig no. 5 remained normal clinically.

Pigs 8 and 9 were euthanatized on day 10 and necropsy examination was conducted. Pig 6 was euthanatized and necropsied later on day 10 because death appeared likely. On day 11, pig no. 10 was found dead, having expired during the night. Necropsy examination was conducted at that time.
Pig no. 7 remained depressed and lame until day 12 and was euthanatized on day 13. Necropsy examination was conducted at that time.

Pig no. 5 remained clinically normal and was euthanatized on day 28 of the trial.

Gross necropsy lesions were observed in all swine in the experimental group. No gross lesions were seen in swine of the control group.

Necropsy lesions observed included anemia, epistaxis, hemoarthrosis, hemorrhage of cervical, iliac and inguinal lymph nodes, subcutaneous ecchymotic or suffusion hemorrhages, intramuscular hemorrhage, abdominal hemorrhage with unclotted blood, renal petechial hemorrhage, and petechiae of the serosa of the ileum and jejunum.

Bacteriologic cultural examination of intestinal contents and tissues did not reveal presence of pathogenic microorganisms.

Histopathologic studies conducted on tissues of affected pigs revealed intramuscular hemorrhage, mild swelling and degeneration of some liver cord cells, and peripheral hemorrhages in the lymph nodes. Specific lesions of necrosis or inflammatory response were not observed.

Casual observation of hematological and SGOT values did not reveal significant differences between control and experimental pigs. Platelet counts were normal in all pigs throughout the trial. The outstanding change found in affected experimental pigs was a prolonged prothrombin time. Prothrombin time results in affected pigs ranged from 24 to 56 seconds compared to control values of from 11.5 to 13.5 seconds.

Prothrombin time requires normal levels of prothrombin, Factor VII (proconvertin), Factor V, Factor X, and fibrinogen. Therefore, plasma samples from pigs 3 (control), 5, 6, and 10 were submitted to a medical laboratory for more detailed factor analysis.* Plasma from pigs 6 and 10, showing severe clinical signs, was found to have prolonged prothrombin time (PT), prolonged partial thromboplastin time (PTT), and normal fibrinogen content. Addition of absorbed plasma rich in Factors VIII, V, VI, and XII to these pig plasmas did not correct PT and PTT. Addition of serum rich in Factors IX, X, XI, XII, and VII to these pig plasmas corrected PT and PTT to normal values. Plasma from pig 5 (mildly effected clinically) produced atypical PT and PTT results but grossly abnormal results. No change in results was noted upon addition of correction plasma or serum. Plasma from control pig 3 had normal PT and PTT results.

The data indicated blood from severely affected pigs to be deficient in Factor X. Because prothrombin times were not corrected to normal range following addition of normal serum, there was probably an associated deficiency of Factor VII. Both Factors VII and X affect the coagulation scheme in the formation of prothrombin conversion factor from tissue thromboplastin. Prothrombin conversion factor causes the conversion of prothrombin to thrombin in the coagulation

*McFarland Clinic, Ames, Iowa.
scheme. Both Factors VII and X are sensitive to coumarin-type drugs and are Vitamin K dependent.¹

The feed components (individually) and the feed itself were analyzed. The absence of warfarin and the aflatoxins has been established by chromatography and instrumental methods including mass spectral analysis. Other mycotoxins are being investigated, particularly rubratoxin, and *Fusaria* toxins, particularly the T2 group.

**DISCUSSION**

Experimental production of the disease syndrome described in field cases was accomplished. Outstanding features of the syndrome observed in this trial include spontaneous hemorrhage, unclotted blood, lack of liver damage, and prolonged prothrombin time with Factor X deficiency and associated Factor VII deficiency. These data would indicate that a Vitamin K deficiency or interference is involved. The condition resembles poisoning by coumarin compounds or their derivatives. However, preliminary chemical analyses indicate that known coumarin compounds are not involved. Thin-layer chromatography of feeds has not demonstrated presence of recognizable amounts of aflatoxin. The aflatoxins also have a structure similar to the coumarin nucleus. Bacteriologic studies, while limited, do not indicate an absence of intestinal flora. All cases investigated have occurred within approximately two weeks of introduction of a different lot of type of feed. This has usually occurred in weanling pigs. Most of the field cases have occurred in association with pelleted feed, but at least two of those investigated have been prepared by grinding and mixing of the natural feedstuffs.

Thus far, data indicate either an interference in the synthesis or utilization of Vitamin K or the presence of some compound (possibly a naturally occurring mycotoxin) which may interfere with production of Factor VII or X. Liver necrosis as determined by SGOT and light microscopy was not observed. This would aid in eliminating aflatoxins as a source of contamination. The elevated clotting time and prothrombin time observed in field cases agrees with the experimental results that a clotting disorder is present. Adequate platelet numbers and normal clot retraction suggests that thrombocytopenia is not a problem in these cases.

A number of feed samples from field cases were examined for mycotic agents, but no consistent isolates were found. The conditions of the pelleting process may have destroyed potential toxin-producing fungi.

Whether this condition is a result of mycotic contamination, toxic changes in the feed ingredients, or simple vitamin deficiency has not as yet been determined.
REFERENCES


SALMONELLA INFECTION IN ONTARIO MARKET SWINE

and D. A. Barnum, D.V.M., D.V.Sc., D.V.P.H.

Many investigators have reported normal market swine to be infected with salmonellae at slaughter\textsuperscript{1,2,3,4,5,6,7}. McDonagh and Smith\textsuperscript{8}, during an investigation in 1954-56, collected evidence to incriminate the abattoir as the most important source of salmonella infection, and swine as the main animal reservoir.

The study reported herein was conducted during a nine month period in 1969, and involved salmonella detection in the following areas: I. market swine in abattoirs, II. slaughterhouse environments, III. swine farm pens, and IV. swine feed.

I. The Occurrence of Salmonellae in Market Swine in Abattoirs

Mesenteric lymph nodes (MLN) from normal swine slaughtered in five abattoirs in Southwestern Ontario designated as A, B, C, D and E were examined for the presence of salmonellae. Eviscerated swine carcasses selected at random on entry into the chill room were examined by internal and external swabs for salmonellae. Rectal swabs were collected in conjunction with MLN samples from the same hogs slaughtered in abattoir E. All samples were examined by the methods outlined by Groves\textsuperscript{9} and two colonies from each specimen were serotyped at the Ontario Department of Health Laboratories, Toronto, Ontario.

Abattoirs A and B were inspected under the “Ontario Meat Inspection Act” where 100-150 hogs in the former and 400-500 hogs in the latter were slaughtered weekly. The hogs were purchased from the “Hog Producer Assembly Yards” and slaughtered within eight hours after removal. In abattoirs C and D, 300-500 hogs were slaughtered under the “Federal Meat Inspection Act” on each of four days per week. In these abattoirs the majority of hogs were slaughtered within eight hours after movement from the “Hog Producer Assembly Yards”. Abattoir E was inspected under the “Ontario Meat Inspection Act” where 80-100 hogs were slaughtered weekly. These animals were purchased directly from local farmers and were not marketed through the “Hog Producer Assembly Yards.”

Of the 462 hogs examined in the five abattoirs, during the period January to September, 1969, salmonellae were isolated from the MLN’s of 20.3 percent of the swine (Table I) with the highest incidence, 33.7 percent, in abattoir E.

In abattoirs A, B and C no salmonellae were isolated from carcasses, while five percent in abattoir D and ten percent in abattoir D were contaminated as shown in Table I.

Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph.
Present address of B. I. Groves — Agricultural Division, Pfizer Co. Ltd., 50 Place Cremazie, Montreal, Quebec.
Rectal swabs and MLN's were cultured from each hog slaughtered and examined in abattoir E; the results are shown in Table II. There was a significant statistical difference at the five percent level between the probability of a salmonella isolation from a MLN sample, 27/33, and that from a rectal swab, 11/33, collected in this abattoir. When the shipping and holding time from sources to slaughter was 18-36 hours, a much smaller difference in the probability of an isolation from a rectal swab, 4/7, and that from a MLN sample, 5/7 was observed. When the results of the MLN and rectal swab examinations are combined, 41.0 percent of the hogs slaughtered in this abattoir were infected with salmonellae.

A total of 17 different serotypes were isolated during this study as illustrated in Table III. S. typhimurium was isolated with the most frequency from swine MLN's. S. heidelberg and S. typhimurium were the only serotypes isolated from the 12 positive carcasses. Ninety-eight salmonella isolates were recovered from 94 MLN's.

II. Salmonellae in Environment of Abattoirs

Environmental samples were obtained from each abattoir on every day when samples were collected from swine. Sewer swabs, fecal samples from holding pens, and wash water from edible pork products were obtained on each collection from each abattoir. In abattoir E samples were procured from water in receptacles used to wash butcher's knives after scapping and trimming out the carcasses. Samples were examined for salmonellae by the above methods. Table IV shows that salmonellae were isolated at least once from environmental samples in four of the five abattoirs.

S. typhimurium was isolated from washings of edible pork products in abattoir D on two of eight days on which samples were collected. S. heidelberg was isolated from washings of edible pork products in abattoir E on two of eight days and from environmental samples on five of eight days. In one instance S. typhimurium was isolated from a sewer swab which was also contaminated with S. heidelberg.

III. Salmonellae in Swine Farm Pens

Farms that served as the source of slaughtered swine with salmonella infected MLN's in abattoir E were investigated. Fecal swabs were collected from swine pens on these farms for the detection of salmonellae. On three of the nine farms sampled salmonellae were isolated in 45 percent, 40 percent and 8.3 percent of the samples respectively. The three farms with salmonellae in swine pens, marketed more than 1,000 hogs per year. Five of the other six, where no salmonellae isolations were made, marketed less than 100 pigs per annum.

The serotypes isolated from the swine pens on the three farms were: 12 isolates of S. heidelberg from one, eight strains of S. muenster from another and five isolates of S. thompson from the third. Only S. heidelberg was found in the slaughtered animals from the respective farm.
IV. Salmonellae in Swine Feed

Feed samples were collected on each of nine farms investigated as reported in Part III. In addition, premix prepared by two major companies was collected from a feed mill which supplied mixed feed to two farms. Feed samples were examined for salmonellae by a method previously described\textsuperscript{10}. Of a total of 102 samples examined, 7.8 percent were contaminated with salmonellae as shown in Table V. \textit{S. heidelberg} was isolated from the feed and swine on farm I as well as from the animals slaughtered in abattoir E from the same farm. \textit{S. muenster} and \textit{S. thompson} were the serotypes found on farms III and IX while \textit{S. binza} was isolated from the premix at the feed mill.

DISCUSSION

Factors that influence the establishment of a serotype within an animal species are those which favour spread of infection such as herding together of animals especially the young which are particularly susceptible\textsuperscript{11}. Once infection has been established on a swine farm, cross infection occurs by the fecal oral route. Investigators\textsuperscript{12} reported a 19 per cent infection rate of salmonellae in older swine and 44 percent infection in healthy one and two day old nursing pigs on an infected farm. A constant source of salmonellae must be present either from feed or feces in the pens to maintain a high infection rate on swine farms. Simth\textsuperscript{13} reported that the longer swine are fed a contaminated feed up to 50 days, the more likely they will become infected in their mesenteric lymph nodes. From the results of this study, the number of swine on an infected farm may have an influence on the degree of infection occurring on the same farm. No isolations were made from random fecal swabs on farms that marketed less than 100 hogs per year and isolations were made from three of four farms marketing more than 1,000 hogs per year.

An important source of salmonellae is the introduction of new serotypes into a swine herd via contaminated feed. Isa \textit{et al.}\textsuperscript{14} and Williams \textit{et al.}\textsuperscript{15} have reported that high percentages of animal feeds are contaminated with salmonellae in North America. Serotypes that are small in numbers in feed may predominate on a farm by selection within swine\textsuperscript{12}. Since swine do not serve as permanent excretors, and if the source of infection is eliminated, with fecal contamination control, salmonella infection in swine will disappear. Pelleting of animal feeds as suggested by Edel \textit{et al.}\textsuperscript{3} and Gibson\textsuperscript{16} would be an aid in lowering the amount of salmonellae contamination of swine feeds. Thus an assurance of salmonellae free feeds combined with sanitary measures on the farms would contribute to the elimination of salmonella infection on swine farms.

The results of this study indicate that market swine slaughtered in abattoirs in Southwestern Ontario are frequently infected with salmonellae. In this nine-month study, 20.3 percent of the MLN's of swine were infected with salmonellae, which is comparable to that reported during a six-month survey on the same abattoirs\textsuperscript{17}.
The results of Kampelmacher et al.\textsuperscript{5} in the Netherlands and Smith\textsuperscript{7} in England showed a 15 percent and 12 percent incidence respectively of MLN's of swine infected with salmonellae. Galton et al.\textsuperscript{4} reported that the skin of 27 percent of 132 carcass sides in the chill room were contaminated with salmonellae. In this study five percent of the carcasses in abattoir D and ten percent of the carcasses in abattoir E were contaminated with salmonellae. McDonagh and Smith\textsuperscript{8} reported on salmonellae isolations from rinse water of pork which is similar to the results reported in this study.

To ensure the production of salmonellae free pork products, swine must be reared in an environment free from salmonellae. It has been suggested\textsuperscript{18} that swine raising centres should be created where salmonellae-free hogs can be produced for market. The pork products from these slaughtered animals would be supplied to groups of individuals relatively more susceptible to salmonellosis, such as hospitals, children's and old people's homes and mental institutions.

**SUMMARY AND CONCLUSIONS**

The occurrence of salmonella infection in market swine, in abattoirs, and on selected swine farms has been studied.

Salmonellae were isolated from 20.3 percent of the mesenteric lymph nodes of market swine examined in five abattoirs over a nine-month period in which \textit{S. typhimurium} was isolated with the highest frequency.

From the farm investigations salmonellae were detected in pigs on 33.3 percent of the farms, in feed samples of 33.3 percent of the farms, also in feed samples collected from a local feed mill. In one instance, \textit{S. heidelberg}, was isolated from feed samples and swine pens on a farm, from swine originating from this farm and slaughtered in an abattoir, from the abattoir environment, carcasses, and washings of edible pork products in the same abattoir.

It has been shown in this study that swine play an important role in the salmonella cycle, by the selection of serotypes from contaminated feed, cross infection in swine pens, in abattoir holding pens and finally in edible pork products. In order to ensure the production of pork without salmonellae contamination, consideration should be given to the establishment of salmonella-free swine farms from which pork could be produced for institutions where individuals are more susceptible to salmonellosis.
SALMONELLA INFECTION IN ONTARIO MARKET SWINE

REFERENCES

7. Smith, H. W.: "The isolation of salmonellae from the mesenteric lymph nodes and faeces of pigs, cattle, sheep, dogs, cats and from other organs of poultry". J. Hyg., 1959, 57: 266.
### TABLE 1
DISTRIBUTION OF SALMONELLA ISOLATIONS IN MARKET SWINE IN ABATTOIRS

<table>
<thead>
<tr>
<th>Abattoirs</th>
<th>M. L. N. Examinations</th>
<th>Carcass Examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>80</td>
<td>15</td>
</tr>
<tr>
<td>B</td>
<td>76</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>112</td>
<td>22</td>
</tr>
<tr>
<td>D</td>
<td>114</td>
<td>20</td>
</tr>
<tr>
<td>E</td>
<td>80</td>
<td>27</td>
</tr>
<tr>
<td>TOTAL</td>
<td>462</td>
<td>94</td>
</tr>
</tbody>
</table>
### TABLE I

SALMONELLA ISOLATIONS FROM RECTAL SWABS AND M. L. N.'S OF SWINE SLAUGHTERED IN ABATOIR E

<table>
<thead>
<tr>
<th>Time (farm to slaughter)</th>
<th>Isolations</th>
<th>Rectal Swabs</th>
<th>M. L. N.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Examined</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>&lt; 6 hrs.</td>
<td>54</td>
<td>26</td>
<td>48.1</td>
</tr>
<tr>
<td>18-36 hrs.</td>
<td>26</td>
<td>7</td>
<td>23.0</td>
</tr>
<tr>
<td>1-26 hrs.</td>
<td>80</td>
<td>33</td>
<td>41.0</td>
</tr>
<tr>
<td>Serotype</td>
<td>Abattoir A No. Isolated</td>
<td>Abattoir B No. Isolated</td>
<td>Abattoir C No. Isolated</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------------------</td>
<td>-------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. muenster</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. anatum</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S. enteritidis</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S. schwazengrund</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>S. thompson</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. california</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. montevideo</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. cerro</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. infantis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. stanley</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. binza</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. derby</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. newport</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S. bareilly</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. sieburg</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
</tbody>
</table>
TABLE IV
DISTRIBUTION OF SALMONELLA ISOLATIONS FROM ENVIRONMENTAL SAMPLES

<table>
<thead>
<tr>
<th>Abattoir</th>
<th>No. Examined</th>
<th>No. Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>35</td>
<td>1</td>
<td>2.8</td>
</tr>
<tr>
<td>B</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>40</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>D</td>
<td>40</td>
<td>4</td>
<td>10.0</td>
</tr>
<tr>
<td>E</td>
<td>40</td>
<td>10</td>
<td>25.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>190</td>
<td>16</td>
<td>8.4</td>
</tr>
<tr>
<td>Farm</td>
<td>No. of swine marketed per year</td>
<td>Premix</td>
<td>Mixed grower and finisher</td>
</tr>
<tr>
<td>------</td>
<td>-------------------------------</td>
<td>--------</td>
<td>--------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pos.</td>
<td>Pos.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1000</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>1000</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>100</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>IV</td>
<td>100</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>V</td>
<td>100</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>VI</td>
<td>100</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>VII</td>
<td>100</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>VIII</td>
<td>1000</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>IX</td>
<td>1000</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Feed Mill</td>
<td>15</td>
<td>20.0</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>28</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.8</td>
<td>4.1</td>
</tr>
</tbody>
</table>
SEROLOGICAL STUDIES IN BLUETONGUE

P. Boulanger, Andre Girard,
G. L. Bannister and G. M. Ruckerbauer*

Bluetongue (BT) has not yet been recognized as a disease affecting livestock of Canada. It is well known that this infection, which, as recently as 1940, was confined to the African continent, spread to Cyprus, Palestine and Syria in 1943, to United States in 1948, to Spain and Portugal in 1956, to Pakistan and Japan in 1959, to Peru in 1962 and to India in 1963. In 1961, concerned by this spread of B.T., we in Canada began to familiarize ourselves with the diagnostic methods then available and attempted to develop “in vitro” tests to supplement the original procedure involving the injection of suspected material into susceptible sheep. We intend to summarise the information gained in our studies.

LITERATURE

The diagnostic procedure recommended by the Institute of Onderstepoort, South Africa and endorsed by FAO consists of the repeated inoculation of blood from suspicious animal into susceptible sheep. After the required observation period, the sera of the inoculated sheep were tested by the group reactive complement-fixation (CF) test, for the presence of group specific antibodies. These antibodies were considered heat-labile by the Onderstepoort workers, consequently in their CF test, they inactivate the sheep serum at only 53 °C for 30 minutes. As the animal inoculation method was time consuming and expensive, this stimulated us to develop the modified direct complement-fixation (MDCF) test which permits the detection of antibodies in both cattle and sheep sera.

In 1967, we reported on the value of the fluorescent antibody technique (FAT) for the demonstration of BT virus in bovine foetal kidney (BFK) cell cultures and in inoculated mouse brain. A few months previously, Pinilla showed by FAT, that BT virus could be demonstrated in tissue culture (TC) cells even though no cytopathic changes were observable. Later in 1968, Pinilla reported on the sensitivity of the indirect FAT for the detection of antibodies in sheep sera.

Another serological method, the agar gel precipitation (AGP) test, was investigated by Klontz in 1962. He claimed that the precipitating antibodies detected by this method persisted longer in the serum of infected animals than the serum neutralizing (SN) antibodies. Jochim in 1969 reported on a micro-AGP test and claimed this test had value for detecting group-specific antibodies to BT virus in sheep and cattle sera. This test was used by Trainer and Jochim in a serological survey for the presence of BT antibodies in a variety of wild ruminants of North America. A total of 1,012 sera were examined and serological reactions were detected in elk, antelope, bighorn sheep, barbary sheep, moose and several species.

*Animal Pathology Division, Health of Animals Branch, Canada Department of Agriculture, Animal Disease Research Institute, Hull, Quebec.
of deer. They claimed that the geographic distribution of BT in wild ruminants paralleled that of the disease in livestock, except in Ontario where reactors were detected in moose and deer. They pointed out that the disease is classed as exotic in Ontario and that an explanation for the occurrence of the AGP reactions in sera from wildlife was not available at the time. In cooperative studies, we have obtained some of the wildlife sera referred to above and tested them by both the MDCF test and the AGP test.

In this paper we will summarize our studies with the MDCF test, with the FAT and the AGP test. Finally we will describe the results obtained in a cooperative study on the Ontario wildlife sera using both the MDCF and AGP tests.

**MATERIALS AND METHODS**

*Complement-fixation Test*

The conventional direct CF test even though widely used in the diagnosis of bacterial diseases in cattle such as brucellosis and in infections due to the psittacosis group of viruses, has failed in the detection of antibodies against smaller viral agents. Such observations were made many years ago by Traub and Mohlmann and by Brooksby during investigation of foot and mouth disease. In 1960 we demonstrated that the MDCF test in which the guinea-pig complement was supplemented with 5 percent selected, normal, non-heat inactivated calf serum, was necessary to detect viral antibodies in cattle serum.

The MDCF test used in our laboratory was based on a 50 percent hemolytic unit of complement. The test sera were inactivated at 60°C for 30 minutes. The BT viral antigen was an aceton-ether extract of infected mouse brain. A BFK tissue culture antigen, concentrated 100-fold by pressure dialysis, has also been used and will be described under AGP test. A normal control antigen was employed in parallel test with each serum. Each reagent in the test, i.e.: the test serum, the complement, the antigen, the amboceptor and the sheep red blood cells were used in 0.1 ml amounts. Four and a half 50 per cent hemolytic units of guinea-pig complement diluted in veronal buffered saline containing 5 percent non-heated normal calf serum was used in the test. This modification was necessary to obtain fixation. The tests were held overnight at 7-9°C to allow for fixation. The following day, after addition of the sensitised erythrocytes the test was incubated 30 minutes at 37°C to allow for hemolysis.

The serotype of the virus used, the volume and the route of inoculation and the description of the experimental animals are given in Tables I and II together with the results.

*Fluorescent Antibody Technique*

Our method used in the preparation of immune sera and their conjugation for the direct FAT has been published. In resume, the specificity and the reactivity of the immune sera used for conjugation were verified by the MDCF test. The globulins were precipitated by dialysis in a 30 percent w/v final concentration of
ammonium sulphate. After the necessary dialysis to remove the excess ammonium sulphate, the buffered pH 9.0 globulins were conjugated in the cold with one part of fluorescein isothiocyanate for 20 parts of globulins containing 10 mg of globulin per ml of solution. After dialysis to remove the excess stain and absorption with appropriate normal tissue powder, the conjugates were verified for specificity and reactivity with infected and normal tissues or tissue cultures. It was then stored, dessicated in small volumes.

**Micro Agar Gel Precipitation Test**

The method used in our studies for the micro-AGP test has been described by Jochim. It consists of a plastic template containing the diffusion wells, which was laid over a thin layer of agar on a microscope slide. The plastic templates (Figure 1) were 1 inch square by 1/8 of an inch thick. They contain a central well and 4 equidistant peripheral wells whose centers were 4 mm from the center of the central well. The top 3/4 of each well had a diameter of 9/64 of an inch whereas the bottom portion of the well had a diameter of 4/64 of an inch. Figure 2 shows the template over the agar diffusion medium on a microscope slide. Figure 3 shows the precipitation lines formed by a positive serum with the antigen. The diffusion media consisted of a 1 percent washed Bacto-agar. The antigen was placed in the center well and the sera in the surrounding wells. The test was incubated at 20°C in a humid chamber for 1 to 5 days. At the end of incubation the templates were removed, the slides washed in distilled water and stained with tiazine red R.

In the present report, we compared the results obtained with three different antigens. First, the isoelectric precipitated antigen produced and used by Trainer and Jochim. The virus was grown in lamb kidney cell (LK) monolayers. The infective virus particles in the supernatant fluid were precipitated by dialysis in phosphate buffer at pH 8.5 and ionic strength of 0.01M. The remaining antigen in the supernatant fluid was concentrated 40-fold by dialysis in carbowax, (polyethylene glycol). The second antigen was prepared by a method commonly used in our laboratory. The TC consisted of BFK primary cells grown in Basal medium (Eagle) with Hank's salts plus 10 percent fetal calf serum. The maintenance medium consisted of Basal medium (Eagle) with Earle's salts. The TC cells were inoculated with 100-500 tissue culture 50 percent infective doses (TCID₅₀) per ml of BT Cyprus strain. The cytopathic effects (CPE) appeared after 24-48 hours and cell destruction was completed in 4-5 days. After centrifugation at low speed (1000G) to remove cells debris, the supernatant was concentrated 100-fold by a pressure dialysis cell* against phosphate buffer pH 8.5 and ionic strength of 0.01M. The concentrated antigen was then clarified by centrifugation at 25,000G for 10 minutes. It was distributed in small quantities and stored in a dry ice chest. The third antigen was supplied by Jochim. In this case the BT virus strain BT-8 had been grown on LK cells in roller bottles. After centrifugation to remove the heavier elements, the supernatant fluid was concentrated about 50 to 100 fold by

---

pressure filtration. This concentrated supernatant TC fluid was then fractionated in a column packed with Sephadex G200.

RESULTS

Complement-fixation Test

No fixation of guinea-pig complement was obtained by the BT antigen and the corresponding antibodies in the sera of calves, sheep and deer when inactivated at 60°C. This confirmed the observations made by the Onderstepoort workers. However antibodies in the heat inactivated sera from these species of animal fixed guinea-pig complement in the MDCF test.

Table I gives examples of the fixation obtained with heat inactivated, experimentally infected cattle sera. It should be noted that antibodies were detectable as early as 28 days after exposure. Titers of 1:80 were reached and persisted at this level for at least 105 days. Also as it was shown previously the MDCF test was group specific, it detected the antibodies against the various serotypes of virus studies.

Table II shows representative results of studies with sheep sera. They were comparable to the ones obtained with cattle sera except that higher titers were obtained and they persisted also for a longer period.

Fluorescent Antibody Technique

As we have reported in previous studies the FAT was group reactive. It permitted the detection of BT virus in BDK cells as early as 3 days after inoculation even in the absence of detectable CPE. Figure 4 shows the appearance of BT virus after 3 days incubation in BFK cells as shown by immunofluorescence.

Micro Agar Gel Precipitation Test

Table III gives the results of the MDCF tests on 19 deer sera from United States, unidentified when tested, and compares the results with those of the AGP test using the 3 different antigens. Ten sera reacted in the MDCF test and 14 reacted in the AGP test using the isoelectric precipitated antigen. However with the ADRI-BFK antigen and the most recent USDA antigen only 9 sera reacted indicating that the isoelectric precipitated antigen had reacted nonspecifically with 5 sera. The serum WD-65-68 gave a 1:10 MDCF titer which was not supported by any of the AGP tests.

Table IV gives the results of the MDCF tests on 30 deer sera from Ontario which were tested unidentified at the same time as those of the United States. The results of the AGP test using 3 different antigens are also given for comparison. None of these 30 Ontario deer sera gave an antibody reaction in the MDCF test, however, 15 were anticomplementary. In the AGP test, with the USDA isoelectric precipitated antigen, 12 sera gave a non-specific reaction. Such reactions were not present in the AGP tests performed with either the ADRI-BFK antigen or with USDA pressure filtration antigen.
DISCUSSION

When assessing serological methods, we are all aware that information obtained from tests conducted with a particular species of animal and a particular antigen are not always applicable to other species or infections. In the case of the direct complement-fixation test it is well established that viral antibodies produced in guinea-pigs, rabbits and pigeons fix complement whereas those produced in cattle, sheep and deer fail to react. Reactions in these species can be obtained by using the MDCF method to titrate such sera.

No explanation is available concerning the non-specific reactivity given by certain deer sera with the isoelectric precipitated AGP antigen. We have evidence that the type of cells either bovine or lamb kidney, used in the preparation of the TC did not influence the results. Also the media used and some of its constituents such as lactalbumin hydrolysate when tested did not cause non-specific reactions. However, the present studies indicate that the reactions obtained with the Ontario deer sera with the isoelectric precipitated antigen were not a result of exposure to BT virus.

ACKNOWLEDGEMENT

The authors wish to acknowledge the cooperation of Dr. Michael M. Jochim and the Director of the Animal Diseases Research Laboratory, United States Department of Agriculture, Denver, Colorado in sending us various lots of antigens for the agar diffusion precipitation test and various other information.

We wish also to thank Dr. D. O. Trainer, Associate Professor, Department of Veterinary Science, University of Wisconsin, U.S.A., for making available the deer sera tested in this comparative study.
Figure 1 – Plastic template used in micro-AGP test.

Figure 2 – Micro-AGP test, showing the template, the diffusion agar and the microscope slide.
Figure 3 — Precipitation lines formed by a positive serum with antigen.

Figure 4 — Appearance after 3 days incubation of bluetongue virus in primary BFK cells as shown by immunofluorescence.
### TABLE I
Results of the Modified Direct Complement-Fixation Test on Sera from Experimentally Infected Cattle

<table>
<thead>
<tr>
<th>Sheep Number</th>
<th>Inoculum</th>
<th>Days of Exposure</th>
<th>Positive Antigen</th>
<th>Normal Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td>5.0 ml sheep blood</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>I.V. and S.C. of BT</td>
<td>45</td>
<td>1:80</td>
<td>1:10</td>
</tr>
<tr>
<td></td>
<td><em>Texas Station strain</em></td>
<td>64</td>
<td>1:160</td>
<td>1:5</td>
</tr>
<tr>
<td></td>
<td>1962</td>
<td>85</td>
<td>1:80</td>
<td>1:5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>113</td>
<td>1:40</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>141</td>
<td>1:10</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>5.0 ml I.V. and 10.0 ml S.C. of blood from calf 29546 <em>B.T. Cyprus</em></td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>1:10</td>
<td>±1:5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>74</td>
<td>1:40</td>
<td>1:5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>88</td>
<td>1:80</td>
<td>1:5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95</td>
<td>1:40</td>
<td>—</td>
</tr>
<tr>
<td>34904</td>
<td>5.0 ml sheep blood I.V. <em>BT California T.10</em></td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56</td>
<td>&gt;1:160</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84</td>
<td>&gt;1:160</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>112</td>
<td>&gt;1:160</td>
<td>—</td>
</tr>
<tr>
<td>Calf Number</td>
<td>Inoculum</td>
<td>Days of Exposure</td>
<td>Complement-Fixation Titres</td>
<td>Normal Antigen</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------------------------------------</td>
<td>------------------</td>
<td>---------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>506520</td>
<td>15.0 ml blood I.V. and S.C. of B.T.</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Cyprus T.3</em></td>
<td>63</td>
<td>1:40</td>
<td>1:20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>94</td>
<td>1:20</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>133</td>
<td>1:20</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>168</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29546</td>
<td>100.0 ml blood I.V. from calf 506520</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>56</td>
<td>1:5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>84</td>
<td>1:10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>105</td>
<td>1:10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>154</td>
<td>1:5</td>
<td></td>
</tr>
<tr>
<td>506602</td>
<td>5.0 ml blood I.V. and S.C. from Calf 29546</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
<td>1:10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>1:40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>56</td>
<td>1:80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>77</td>
<td>1:40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>84</td>
<td>1:20</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>98</td>
<td>1:10</td>
<td></td>
</tr>
<tr>
<td>506226</td>
<td>3.0 ml calf blood I.V. daily for 3 days</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B.T. <em>California T.10</em></td>
<td>35</td>
<td>1:80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>105</td>
<td>1:80</td>
<td></td>
</tr>
</tbody>
</table>
TABLE III
Results of Complement-Fixation and Micro-Agar Gel Diffusion Tests
with 19 Deer Sera from the United States of America

<table>
<thead>
<tr>
<th>Deer Number</th>
<th>Complement-Fixation Test</th>
<th>U.S.D.A. Isoelectric Antigen (1)</th>
<th>A.D.R.I. BFK Antigen (2)</th>
<th>U.S.D.A. Pressure Filtration Antigen (3)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>WD-62-68</td>
<td>1:10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 63&quot;</td>
<td>ac 1:5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AGD-failure?</td>
</tr>
<tr>
<td>&quot; 64&quot;</td>
<td>1:80 ac 1:10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AGD-failure?</td>
</tr>
<tr>
<td>&quot; 65&quot;</td>
<td>1:10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AGD-failure?</td>
</tr>
<tr>
<td>&quot; 66&quot;</td>
<td>1:40</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AGD-failure?</td>
</tr>
<tr>
<td>&quot; 67&quot;</td>
<td>1:10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AGD-failure?</td>
</tr>
<tr>
<td>&quot; 68&quot;</td>
<td>ac 1:10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 69&quot;</td>
<td>ac 1:10</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 70&quot;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 71&quot;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 72&quot;</td>
<td>+1:5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 75&quot;</td>
<td>1:10*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 77&quot;</td>
<td>ac 1:20</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 78&quot;</td>
<td>1:10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 81&quot;</td>
<td>1:10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 85&quot;</td>
<td>1:160 ac 1:10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 86&quot;</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 87&quot;</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>&quot; 88&quot;</td>
<td>1:40</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
</tbody>
</table>

Reactors: 10
False reaction: 14
Failure?: 9

ac : serum anticomplementary
n.sp. : non-specific
* : reacted slightly with the normal antigen.
### Results of Complement-Fixation and Micro-Agar Gel Diffusion Tests with 30 Deer Sera from Ontario

<table>
<thead>
<tr>
<th>Deer Number</th>
<th>Complement Fixation Test</th>
<th>U.S.D.A. Isoelectric BFK Antigen (1)</th>
<th>A.D.R.I. Pressure Filtration Antigen (2)</th>
<th>U.S.D.A. Pressure Filtration Antigen (3)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>ac 1:10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>ac 1:20</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>AGD-(1) n.sp reactivity</td>
</tr>
<tr>
<td>38</td>
<td>ac 1:20</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>ac 1:20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>ac 1:10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>ac 1:20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>173-29</td>
<td>ac 1:5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>198</td>
<td>1:5*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp reactivity</td>
</tr>
<tr>
<td>199</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>206</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>207</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>702 N. Island</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>60-1-6-900 WTD</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>62-18</td>
<td>ac 1:5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>62-27</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>62-35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>62-37</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>62-38</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SC-279-WTD</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6310-11-0100-WTD</td>
<td>ac 1:5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6310-11-0300</td>
<td>ac 1:5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6310-11-0500</td>
<td>ac 1:20</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>6311-5-1400</td>
<td>ac 1:5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6311-15-946</td>
<td>-</td>
<td>-</td>
<td>IS</td>
<td>IS</td>
<td></td>
</tr>
<tr>
<td>6312-17-1507</td>
<td>ac 1:20</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>6312-17-1513</td>
<td>ac 1:5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6312-17-1516</td>
<td>ac 1:40</td>
<td>-</td>
<td>IS</td>
<td>IS</td>
<td></td>
</tr>
<tr>
<td>Reactors</td>
<td>1 (15 ac)</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>12 AGD-(1) n.sp. reactivity</td>
</tr>
<tr>
<td>False reaction</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* : reacted with normal antigen to the same degree
ac : serum anticomplementary
n.sp. : non-specific
IS : Insufficient serum
REFERENCES

FAT SOLUBLE TOXICANTS
INTOXICATION AND DETOXICATION

by
D. J. Wagstaff and J. C. Street

Toxicology is a discipline which is seldom fully utilized by veterinary diagnostic laboratories. There are at least two reasons for this. First, a rather large capital outlay is required for instrumentation. And second, there is a lack on the part of veterinarians of an appreciation for and training in toxicology. Although more time is allotted in preveterinary curricula to chemistry than any other subject, there is a general avoidance of chemistry and its applications by veterinary students and graduates. This latter problem can be solved by educating students, faculty members, diagnosticians and administrators in the general methods and usefulness of toxicology.

The duties of the laboratory diagnostician involved in toxicology (fig. 1) include (1) rendering a specific diagnosis based on the materials and information presented to him, (2) interpreting that diagnosis, and (3) making recommendations. A chemical analysis can assist in making toxicologic diagnosis but other factors such as history and pathology reports are also required in most cases. Interpretation includes mental reconstruction of the intoxication process in each case and rendering a prognosis. Based on the diagnosis and interpretation, recommendations such as therapy, prophylaxis, and ecologic or legal considerations can be made. An analytical chemist in a diagnostic laboratory or outside facility can perform chemical assays but a veterinary toxicologist is best qualified to make the diagnosis, interpretation and recommendation. For example, a finding of 100 ppm DDT in the body fat of an animal may bear no relationship to an acute nervous disorder. This chemical analysis if misinterpreted could even mislead and confuse the clinician.

This presentation will deal mainly with examples of principles involved in interpreting histories and analytical reports. Because all substances can be toxic under certain conditions, it is necessary to limit consideration here to one class of compounds — those which are soluble in lipids.

A vast number of potentially toxic compounds are soluble in fat. The group is exemplified by DDT, but other common compounds of the class are found in drugs, explosives, industrial chemicals, and even toys. Many lipid soluble substances are of course nontoxic and are normal components of the animal body or his diet.

Foreign lipoidal compounds when ingested are absorbed along with dietary fat. The dietary fat, the major component of which is triglyceride, is repeatedly cleaved and oxidized until only carbon dioxide remains (fig. 2). Energy is derived from the process and the residual atoms of the fat molecule are expelled as carbon dioxide.

1School of Veterinary Medicine, Univ. of Mo., Columbia, Mo. 65201
2Dept. of Animal Science, Utah State Univ., Logan, Utah 84321
and water. However, foreign lipoidal compounds as a general rule cannot be cleaved nor do they yield energy. They possess chemical structures such as aromatic rings or halogenated carbons which resist biological cleavage. To illustrate, DDT (fig. 3) has both aromatic rings and carbon-halogen bonds. Because cleavage of DDT is virtually nonexistent, degradation must proceed by noncleavage mechanisms. These alternate means are often not rapid enough to offset intake and as a result DDT is partitioned into body fat. If both degradation and storage are inadequate, then intoxication occurs.

Fortunately the homeostatic mechanism of the body is equipped to deal with these compounds in a different way from the dietary fat. Basically it is a process of altering their chemical structures by means other than cleavage to enable them to become water soluble and be excreted in urine or feces. The process appears to be controlled mainly by enzymes located in the microsomal fraction of liver cells. Thus, these enzymes are called hepatic microsomal enzymes. Their activities can be divided into two basic functions. First, creation of an active site on the molecule. Second, using this active site as a point of attachment, conjugation of the compound with a water soluble compound. The entire complex can then be excreted in urine or feces. Sometimes excretion occurs after the first step if sufficient water solubility is created by the active site. As an example, benzene (fig. 4) is a foreign compound of ring structure which resists cleavage. Hepatic microsomal enzymes create an active site, a hydroxyl group, on the ring and the product formed is phenol. Some phenol is excreted in urine. Other microsomal enzymes then form a conjugate of phenol and glucuronic acid. This conjugate is then excreted in the urine.

Animals can adapt to increased exposure to fat soluble toxicants by enhancing the activity of microsomal enzymes through the process of induction. Rats exposed to DDT produce increased quantities of DDT-metabolizing enzymes (Morello, 1965). The induction process is superficially analogous to immunologic reactions (fig. 5), but there are two major differences. First, the increased toxicant-metabolizing activity disappears completely within a short time after the stimulation ceases and there is no anamnesis. Second, the enzymes induced are active against a very large number of compounds. Animals exposed to DDT are less susceptible to phenobarbital and other drugs and poisons.

Induction requires from a few days up to a week before the maximum rate of activity is achieved. Therefore, initial exposure may produce intoxication but continued exposure may produce no harm as the organism adapts. Although for many years it was not recognized, there is evidence that animals adapt to DDT. More recently the role of hepatic microsomal enzymes capable of metabolizing DDT has been recognized (Morello; 1965).

A dramatic example of the relationship of intoxication and detoxication by microsomal enzymes was provided by feeding the organochlorine pesticide endrin to rats. We fed adolescent female rats diets containing 25 ppm endrin for 15 days (fig. 6). During the first day, the animals appeared to be normal. By the third day however, their feed consumption and body weight gains were much depressed.
During the latter part of the first week, a number of rats were observed in convulsions typical of organochlorine pesticides. No animals died in the experiment illustrated but in parallel experiments some rats did die. During the second week endrin-fed rats regained a normal appearance. Their body weight gain and feed consumption also returned to normal. These animals had adapted to the endrin in their diets and appeared clinically normal even though consuming a toxic dose. Determinations of microsomal enzyme activity were performed at the end of the feeding period (P-nitroanisole O-demethylase according to Kinoshita et al. 1966). Enzyme activity in animals consuming endrin was elevated to 233% of control values.

It was theorized that the tolerance to endrin was a direct result of stimulated activity of hepatic microsomal endrin-metabolizing enzymes. To test this theory, rats were induced by being fed a diet containing 25 ppm dieldrin (a potent microsomal inducer) for 5 days prior to feeding a diet containing endrin for 10 days. O-demethylase activity was elevated to 187% of control values by consumption of this level of dieldrin for 5 days in a parallel experiment. Rats pretreated with dieldrin consumed more endrin-containing feed but lost less body weight than those not pretreated (fig. 7 and 8). Mortality was 0/4 among pretreated rats and 2/4 among those not pretreated. In spite of increased dose of endrin the pretreatment partially protected against endrin intoxication. In other experiments pretreatment with another inducer compound, phenobarbital, was followed by decreased mortality among endrin-fed rats. Therefore, induction of microsomal enzymes prior to endrin exposure afforded some protection against intoxication by endrin. However the degree of tolerance to endrin was less than that following consumption of endrin per se. Because of this incomplete protection it is not possible to completely rule out all nonmicrosomal processes in endrin tolerance.

Let me relate this to the diagnostian by using another analogy to microbiology. If an immunologic procedure is performed only once in an outbreak, little information is gained upon which to base a diagnosis or prognosis. Likewise, only one sample assayed for endrin may tell you little; the animal may have received a lethal dose or may be tolerant.

Environmental problems have been created when certain species such as some birds and fish are exposed to such high levels of these toxicants that the adaptive detoxication processes are swamped. A well known example is the accumulation of DDT in the food chain of certain wild species.

Detoxication of natural fat-soluble toxicants is similar to that for synthetic compounds. A neutral product of recent interest in veterinary medicine is aflatoxin. We fed aflatoxin B1 to rats at various dietary levels (fig. 9). There was marked weight loss corresponding to dose of the toxicant during the first 3 days. After that, weight gains and appearance returned to normal. Exposure of rats to aflatoxin causes induction of microsomal enzymes which degrade aflatoxin itself but other microsomal enzymes degrading other toxicants are not induced and may even be inhibited. Scabort and Steyn (1969) reported that in rats injected with aflatoxin there was an increase of aflatoxin hydroxylase activity to 353% of control.
However, we observed that O-demethylase activity decreased to 73% of control in aflatoxin-fed rats. Aflatoxin therefore may be in the class of toxicants which is an exception to the general rule and induces only a narrow spectrum of enzymes. Other inducing agents also stimulate aflatoxin-metabolizing enzymes. Animals treated with DDT are protected against aflatoxin (McLean and McLean, 1967). The spectacular carcinogenic action of aflatoxin in certain fish may be due to their inability to adapt.

One of the groups of poisons in veterinary medicine possessing the highest degree of lipid solubility is the chlorinated naphthalenes, the cause of bovine hyperkeratosis. We have recently observed that these compounds induce microsomal enzymes. O-demethylase values were increased to 235% of control in rats fed diets containing 5000 ppm of 1,4-dichloronaphthalene for 15 days. However, the relationship of induction of microsomal enzymes to the natural disease is unknown.

Detoxication is not a process reserved for that rare dramatic moment but rather is a process which is constantly functioning and adapting to enable life to continue day after day. Detoxication is affected by a number of biologic factors including species, age, sex, and nutrition. In regard to the last of these, it is interesting that even seemingly small differences between nutritionally adequate diets can markedly alter detoxication rates. O-demethylase activity in rats fed a vitamin A test diet (USP XII) fortified with vitamin A was only 57% of that in rats fed Purina Laboratory Chow.

In the field of microbiology, determinations of detoxication activity, which in that context are known as immunologic or serologic reactions, are performed routinely in diagnostic laboratories. Perhaps in the future toxicologists will routinely determine detoxication activity in cases of intoxication to augment chemical analyses and biologic assay procedures.

It is hoped that members and administrators of veterinary diagnostic laboratories will view toxicology in all its aspects in a sophisticated manner. There is no more reason to "farm out" specimens for toxicologic examination than for sending out specimens for pathologic or microbiologic examination. Being realistic, I believe that only a few veterinary diagnostic laboratories will be able to quickly acquire the expertise and instrumentation required to perform modern "in house" toxicology. However, we should maintain a goal of complete toxicologic capabilities within the diagnostic laboratories. Even though some analyses may have to be performed by other laboratories while we grow, we should not delay the acquisition and employment of toxicologic expertise in diagnostic laboratories for rendering high quality toxicologic diagnoses, interpretations, and recommendations.
1. Diagnosis
   a. History
   b. Chemical Analysis
   c. Pathology Report
2. Interpretation
   a. Reconstruct Development of Intoxication
   b. Prognosis
3. Recommendation
   a. Therapy
   b. Prophylaxis
   c. Ecologic and Legal Considerations
Figure 2

NORMAL fat soluble compounds
(endogenous and dietary)

\[(\text{CH}_2\text{O})_x \xrightarrow{\text{O}_2} \text{repeated cleavage} \rightarrow \text{energy} + \text{CO}_2 + \text{H}_2\text{O}\]

Figure 3

Detoxication

(noncleavage means)

\[\text{DDT} \rightarrow \text{Storage in body fat} \rightarrow \text{Intoxication}\]
**Figure 4**

FAT SOLUBLE TOXICANTS

![Diagram showing the metabolism of benzene](image)

- **Benzene** is oxidized by hepatic microsomal enzymes in the presence of oxygen to form **phenol**.
- Phenol is then conjugated with glucuronic acid to form **phenyl glucuronide**.

**Figure 5**

- **Lipoidal toxicant** undergoes induction to form hepatic microsomal enzymes, leading to tolerance.
  - a. nonspecific
  - b. short lasting

- **Bacterial toxin** induces the formation of **antibodies** (antitoxins), leading to immunity.
  - a. specific
  - b. long lasting
Figure 6

Body wt. gain (% of control)

-100%

100%

0%

Control

25 ppm endrin

3 6 9 12 15 days on feed
Figure 7

- Control
- Dieldrin pretreatment
- Endrin

Deaths of endrin-fed rats

Feed consumed (% of control)

Days on feed

2 4 6 8 10
Figure 8

Body weight gain (% of control)

Control

dieldrin pretreatment

endrin
days on feed

deaths of endrin-fed rats

-300%
-200%
-100%
0
100%

2 4 6 8 10

days on feed
Control

Figure 9

Body wt. gain (as of control)

-500%  -400%  -300%  -200%  -100%  0  100%

3  6  9  12  15  days fed aflatoxin

X-X .5 ppm  O-O 1 ppm  --- 5 ppm
REFERENCES


ISOLATION AND IDENTIFICATION OF OBLIGATE ANAEROBIC BACTERIA

A. R. Dommert, D.V.M., Ph.D.*

This subject is obviously too complex for anyone to cover thoroughly in the time allotted here. Therefore, the purpose of this paper is to discuss some of the problems which must be overcome in work with anaerobic bacteria and some of the methods which are available for anaerobic work. Through this I hope that you will be encouraged to improve your diagnostic activities with anaerobic bacteria and that you will know where to look for details of methods to be used.

INTRODUCTION

Anaerobic microorganisms were probably among the earliest living forms in the world. They were probably able to grow even before the processes of photosynthesis began to liberate oxygen into the atmosphere. Anaerobic ecosystems continue to exist in skin pores, decomposing tissues, body cavities, soil, decaying vegetation, lake and sea bottoms. Anaerobic bacteria abound in these anoxic locations. Human skin and mouth may have 30 to 1 anaerobic over aerobic bacteria (Rosebury, 1962). Anaerobic bacteria outnumber aerobes more than 1000 to 1 in the human intestinal tract (Smith and Holdeman, 1968). Recent developments in anaerobic bacteriology have demonstrated that anaerobes in pure or mixed culture are present in over 78 per cent of the clinical abscesses in human and animal organs or tissues (Cato et al., 1970). Most of the potentially pathogenic anaerobes are found in the body along with the nonpathogens. Anaerobic members of the normal flora appear to require special conditions before their pathogenicity may be manifested. Thus, they seem to be opportunists and infections are of endogenous origin.

EFFECTS OF OXYGEN

A number of explanations have been offered as to why anaerobic bacteria are sensitive to oxygen. One theory was that anaerobes could not produce catalase to break down hydrogen peroxide which was produced in the presence of oxygen. Subsequently, studies indicated that the formation of catalase was not the primary factor determining whether an organism could grow in the presence of air (Smith and Holdeman, 1968).

The oxidation-reduction (O-R) potential of medium is one of the more important factors affecting growth of anaerobes (Smith and Holdeman, 1968). This potential, expressed as the Eh value in millivolts (mv), is the tendency of a system

*Associate Professor, Department of Veterinary Microbiology, School of Veterinary Medicine, University of Missouri, Columbia, Missouri 65201.
to give up electrons when compared to a hydrogen half-cell. The Eh of a system is a function of its reducing tendency and its hydrogen ion concentrations. Eh is reduced to 60 mv if the pH is increased one unit. This must be kept in mind when preparing media for anaerobic bacteria. Perhaps this is responsible for some of the discrepancies which occur between different laboratories using the “same” media.

Eh may be measured electrically or it may be determined by the ability of a system to reduce certain dyes to their colorless bases. The color changes take place at rather definite Eh values. Methylene blue, a commonly used anaerobic indicator, changes at about +11 mv. Resazurin is incorporated into some anaerobic media as an indicator of O-R potential and it changes at -42 mv. Lower O-R potentials may be indicated by phenosafranin which changes at -252 mv.

The limiting Eh values or upper potentials permitting growth have been determined for several of the clostridia and a few of the other anaerobes. The upper limits for clostridia range from about +150 mv down to +90 mv. Gram-negative anaerobic bacteria and the more fastidious Gram-positive anaerobes may have upper limits of +40 mv down to -300 mv. Cato et al. (1970) prepare pre-reduced media with an O-R potential of less than -150 mv. The media of Aranki et al. (1969) were reduced to -300 mv. An O-R potential of -359 mv will grow even the most oxygen-sensitive bacteria known.

Anaerobic bacteria inoculated into otherwise satisfactory medium with a high Eh respond by an exaggerated lag period if they are not killed first (Smith and Holdeman, 1968). During the long lag period, the bacteria apparently lower the Eh of their immediate surroundings by metabolizing nutrients carried over in the inoculum. When the Eh locally becomes sufficiently reduced, lag phase growth occurs at the usual rate.

From this it should be apparent that casually inoculating blood agar plates from the shelf and placing them in a closed container with a hydrogen generator will not suffice for culturing oxygen-sensitive anaerobes. Exposure of sterile medium to air, such as the pouring of plates or the inoculation of screw cap tubes that are later placed in anaerobic jars, produces organic peroxides in the medium and local increases in O-R potential that may preclude the growth of a major population of anaerobic bacteria. Consequently, a medium should be freed of oxygen when it is prepared and it should be kept that way during inoculation and incubation if possible. Smith and Holdeman (1968) wrote that, “Ideally, neither organisms nor media should ever be exposed to oxygen . . .”

Reduction of media usually is achieved using a combination of physical and chemical methods. Heat from boiling or autoclaving drives off oxygen dissolved in the media and helps reduce it. Usually chemical reducing agents such as sodium thioglycollate, cysteine, sodium sulfite, sodium sulfide (0.01 to 0.1%) or materials such as metallic iron and chopped meat are also added to the medium. Thioglycollate is apparently toxic for many fastidious anaerobes, because significantly fewer of them are cultured when media are reduced with thioglycollate than when cysteine is used (Smith and Holdeman, 1968).
ROLL-TUBE TECHNIQUE

The first effective method of culturing fastidious anaerobic bacteria was developed by Hungate (1950) to isolate rumen bacteria. He used an anaerobic roll tube method which was modified by Moore (1966). Pre-reduced, anaerobically sterilized (PRAS) media are used in this effective system. Media are prepared as usual, freed of oxygen by boiling and cooled in an ice bath while being perfused with oxygen-free gas. Cysteine is added as the reducing agent, the pH is adjusted and the media are dispensed into tubes swept free of oxygen with oxygen-free nitrogen. The tubes are stoppered with rubber stoppers; the stoppers are clamped firmly in place and the tubes are autoclaved. Tubes are inoculated while a stream of sterile, oxygen-free gas is passed into them to keep them anaerobic. Resazurin in the medium turns pink if the O-R potential gets above -42 mv at any point. Details of these procedures and recent improvements are described by Cato et al. (1970) in their *Outline of Clinical Methods in Anaerobic Bacteriology*.

The roll-tube method with PRAS media has not been used extensively in clinical work, but the efficacy is clearly reported by McMinn and Crawford (1970). They found that PRAS medium, used with rigid adherence to the cultivation techniques of Moore (1966), grew more than twice the number of anaerobic bacteria from clinical specimens that could be recovered by the conventional use of fluid thioglycollate medium and of blood agar plates incubated anaerobically with hydrogen generation packets.

ANAEROBIC CHAMBER TECHNIQUE

An alternate to the anaerobic roll-tube method is the use of an anaerobic glove box (Draser, 1967; Aranki et al., 1969). Media are usually prepared in the conventional manner and placed in anaerobic chambers through an airlock soon after autoclaving. Chambers are filled with anaerobic gas containing some hydrogen and the atmosphere is circulated over palladium coated catalyst to continually remove traces of oxygen. This catalytic system uses the same materials incorporated in the Torbal or Baird-Tatlock jars. Aranki et al. (1969) report that an overlay of trace amounts of palladium black catalyst reduce plated agar media to an O-R potential of -300 mv within 2 days after introduction into the glove box. This system apparently matches or excels the roll-tube method in attaining low O-R potentials and it permits the use of conventional plates and tubes for culturing.

We have used three such chambers during the last several years. Two have been made of flexible vinyl plastic with rigid airlocks and one has a controlled atmosphere fiberglass glovebox. One chamber was a converted gnotobiotic isolator to which a rigid airlock was added. We use a gas mixture of 10% hydrogen, 10% carbon dioxide and 80% nitrogen. A thermostatically controlled heating element maintains temperature at 37 C in order for us to use the chamber as an incubator also. A blower continuously circulates the gas through seives containing the catalyst and silica gel to maintain anaerobiosis. Resazurin and phenosafranin are used to
indicate the O-R potential. We like this system for a number of reasons, but primarily because the materials are not exposed to oxygen during manipulations in the chamber and because conventional bacteriologic methods can be used.

ANAEROBIC JAR TECHNIQUE

Clinical laboratories usually use anaerobic jars more extensively than any other method for obtaining anaerobiosis. Three types of jars are commonly used. The Brewer jar is the type that is evacuated and filled with a combustible gas with which the heat activated catalyst combines oxygen. Torbal and Baird-Tatlock jars are evacuated and refilled with gas containing hydrogen. The hydrogen combines with oxygen in the presence of a palladium catalyst. Gas-Pak jars (BBL Labs, Baltimore, Md.) are not evacuated, but contain palladium catalyst and individual generators of hydrogen and carbon dioxide. A jar which is evacuated is preferable because oxygen is removed more quickly.

Anaerobic jars will not achieve or continuously maintain the degree of anaerobiosis that is possible in an anaerobic chamber or roll-tube; however, when properly used they are effective for culturing many of the anaerobic bacteria encountered in clinical cases. All factors must be kept at their attainable limit in order for the system to be effective. The catalyst must be sufficiently active to remove oxygen. The gas must contain sufficient hydrogen or oxidizable ingredients to combine with all of the oxygen. Most important of all, media must be reduced prior to inoculation. This may be achieved by pouring plates just prior to use or by storing the plates in anaerobic jars until they are inoculated. Plates which are prepared and quickly placed in an anaerobic jar should be reduced to the limit of this system after 24 to 48 hours of storage. They should be removed, immediately streaked with a platinum or stainless steel inoculating loop and returned to an anaerobic jar within ten minutes. Liquid media must be freshly prepared just prior to use or boiled for 20 minutes and quickly cooled just prior to inoculation.

LESIONS ASSOCIATED WITH ANAEROBES

Many of the obligate anaerobic bacteria have been ignored or disregarded in veterinary medicine. This is probably due to the strict growth requirements of the organisms and due to the fact that they usually occur in association with other bacteria. Biberstein et al. (1968) recognized this and subsequently studied 2,164 animal disease samples. They reported 102 isolates of Bacteroides melaninogenicus. Abscesses were the usual sources; however, isolates were also made from suppurative lesions of practically all other parts of the body. The incidence of infection was highest in cats and lowest in horses.

Other Gram-negative obligate anaerobes such as Fusobacterium necrophorum and Bacteroides nodosus have been associated with foot rot and other necrotic processes of cattle and sheep. Liver abscesses in cattle are usually attributed to infections with fusobacteria and/or clostridia. We are all familiar with the common
disease processes associated with clostridial infections.

COLLECTION OF SAMPLES

I have already stated the effectiveness of anaerobic culturing depends on the quality of anaerobic techniques used, but it is equally dependent upon the method of collection and method of handling the sample prior to isolation. Every effort must be made to minimize exposure of the sample to air. Where possible, fluid pus from abscesses should be collected in a syringe. Deep tissue samples should be collected for culturing from other necrotic lesions. Cotton swabs are usually less effective for obtaining good anaerobic samples. If delay before culturing is unavoidable, the sample should be places in anaerobic media and held at room temperature.

ANAEROBIC MEDIA

The choice of media for anaerobic bacteria is probably even more arbitrary than the choice of media for the enteric bacteria. Anaerobic bacteria require nutritionally rich media and media to be stored under anaerobic conditions should have 0.05% L-cysteine HC1 added after it is autoclaved and cooled. Several acceptable basal media are listed in good technique manuals such as the Manual of Clinical Microbiology by Blair et al. or the Outline of Clinical Methods in Anaerobic Bacteriology by Cato et al. Certain ingredients must be added to such media to make them selective or suitable for growing some specific bacteria. Bacteroides melaninogenicus requires hemin which may be provided with laked blood and some strains require menadione (Gibbons and Macdonald, 1960). Bacteroides nodosus from foot rot of sheep requires sheep hoof in the media for growth.

Brain heart infusion agar (BHIA) is a good isolation media for general use. Reinforced clostridia medium (RCM) is another commercially available dehydrated medium which supports growth of many anaerobic bacteria. Whole blood, laked blood or selective agents such as neomycin, kanamycin and vancomycin may be added to these media. Finegold (1970) recommends inoculating blood agar and prerduced chopped meat-glucose medium as a minimum for isolation of anaerobes from clinical specimens. The desirable list to be inoculated include eight other enrichment or selective media. Freshly prepared chopped-meat medium is probably one of the best media for supporting growth of all anaerobic bacteria.

IDENTIFICATION OF ANAEROBES

Single colonies must be picked as in aerobic bacteriology, but two or three platings may be necessary to obtain a pure culture. Pure cultures should be transferred to chopped-meat, RCM broth or other enriched anaerobic broth to determine the Gram reaction, morphology, flagella location and spore formation. The Kopeloff modification of the Gram stain is recommended for anaerobic
bacteria (Cato et al., 1970). Phase contrast examination is also helpful in determining the cell morphology, motility and spore formation.

Clostridia are usually identified on the basis of Gram reaction, spore formation, fermentation of common sugars, reaction on milk and the production of several enzymes. Keys for identification of anaerobic bacteria are presented in the ASM Manual of Clinical Microbiology, in the V.P.I Outline of Clinical Methods in Anaerobic Bacteriology and in the upcoming 8th Edition of Bergey's Manual of Determinative Bacteriology. Volatile fatty acids and alcohols produced from the fermentation of glucose are particularly useful in the identification of Gram-negative bacteria. These products are usually detected by gas chromatography. Most nonmotile, Gram-negative, nonsporeforming rods belong in the genera *Bacteroides* of *Fusobacterium*. These organisms were once separated on the basis of morphology, which was difficult due to the many intermediate strains. The genera are now differentiated on the basis of fermentation acids and other metabolic products (Holdeman and Moore, 1970). Organisms that produce butyric acid as a major product from glucose fermentation are placed in the genus *Fusobacterium*. Organisms that do not produce butyric acid but produce acetic and succinic acids, often with propionic or formic acids, as the major products are placed in the genus *Bacteroides*. Organisms formerly in the genus *Sphaerophorus* are placed in the genus *Fusobacterium* or the genus *Bacteroides*.

**SUMMARY**

Thus you can see, practical anaerobic methods are now available for isolating even the most demanding anaerobes and recent information about the characteristics of anaerobes now makes their identification a much simpler task.

I. Anaerobic bacteria are associated with numerous disease processes in animals.

II. Anaerobic bacteria have different limiting Eh values and the quality of anaerobic technique used will limit the organisms which may be isolated.

III. New clinical manuals provide valuable information for identification of anaerobic bacteria.
REFERENCES


Sufficient laboratory tests and field observations have now been conducted to indicate that the immunodiffusion test for equine infectious anemia (EIA) is a reliable indicator of EIA infection in the horse. Data was presented at the American Veterinary Medical Convention this year in Las Vegas which showed a direct correlation between positive test results and infectivity as determined by animal inoculation. It was concluded that the test is at least 95% accurate for the diagnosis of EIA. Since then twelve additional animal inoculations have been made, four epizootics of EIA have been investigated, and several thousand survey samples have been examined. The immunodiffusion test is performing satisfactorily in the field. Furthermore I understand that Pearson et al. at the National Animal Disease Laboratory have tested several hundred serums and have obtained equally satisfactory results.

Since the technique was described only briefly in the original article and in response to numerous requests for more detailed information on the procedure, the test is described more fully in this paper.

PREPARATION OF ANTIGEN

Specific EIA antigen is obtainable from the spleen of a pony or horse acutely infected with any of several virulent isolates of EIA virus. The most satisfactory antigens have been obtained from animals showing a severe febrile reaction after a short incubation period of 3 to 5 days and when the spleen was harvested at 9 to 11 days after inoculation of virulent EIA virus. Antigens have been produced by the inoculation of ponies intravenously with 1 ml. of serum containing Wyoming EIA virus (Serum taken at 9 days postinoculation that has a titer of $10^7$ pony infectious doses per ml.) but larger volumes of infective serum (10 to 100 ml.) appear to more consistently produce short incubation periods. It has been found that a sub-inoculation of acute stage blood in an amount of about 300 ml. has helped to shorten the incubation period and cause a more severe illness. The spleen from one infected horse became greatly enlarged and engorged with blood so that the antigen was too dilute. Antigens cannot usually be diluted beyond 1:2 or 1:4. It was possible to concentrate the antigen by precipitation with half-saturated ammonium sulfate and reconstitution in a smaller volume.

Spleen is collected sterilly, stored frozen at minus 20 C and is thawed and frozen several times before using. Aging the spleen in the freezer appears to aid in the release of the antigen also. The essential points in antigen preparation are:

1. Select a spleen from a horse showing a very severe acute reaction to EIA
IMMUNODIFFUSION TEST FOR E.I.A.

2. Harvest the spleen at 9 to 11 days post inoculation.
3. Freeze and thaw the spleen several times before using and avoid dilution in anyway.

PREPARATION OF ANTISERUM

A positive reference antiserum can be chosen from a horse surviving EIA infection. Long term carriers often have been found to have satisfactory serums. The serum should give only one dense, distinct precipitin line when tested with the EIA antigen and the line should form approximately midway between the serum and antigen wells with no tendency to broaden or fade with time. Thyprecipitin line must be shown to be specific for EIA by horse infectivity tests or by forming a line of identity with an antiserum which has been shown to specific for EIA by animal inoculation tests. Serums with excess antibody in relation to antigen concentration tend to form broad bands rather than a narrow distinct line which is essential for the accurate determination of line of identity. It is much easier to see the suspect line coalesce with the reference line if the latter is a dense, narrow line. Discrete precipitin lines can be obtained with the higher titered serums but they must be diluted and they seem to be less dense. Avoid serums that give a cloudy ring around the well.

The essential point in antiserum preparation is: Select a serum from a horse surviving EIA infection that give only one dense, distinct precipitin line that is specific for EIA.

PREPARATION OF IMMUNODIFFUSION TEST

Immunodiffusion reactions are carried out in 85-mm plastic petri dishes. Because of occasional seepage of serum underneath the agar, a base layer of 5 ml. of 2% Noble's special agar in a borate buffer is used. When this layer has hardened a top layer of 15 ml. of 1% Noble's special agar in the same buffer is added. The borate buffer is prepared by mixing 2 gm. of NaOH and 9 gm. of H₃BO₃ in a liter of distilled water. No preservative or sodium chloride is used. The pH of the buffer should be about 8.6. It is suggested that the agar be dissolved and melted by boiling because autoclave temperatures cause some discoloration. The agar is allowed to harden in petri dishes at room temperature with the lids partly open to allow moist air to escape. Excess water in plates can dilute the antigen and should be avoided. Fresh plates are poured daily as old ones tend to become cloudy.

A template with seven circular cutters is used to cut six wells, 7 mm in diameter and spaced 3 mm apart, around a central reservoir of the same diameter. Four of these patterns can be cut in each petri plate. A cork borer can be used to cut the wells as outlined on a paper underneath the petri dish. Care should be taken to lower the cutters through the 1% agar until the resistance of the 2% layer is felt. Agar in the wells is then removed with a short pipette attached to a suction pump.
The 2% agar base layer should be left intact.

Splenic pulp is teased from the connective tissue and packed in the central reservoir. Care should be taken not to leave air pockets in the bottom of the well or to over-fill because the tissue tends to swell in some cases. A positive reference serum is placed in two wells on the periphery directly opposite each other and the four remaining wells are filled with suspect serum samples. With this arrangement each suspect serum is next to a reference serum which facilitates easy determination of lines of identity. Plates are incubated at room temperature (20°C) in an inverted beaker containing a wet towel.

**READING THE IMMUNODIFFUSION TEST**

The immunodiffusion reactions are observed over a strong narrow beam of light and against a black background at 24 and 48 hours. Distinct precipitin reactions are normally visible at 24 hours but weaker reactions sometimes form between 24 and 48 hours. The patterns do not usually change after 48 hours. Doubtful reactions should be retested in duplicate and set up in various patterns to see if they are reproducible. Broad precipitin bands which often occur with carrier serums are not very difficult to recognize because they cause the reference line to stop abruptly about half-way across its normal position and such reactions can be confirmed as specific for EIA by diluting and obtaining a more distinct line. Weakly reacting serums, such as are found in the early stages of EIA infection and in foals with maternal antibody, are more of a serious problem. Such serums usually have to be retested in duplicate and with variations of the patterns. Since the reference precipitin line bends slightly toward the serum well, deviation of this line toward the antigen well as it nears the suspect serum well indicates a weak antibody reaction. It is advisable to recommend that these animals be bled again especially if they are only recently infected. Stronger reactions are commonly seen with serums taken a week or so later. Accurate drawings of the precipitin reactions for future reference is suggested.

**INTERPRETATION OF THE TEST**

The immunodiffusion reaction has been shown to be an accurate and reliable test for the detection of EIA infection in the horse except for animals in the early stages of infection and foals of infected dams. Since the test measures antibody produced as a result of EIA infection, horses in the first two to three weeks of infection and before antibody is produced will test negatively. Such animals should be bled again in a week or two. In order to make a diagnosis in a young foal it is necessary to determine the antibody status of the dam. If the mare is negative then her positive reacting foal can be declared infected. If the mare has EIA antibody then the foal may or may not be infected. Only a gradual decline and eventual loss of the antibody over a period of several months will indicate absence of infection. A few foals have maintained their maternal antibody for 4 and 5 months.
The EIA immunodiffusion test is not a foolproof technique and requires a degree of expertness in order to interpret the results accurately. Such competence can only be obtained by experience with the test and by observing known positive and negative reactions of varying degrees as well as the non-specific precipitin lines that are occasionally seen. It is felt that initially operators should send duplicate samples to a competent laboratory for an evaluation of their test readings before making routine diagnosis. In addition all reagents should be checked for specificity against known EIA reference antigen and antiserum. Many laboratories will want to do animal inoculation tests to confirm their findings.

Although the presence of precipitating antibody is closely correlated with the presence of EIA virus in the horse, there is no consistent correlation with existing clinical signs of EIA. Many carriers show chronic emaciation, anemia, and periodic febrile illness but some are completely normal clinically. In attempts to demonstrate EIA virus in the inapparent carrier it has become increasingly evident that the virus titer may be very low and that the infective virus may only be found in whole blood and not in the serum. Thus an immediate transfusion of a large volume of whole blood in an experimental test animal may be necessary to transmit the infection and produce a clinical response.

REFERENCES

ABORTION IN LIVESTOCK

E. J. Bicknell, D.V.M., Ph.D.; and W. U. Knudtson, M.S.

Results of research indicate that of the 2,000,000 cows in South Dakota, approximately 20,000 will abort or produce stillborn calves each year. A recent survey in the state indicated that it costs from $100.00 to $125.00 to maintain a beef cow through one year, under average conditions. When such a cow fails to produce a viable calf, this maintenance cost becomes a total loss. Failure of a dairy cow to produce a normal offspring can be even more costly. The total loss from abortion and stillbirth of calves in South Dakota appears to be greater than $2,000,000 per year. Sizable losses also occur due to abortion and stillbirth in swine and sheep.

Two years ago a research project was initiated at the South Dakota Animal Disease Research and Diagnostic Laboratory to identify the major causes of abortion and stillbirth, to develop and improve diagnostic procedures, and to devise methods to control major causes of abortion and stillbirth in domestic farm animals. Between July 1, 1968 and June 30, 1970, abortions and stillbirths were investigated from 798 cows, 75 sows and 74 ewes. The number and type of specimens available from each case varied. In some instances only placenta was presented; in others the fetus, placenta, blood from the dam, and feed and water samples were available. As many as 30 individual laboratory examinations were often completed in investigating a single case. Despite this effort, between 70 and 75 per cent of abortion and stillbirth cases remain undiagnosed. This emphasizes the difficulty encountered in determining causes of abortion.

While there are numerous well known infectious abortifacient agents, it seems likely that there are many more which remain unrecognized. Most of the noninfectious abortifacients, such as environmental conditions, nutritional deficiencies and imbalances, toxins of all kinds, endocrine imbalances, genetic improprieties, as well as in utero trauma, are often poorly defined and, for the most part, go undiagnosed as causes of abortion.

There are no well-established, reliable means of diagnosing many of the known causes of abortion and in many instances, no diagnostic lesions or agents are to be found in the fetus or placental tissue. Thus the diagnostician is presented with many problems when he attempts to determine the cause of abortions and stillbirths.

For the most part, the practitioner who submits an abortion case to the diagnostic laboratory has little on which to establish a clinical diagnosis. Therefore, his request for help from the laboratory is in a general form, often with no specific disease entity in mind. Should the diagnostician fulfill only the specific request of the submitting clinician, the likelihood of arriving at a diagnosis is very small. Because of the wide variety of possible abortifacients, the diagnostic laboratory must establish a protocol which will reveal as many of the known causes of abortion as possible. At the same time the number of examinations performed is
limited by practical factors of time and economics.

At the South Dakota laboratory the procedure of investigating an abortion case begins with examination of the history. A necropsy is then performed on the fetus or fetuses and results of the examination are recorded. If placenta is available it is always examined for gross lesions. Lung, liver, spleen, kidney, brain and stomach contents of ovine and porcine fetuses are routinely cultured for bacteria.

After examination of more than 500 bovine abortion specimens it became evident that bacteriologic examination of stomach contents would reveal over 90% of bacterial causes of abortion in this species. The amount of time, effort and expense necessary to examine several other tissues for bacteria does not seem to be warranted by the relatively few additional diagnoses which might be made. Bacteriologic examination of placental tissue is severely complicated by many contaminate organisms ordinarily present. For the most part such examination provides little information which cannot be gained through other procedures.

Stomach contents are routinely cultured for fungi. Results of this procedure indicate that it is of more value as a research tool than as a diagnostic procedure. The results appear to require considerable interpretation to be of diagnostic value.

Histopathologic examination is routinely completed on lung, liver, spleen, kidney and brain of all fetuses. In addition, other tissues with obvious gross lesions are also examined microscopically. The placenta, when available, is always examined for microscopic lesions.

Lung, liver, spleen and kidney tissues are ordinarily pooled and inoculated onto embryonic kidney tissue culture of the same species as the aborted fetus. When placenta is available, it is cultured separately because of the gross contamination usually present in this tissue.

In addition to cytopathic effects, tissue cultures from porcine fetuses are examined for hemagglutinating properties after the second passage. The direct fluorescent antibody test for hog cholera virus is done on tissues from each aborted porcine fetus.

When blood from the dam is submitted, the microagglutination test for leptospirosis is performed with at least three serotypes. The plate agglutination test for brucellosis is routinely done.

From time to time the protocol has been changed to accomodate new or improved procedures or to eliminate some found to be unsuitable or unproductive. Culture of the stomach contents and tissues of all fetuses for mycoplasma has recently been included in the protocol. In the future it is anticipated that fetal tissues will also be examined for anaerobic bacteria.

It has been found that routine examination of aborted porcine fetuses for bacteria, viruses and microscopic lesions provides few diagnoses. Dark field microscopic examination of the pericardial fluid and fluorescent antibody examination of pericardial fluid and frozen tissue sections have disclosed several cases of leptospiral abortion which had been missed on routine examination. At present, kidney and liver homogenates from each porcine fetus is inoculated into gerbils. It is anticipated that the efficacy of microscopic and fluorescent antibody
examinations in disclosing leptospirosis can be determined by animal inoculation.

Table 1 lists diagnoses made from 798 cases of bovine abortion over a period of two years. It is anticipated that the study will continue one more year. There has been no attempt to refine the data to this point. What is presented represents crude data subject to change and refinement. It should be noted that the results reflect not only the incidence of the particular abortifacient in the area but to some extent the efficacy of diagnostic procedures for identifying that agent.

Infectious bovine rhinotracheitis (IBR) is the most commonly diagnosed cause of bovine abortion in South Dakota. Results thus far indicate that IBR causes at least one out of every 10 bovine abortions. Diagnosis of this disease has been based mainly on presence of the characteristic microscopic lesion (focal necrosis) which occurs most frequently in the liver, but occasionally also in the spleen and kidney. The virus can be isolated from tissues from approximately one-third of the fetuses with lesions. When the placenta is available, the number of virus isolations increases approximately 50 per cent. Recently an immunofluorescent technic applied directly to fetal tissues has proven to be very effective. The technic and the evaluation of it are the subject of another presentation at this conference.

Mycotic placentitis is the second most commonly diagnosed cause of bovine abortion in South Dakota. Gross lesions of this disease consistently occur in the placenta and occasionally in the skin of the fetus. Positive diagnosis is made by demonstrating the presence of mycotic elements in the placental tissue or skin. Occasionally the fungus may cause a fetal pneumonia. Stomach contents of calves aborted due to mycotic placentitis frequently contain large quantities of fungi, usually *Aspergillus* sp. The presence of a few fungal elements in the stomach contents does not provide firm evidence that this organism was involved as the cause of the abortion.

Vibriosis is the third most commonly diagnosed cause of bovine abortion in South Dakota. *Vibrio fetus var. venerealis* and *V. fetus var. intestinalis* are both associated with this problem. Since there is considerable difference in the syndrome produced by the different varieties of this organism, it is important that they be distinguished from each other. Table 2 illustrates the definitive characteristics presently being utilized in our laboratory. It is realized that this method has limitations but it appears to be accurate enough for diagnostic purposes.

Isolation of the organism from stomach contents of aborted fetuses provides the most definitive means of diagnosis of vibriosis. A reliable fluorescent antibody technic would have the tremendous advantage of being able to detect organisms which have been inactivated in the process of transporting the specimen. Not all *V. fetus* conjugates in use at present appear to be reliable.

*Corynebacterium pyogenes* is associated with a surprisingly large number of bovine abortions in South Dakota. Its presence is frequently associated with inflammatory changes, especially suppurative bronchopneumonia. This lesion is not definitive since it is commonly seen in fetuses aborted due to brucellosis, vibriosis, and other bacterial as well as mycotic infections. *C. pyogenes* abortions most often occur sporadically, but occasionally epizootics occur. Very little is known
concerning the pathogenesis and epizootiology of this disease.

Leptospirosis is the most commonly diagnosed cause of swine abortion in South Dakota. An insufficient number of isolates have been obtained to indicate the most common serotypes.

Occasionally *Staphylococcus* sp. has been isolated in pure culture from stomach contents and tissues of several porcine fetuses aborted from the same sow. This provides presumptive evidence that these organisms were involved as a cause of the abortion. In one case *Erysipelothrix insidiosa* was isolated from pigs aborted from the same sow.

Recently several hemagglutinating viruses have been isolated from aborted pig fetuses. These agents have not been identified and the significance of their presence is unknown.

Several mycoplasma have been isolated from aborted porcine, ovine and bovine fetuses. These isolates are in the process of being identified. Again, the significance of their presence is unknown. At least one of the bovine fetuses from which a mycoplasma was isolated had gross and microscopic lesions similar to those described for epizootic bovine abortion (EBA).

The most commonly diagnosed cause of ovine abortion in South Dakota is vibriosis. Occasionally the characteristic gross and microscopic liver lesions are found but diagnosis is based upon isolation of *V. fetus var. intestinalis* from the stomach contents and tissues. This organism seems to be more hardy and easier to recover from fetuses than the venereal variety.

Examination of ovine placentas for elementary bodies characteristic of epizootic abortion in ewes (EAE) has so far failed to reveal this disease.
TABLE 1
Diagnoses made investigating 789 cases of bovine abortion or stillbirth over a 2 year period.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>% of total cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBR</td>
<td>85</td>
</tr>
<tr>
<td>Vibriosis</td>
<td>25</td>
</tr>
<tr>
<td>Mycotic placentitis</td>
<td>22</td>
</tr>
<tr>
<td>C. pyogenes</td>
<td>18</td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>10</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>4</td>
</tr>
<tr>
<td>EBA</td>
<td>3</td>
</tr>
<tr>
<td>BVD</td>
<td>2</td>
</tr>
<tr>
<td>Listeria</td>
<td>2</td>
</tr>
<tr>
<td>Anomaly</td>
<td>2</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>1</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>1</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>1</td>
</tr>
<tr>
<td>Yeast</td>
<td>1</td>
</tr>
</tbody>
</table>

Total diagnoses 177
% of total cases 22

TABLE 2
Differentiation of Vibrio Isolates Based on Reactions in FeSO₄ Medium*

<table>
<thead>
<tr>
<th>ISOLATE</th>
<th>H₂S PRODUCTION</th>
<th>GROWTH IN 1% GLYCINE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. fetus intestinalis</em></td>
<td>--</td>
<td>+</td>
</tr>
<tr>
<td><em>V. fetus venerealis</em></td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>V. bubulus</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*After Ringen & Frank
PRELIMINARY STUDIES IN INTERFERON INDUCTION ON THE RESPIRATORY TRACT OF CATTLE

A. B. Angulo* and M. Savan
Veterinary Microbiology and Immunology
Ontario Veterinary College, Guelph, Ontario, Canada

INTRODUCTION
There are many reports in literature on the effectiveness of interferon for protection or prevention against many virus infections. In most of these studies an in vitro cell culture system or small laboratory animals such as mice and rats are utilized. Large animals like cattle are not commonly used because of the need for a large dose of interferon or inducers that are toxic in large amount, and the difficulty of detecting interferon response in the various tissues. Recently however it has been reported\textsuperscript{1,2} that the bovine species is capable of producing circulating interferon after parenteral administration of suitable inducers.

Based on the current concept that a local state of immunity in the respiratory tract is produced after exposure with infectious agents and that this immunity is a better index of resistance to reinfection, a similar approach was tried for the induction of interferon. Attempts were made to induce interferon locally on the respiratory tract of cattle using a synthetic polynucleotide complex with the objective of producing a temporary state of broad spectrum nonspecific resistance to many respiratory viruses.

MATERIALS AND METHODS

Cell Culture and Medium
Primary embryonic bovine kidney cell cultures (EBK) were prepared following the methods of Madin et al\textsuperscript{3}. The cultures were grown and maintained in Eagles Minimal Essential Medium (EMEM) containing 10\% and 5\% fetal calf serum respectively.

Preparation of Poly riboinosinic - polyribocytidylic (Poly rI-rC) nucleotide complex.

The procedure described by Fields et al.\textsuperscript{4} was followed in preparing the poly rI-rC complex. The potassium salts of polyriboinosinic and polyribocytidylic acids\textsuperscript{**} were used at a concentration of 1 mg/ml and were mixed in an equimolar concentration in phosphate buffered saline (PBS) pH 7.1 (0.01 M sodium phosphate, 0.15 M sodium chloride). The preparation was incubated at 37\textdegree C for 20 minutes or until the formation of double stranded poly rI-rC complex was indicated by its hypochromic effect.

---

*The senior author is supported by a Colombo Plan Scholarship through the Canadian International Development Agency. The investigation was partially supported by a NRC Grant (A-1389).

**Obtained through a Sigma Co-operative Allowance Policy. Sigma Chemical Company.
Collection of Nasal Secretion

Following the technique described by Rouse and Angulo\(^5\) pre and post exposure nasal secretions were collected. Briefly this was done by inserting a sterile gauze swab attached to a flexible rubber covered wire support into the ventral meatus of the nasal cavity, in a manner similar to the insertion of a stomach tube. Another swab was also placed in the other nasal cavity and both were left in situ for 10-15 minutes. The secretions were collected by aspiration into a plastic syringe.

Determination and Assay of Interferon in the Nasal Secretion

The secretions were first dialysed against HCl - KCl buffer, pH 2 at 4\(^\circ\) C for 24 hours and back dialysed to pH 7.1 PBS, centrifugation at 3,000 rpm for 20 minutes was done to remove cell debris, and the secretions were filtered through .8u and .45u millipore membranes. Serial two fold dilutions from undiluted to 1:80 were made with PBS and 0.5 ml of each was inoculated into each of 3 EBK tubes. The cell cultures were incubated overnight at 37\(^\circ\) C, then washed with PBS, challenged with 0.1 ml 100 CCID\(_{50}\) (cell culture infectious dose\(_{50}\) ) Vesicular Stomatitis Virus (VSV) and refed with fresh medium. Uninoculated and VSV infected cultures were included as controls. The cell cultures were incubated at 37\(^\circ\) C and were observed daily for cytopathic effect (CPE) up to 5 days. The presence of interferon was indicated by absence of VSV CPE and the titer was expressed as the highest dilution showing complete inhibition of CPE. The presence of 25-50% CPE on the cell monolayer was considered as partial inhibition. The virus controls, showed complete cell monolayer destruction while the uninoculated cell cultures no apparent change. The nasal secretions showing inhibitory activity against VSV were further characterized for some physical and biological properties. The procedures for the different tests are cited and described in Interferons\(^6\). The following criteria were satisfied in order to establish the presence of interferon in the nasal secretion (Table 1).

| TABLE 1 |
| Criteria for Establishing the Presence of Interferon in Nasal Secretion |

1. Activity stable at pH 2, 4\(^\circ\) C for 24 hours
2. Activity stable at 56\(^\circ\) C for 30 minutes
3. Absence of toxicity to cell culture
4. Retention of antiviral activity after thorough washing of the interferon treated cell monolayer prior to inoculation with VSV.
5. Absence of direct inactivating effect to VSV.
6. Inhibitory activity against heterologous viruses (e.g. IBR and VSV).

Interferon Induction with Various Doses of Poly rI-rC

Four healthy male hereford calves 3-4 weeks old were designated as A, B, C and D and confined in isolation rooms for the duration of the experiment. Calves
A, B, C and D were exposed intranasally by means of DeVilbiss No. 40 Nebulizer to 0.5, 1.0, 2.0 and 3.0 mgs. of Poly rI-rC respectively. Pre and Post exposure nasal secretions were collected at 0, 12, 24, 72 and 96 hours for interferon determination.

*Interferon Induction following Repeated Exposure to Poly rI-rC*

Three female hereford 3-4 weeks old were used in this experiment. One calf was exposed to 0.5 mg of poly rI-rC and without collecting the nasal secretion, a second dose of 0.5 mg of the inducer was made 12 hours later. The 2nd and 3rd calves were given 1 and 3 mgs of inducer respectively. The secretions were taken at the same time intervals described earlier. Following the collection at 72 hours, the 2nd and 3rd calves were again exposed twice to the same doses at 72 hour intervals. The nasal secretions of these 2 calves were then further collected at 96, 120, 144 and at 192 hours.

*Effect of the Frequency of Nasal Secretion Collection on the Interferon Titer*

Two Holstein-Friesian bull calves 3-4 weeks old were each exposed to 2 mgs of Poly rI-rC. In one of the calves the nasal secretions were collected at 12, 24 and 48 hours while only one sample at 48 hours was taken from the other calf.

**RESULTS**

An inhibitor with physical and biological properties similar to interferon (Table 1) was detected in some of the post exposure nasal secretion samples. The pre-exposure samples did not inhibit VSV infection of the cell cultures.

The response of the calves to the induction of interferon with various doses of poly rI-rC are presented in Table 2. No detectable interferon was found in the nasal secretions of the calf exposed to 0.5 mg of inducer. With 1 mg dose, interferon was demonstrated in the undiluted secretions collected at 12, 24, and 72 hours. At 48 hours the interferon titer was 10. No inhibitory activity was present in the 96 hour sample. In the nasal secretions of the calf given 2 mgs of the drug, interferon activity was present in the undiluted secretions taken at 12 and 24 hours while titers of 20 and 5 were demonstrated in the 48 and 72 hours samples respectively. No activity was found in the 96 hour sample. The calf dosed with 3 mgs of inducer had detectable interferon in the undiluted, 1:5, 1:20 and 1:10 dilutions of the secretions at 12, 24, 48 and 72 hours respectively. There was a 50% inhibition of virus CPE at 96 hours.

The results of repeated exposures to poly rI-rC are presented in Figure 1. Interferon was detected in the nasal secretions at 24 and 48 hours following successive exposures of 0.5 mg of the inducer at 12 hour intervals. With 3 exposures of 1 and 3 mgs of poly rI-rC at 72 hour (3 day) intervals an interferon response was shown at 12 hours persisting up to 192 hours (8 days) at which time the final sample was collected. The interferon titers of the secretions varied from undiluted to as high as 40. Furthermore, upon subsequent inductions the response did not diminish compared to the titer on initial stimulation.
In the third experiment no significant difference was observed between the interferon titers of the nasal secretions from the 2 calves exposed to 2 mgs of inducer. Interferon was present in the undiluted secretions collected from one calf at 12 and 24 hours and in a titer of 10 at 48 hours. On the other hand the only sample taken from the other calf at 48 hours had only a titer of 5.

**TABLE 2**

Interferon Titer* in the Nasal Secretions of Calves Given Various Doses of Poly v1-rC.

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>undiluted</td>
<td>undiluted</td>
<td>10</td>
<td>undiluted</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>undiluted</td>
<td>undiluted</td>
<td>20</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>undiluted</td>
<td>5</td>
<td>20</td>
<td>10</td>
<td>+</td>
</tr>
</tbody>
</table>

*Based on .5 ml nasal secretion/CPE inhibition of .1 ml 100 CCID50 VSV challenge

**DISCUSSION**

Interest in the study of interferon stems from the prospect that it could provide a means for a broad spectrum prophylaxis against many viral diseases, particularly those responsible for respiratory infections in man and animal. In cattle, the majority of the virus respiratory diseases have short incubation periods and are most often precipitated by stress such as weaning, overcrowding and poor management, or during transfer to feed lots. The losses from these diseases are considerable in spite of the intensive vaccination practices against many of the virus agents. The control and prevention of virus respiratory diseases through immunization procedures is difficult because of the need for more effective vaccines. Furthermore the development of potent vaccines is formidable because of the large number of strains and occurrence of antigenic shifting in some virus agents.

In this connection the building up of a temporary state of broad spectrum resistance by induction of interferon would be beneficial; at the time when the animals are subject to most stress; by the inhibition of viral multiplication at the portal of entry; or by limiting the virus spread.

Results of the preliminary studies in cattle indicate that interferon could be induced in the respiratory tract using poly r1-rC. This finding also confirms the earlier observation\(^1,2\) that interferon could be successfully induced in cattle by providing a suitable dose of an inducing agent. Furthermore, it is also significant in
the report\(^2\) that following the parenteral administration of live IBR virus the interferon titer persisted for a few days and was closely related to the presence of viremia. Unlike the systemic induction of interferon, localized induction in the respiratory tract requires only a small dose of inducer and thus the problem of toxicity inherent to some types of inducer is minimized. In addition the presence of interferon at the portal of entry is more effective in preventing the infection.

Following repeated stimulation of the respiratory tract with poly rI-rC interferon activity was detected in the nasal secretions for 8 days at which time the final sample was taken. A similar result of prolonged interferon level was obtained in mice following repeated parenteral administration of maximum doses of poly rI-rC\(^7\). Unlike the results in mice where the interferon titer diminished with subsequent stimulation, no evidence of hyporeactivity or refractiveness was observed in cattle. It is suggested that this maybe due to the fact that since the dose administered in cattle was small, only a portion of the cells in the respiratory tract were exposed during each induction.

Although it was shown that an interferon response was obtained after successive administration of subminimal doses, no work however was undertaken to explain this finding. It is possible that an additive effect or a primed state of exposed cells had been established.

The present study has shown that it is possible to induce interferon in the respiratory tract of cattle with poly rI-rC and indicates the prospect of a clinical application. The next step is for a better understanding of the mechanisms involved.
Fig. 2. Interferon TITER in the nasal secretions of cattle following repeated exposure to Poly rl-rC. * Based on CPE inhibition of 0.1 ml 100 CCID_{50} VSV.

- 0.5 mg,
- 1 mg, and
- 3 mg. Poly rl-rC.
REFERENCES


THE FLUORESCENT ANTIBODY TECHNIQUE IN THE
DIAGNOSIS OF BOVINE RESPIRATORY VIRUS DISEASES

by
L. van der Heide, D.V.M., Ph.D.
Department of Animal and Veterinary Sciences
University of Maine, Orono, Maine 04473

INTRODUCTION

An increasing number of cases with acute and chronic respiratory disease
problems, anamnestically related to the incidence of abortion, calf unthriftiness,
and cow and calf mortality are being observed in dairy herds in Maine.

Consequently, there has been an increase in the number of requests by
veterinary practitioners for an adequate etiological diagnosis. Vaccination programs
or other measures against Infectious Bovine Rhinotrachitis (IBR), Para Influenza 3
(PI3), and Bovine Virus Diarrhea (BVD) are dependent on such a diagnosis. Especially in regard to vaccination against these three virus diseases, an umbrella
type approach is not advocated in the Northeast.

Of the different diagnostic laboratory procedures that have been adopted so far
for differential detection of IBR, PI3, and BVD, the direct fluorescent antibody
(FA) technique is recognized as the quickest and cheapest method.

FA testing of nasal swabs, vaginal swabs, aborted fetuses or other tissues from
affected animals have been reported as successful by various authors. Usually
bovine cell culture systems were inoculated with suspensions from infected tissues,
after which the FA reactions were demonstrated in the infected tissue cultures.
(Fluorescent antibody cell culture (FACC) method). Smithies and Robertson
(1969) have shown that BVD infections can be diagnosed by the FACC method; 9.3% of 182 nasal swabs tested were FA positive.

Brown et al (1968) reported favorable results with the FACC method for the
detection of IBR, PI3, and BVD infections.

If nasal swab smears would show reliable FA reactions when directly reacted
with anti-virus conjugates, without the use of cell culture inoculations, this would
simplify the diagnostic procedures considerably and thus would make the FA
testing for bovine respiratory virus diseases feasible for more diagnostic labora-
tories.

The FA testing of nasal swabs, as opposed to internal tissues, permits testing of
herds without the necessity of sacrificing animals for the purpose of obtaining
tissue samples. In affected herds without mortality, this is important from an
economic standpoint.

Since January 1969, the Animal Disease Diagnostic Laboratory of the
University of Maine has tried to obtain an insight into the accuracy and reliability
of the direct FAT examination of nasal swabs and other tissues obtained from
problem herds.
MATERIALS AND METHODS

Conjugates

Anti-IBR, PI3 and BVD conjugates* were used, along with specific antisera against IBR and PI3 and normal SPF calf serum.**

Cellsmsears

Fresh nasal swabs were obtained by rubbing a cotton swab against the inner nasal septum of suspected animals. Other tissues, such as fetuses, placentas, etc. were brought in from the field as fresh as possible. Cell smears were made directly from tissues or nasal swabs on glass slides and air-dried. After fixing in acetone for 10 minutes at 0°C and air-drying, the cell smears were reacted with specific conjugates mixed 1:1 with antiserum or normal calf serum, (one-step inhibition test).

During the first months of 1969, the FA tests were run with specific conjugates only. From then on, FA tests for IBR and PI3 were performed with the one-step inhibition test.

After reacting the smears for ½ hour at room temperature in a humidified atmosphere, the slides were washed in PBS (pH 7.4) for 20 minutes, then rinsed in distilled water for 2 minutes.

After air-drying the smears were covered with 20 X 20 mm coverslips with phosphate buffered glycerine (pH 7.4) as a mounting medium. The smears were examined with an AO Spencer or a Leitz fluorescent microscope with an Osram HbO 200 mercury arc light source, UG 1 exciter filter, OG 1 barrier filter and an oil immersion darkfield condensor.

RESULTS

From January 1969 until July 1970 a total of 497 FA tests were performed. The results of all the tests are depicted in Tables 1, 2, 3, and 4.

The FA tests on nasal swabs were positive for IBR in 41.6% of 166 tests, positive for PI3 in 17.8% of 157 tests and positive for BVD in 4.4% of 90 tests.

Nasal swabs that were positive for IBR were left at 4°C for 24 hours. Then new smears were made from the same nasal swabs, at which time they were FA negative for IBR.

Nasal swabs sent in by mail were consistently negative for IBR, PI3 and BVD.

It is therefore recommended now to bring the samples into the lab. on the same day they were taken from the animals, while kept cool. The nasal swabs should be taken preferably from animals showing nasal discharge.

Loose mucus taken from the nostrils of such animals appeared not to contain live epithelial cells, only cornified and sloughed off degenerated cells could be

*Kindly provided for by Dr. W. Bolton, University of Vermont, Burlington, Vermont, and Dr. E. A. Carbrey, NADL, Ames, Iowa.

**Kindly provided for by Dr. E. A. Carbrey, NADL, Ames, Iowa.
found, which did never show specific immunofluorescence. Therefore the swabs should be obtained by directly rubbing the inner nasal septum.

Experience has shown that it is preferable to test several cows from the same herd; quite often only one in 5 or 6 cows showed positive FA results, even when all the tested animals were showing clinical symptoms. Nasal swabs from 5-10 cows are now pooled routinely and tested on 1 slide.

The direct FA examination of fresh nasal swabs appeared to yield better diagnostic results than the FA testing of other tissues, such as fetuses, placentas, etc. This might be caused by the fact that fetal tissues were hardly ever fresh, when received in the laboratory for testing.

SUMMARY

During an 18 month period (January 1969 - June 1970), several herds of cattle with respiratory and other disease problems were examined with the direct FA method for the presence of IBR, BVD and PI3 infections. Direct FA testing of fresh nasal swabs, without the use of tissue culture inoculations, showed 41.6% positive results for IBR, 17.8% for PI3 and 4.4% for BVD. These percentages were higher than for any other tissue tested, such as placenta, fetal tissue, etc.. A total of 497 tests on various tissues were performed.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. John Woodcock, Division of Animal Industry, Augusta, Maine, for his kind cooperation in supplying many field samples, and to Miss F. Fling, Mrs. R. Carr, Mr. J. Rossingno1 and Mrs. R. Yerxa for their excellent technical assistance.
### TABLE 1
RESULTS OF FA TESTS FOR IBR VIRUS ON FIELD CASES
JANUARY 1969 TO JUNE 1970

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Total Number</th>
<th>FA Tested</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placenta</td>
<td>6</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>Fetal Tissue</td>
<td>20</td>
<td>2</td>
<td>10.0</td>
</tr>
<tr>
<td>Nasal Swabs</td>
<td>166</td>
<td>69</td>
<td>41.6</td>
</tr>
<tr>
<td>Other Cow Tissue</td>
<td>11</td>
<td>4</td>
<td>36.4</td>
</tr>
</tbody>
</table>

### TABLE 2
RESULTS OF FA TESTS FOR BVD VIRUS ON FIELD CASES
JANUARY 1969 TO JUNE 1970

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Total Number</th>
<th>FA Tested</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placenta</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Fetal Tissue</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Nasal Swabs</td>
<td>90</td>
<td>4</td>
<td>4.4</td>
</tr>
<tr>
<td>Other Cow Tissue</td>
<td>9</td>
<td>1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

### TABLE 3
RESULTS OF FA TESTS FOR PI3 VIRUS ON FIELD CASES
JANUARY 1969 TO JUNE 1970

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Total Number</th>
<th>FA Tested</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placenta</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Fetal Tissue</td>
<td>15</td>
<td>2</td>
<td>13.3</td>
</tr>
<tr>
<td>Nasal Swabs</td>
<td>157</td>
<td>28</td>
<td>17.8</td>
</tr>
<tr>
<td>Other Cow Tissue</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 4
POSITIVE FA-IBR RESULTS OF NASAL SWABS IN RELATION
TO OTHER FA TESTS PERFORMED

<table>
<thead>
<tr>
<th>IBR</th>
<th>PI3</th>
<th>BVD</th>
<th>Number of Tests</th>
<th>% of Total IBR Pos. Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>NT*</td>
<td>NT</td>
<td>3</td>
<td>4.4</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>NT</td>
<td>22</td>
<td>31.9</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>37</td>
<td>53.9</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>3</td>
<td>4.4</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>3</td>
<td>4.4</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>1</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*NT = Not Tested.

REFERENCES


OBSERVATIONS ON THROMBOEMBOLIC MENINGOENCEPHALITIS (TEM) IN CATTLE IN INDIANA FEEDLOTS

Harvey J. Olander, D.V.M., Ph.D., A. M. Gallina, D.V.M., Ph.D., Dietra Beckwith and Mary Morrow, B.S., M.S.
Purdue University
Lafayette, Indiana 47907

In the early descriptions of thromboembolic meningoencephalitis (TEM) the disease was recognized primarily as a cause of sporadic nervous signs and deaths in feedlot cattle. Since then the disease has been demonstrated to be a septicemia regularly effecting tissues throughout the body, but clinically manifesting itself most strikingly when localization occurs in the central nervous system. In one report, serologic evidence of a high incidence of inapparent infection by the causative agent, a Haemophilus-like organism, was demonstrated. The reasons for the low incidence of clinical disease of the nervous system, and the nature of spread of the infection within a herd have not been elucidated.

The earliest cases of TEM were reported from western regions of the United States, but the disease has become prominent in the midwest, and it must be a major consideration in the differential diagnosis of nervous signs in cattle feedlots wherever they are located.

Feedlots in Indiana have not been exempted from this disease. However, because cattle feeding establishments are generally small and widely scattered, often at some distance from a diagnostic laboratory, the importance of the disease in Indiana is difficult to ascertain.

The occurrence of the disease in a small feedlot near Purdue University in the winter of 1969 prompted an investigation into the etiology and epidemiology of the disease in Indiana. A review of cases of central nervous diseases diagnosed in cattle at Purdue University indicated that the first case of TEM was received in October of 1968, and that the diagnosis has been made 6 times at the laboratory. By comparison, polioencephalomalacia has been diagnosed 10 times, lead poisoning twice and listeriosis once in feedlot cattle during the same period. During the preceding 2 years the same diagnoses had been made 12, 10 and 0 times, respectively.

MATERIALS AND METHODS

Retrospective inquiries were made into the herd histories of each of the 6 feedlots in which TEM had been diagnosed at Purdue. Necropsies were performed on 2 animals from Case V and one animal each from Cases I, II and III. Only the

A From the Animal Disease Diagnostic Laboratory, School of Veterinary Science and Medicine, Purdue University, Lafayette, Indiana. Paper No. 4222 journal series, Purdue University, Agricultural Experiment Station, Lafayette, Indiana.

589
head of a steer was received from Case IV and 2 brains fixed in formalin were received from Case IV.

Cultural characterizations and pathogenicity studies were made using a bacterial strain (IN1) isolated from a steer in an Indiana feedlot (Case V). The cultural characteristics were compared with those of a California isolate, *H. oakley*, which was obtained from Dr. E. L. Biberstein, University of California, at Davis. Antigens for plate agglutination tests were made by growing large quantities of IN1 on tryptose agar with yeast extract added and of *H. oakley* on chocolate agar. The bacteria were washed from the media with physiologic saline, killed by addition of 0.5% formalin, and homogenized by repeated flushings through a 20 gauge needle on a syringe. The suspension was allowed to settle at 4 C for 24 hours, and the turbid supernate was used as the antigen. Plate agglutination tests were performed by adding one drop of antigen to one drop of serum, stirring immediately, and observing 10 minutes later.

The inocula used in pathogenicity studies were saline suspensions of 24 hour cultures of IN1 on sheep blood agar (BA). In each instance heavily seeded culture plates yielded about 1 ml of inoculum. In experiments using calves, the inocula were second passage cultures from the brain of a steer in Case V. In experiment 1 the brain had been frozen at -10 C for 2 months prior to use. In experiment 2 the same brain had been frozen for 3 months, and had been partially thawed in preparation for experiment 1.

**Herd Histories**

Since the feedlots involved were small and usually managed by a stable workforce, retrospective histories were reasonably complete. Some of the details of the outbreaks appeared to offer insight into the epidemiology of the disease, so they are included in this report.

CASE I (Accession 3186-68): At the time of onset of TEM, October 20, 1968, there were 589 feeder cattle on this farm located in east central Indiana. The effected group (A) consisted of 267 yearling Angus, Hereford, and Angus-Hereford calves which had been brought to the farm from Florida 45 days previously. The animals were in a corn stubble pasture and being fed a commercial supplement and corn silage. The affected cattle were in good condition. The first signs of illness were sluggishness and occasional circling. The animals were prostrate within 24 hours and usually died within 3 days. Some treated with antibiotics lived up to 14 days. Within 2 weeks 38 cattle were affected, 30 died, and 8 responded to antibiotic therapy.

On November 15, after the losses had abated, the remaining cattle were moved into one half of a barn where they had fence contact and common feeding of silage with 300 market cattle (group B). These cattle had originated in Tennessee, Georgia, and Florida. No signs of disease were observed in group B prior to slaughter in December.

However, on December 15, a group of 28 calves (group C), which had been raised locally and maintained in an isolated woodlot on the farm for 2 months,
were placed on the pasture from which group A had been removed. Within 36 hours 2 calves were affected with TEM and became prostrate. They recovered following treatment with antibiotics.

Except for one calf in group A which had been treated for pneumonia, the owner had not observed previous signs of illness. All purchased calves had been "preconditioned" prior to shipment. The owner felt that the survivors of clinical illness had lost 30-45 days gain, but the rate of gain of other animals in the lot had been unaffected. The weather had been cold but not freezing.

CASE II (Accession 3847-68): This feedlot was located in northeastern Indiana. The herd consisted of 290 yearling Angus cattle that had been maintained in one drylot, being fed corn silage for 8 weeks prior to the onset of signs. Illness was first noted on December 25, 1968. All animals were normal in the morning, but by noon one was circling and it died during the night. During the next week 3 animals were observed circling and became prostrate. These were treated successfully with antibiotics and recovered completely after a convalescence of 8 weeks.

The animals had originated from several premises in Tennessee. All had been shipped immediately after weaning with no preconditioning. Affected and unaffected animals were in poor condition at the time of onset of TEM. During the following Fall pinkeye was again a major problem in the new cattle and one calf developed TEM and responded to treatment with antibiotics.

CASE III (198-69): There were 1500 cattle sorted by size as they were being maintained in 7 pens in this drylot in central Indiana. The diet consisted of corn, cobmeal and commercial vitamin and mineral supplement. The disease occurred in January 1969 in 2 lots with fence contact between them and the unaffected adjacent lots. Beginning 8 weeks after arrival at the lot, and after 5 weeks on a full feed ration, 16 cattle weighing 900 to 1200 lbs. died within 3 weeks. Except for one steer, which was seen to stagger and fall, the affected cattle were found prostrate. All died within 2 days. After a diagnosis had been made, antibiotic levels in the feed were increased for 2 days and the losses ceased.

These cattle had been purchased in South Carolina, Kentucky, Georgia, and Indiana, and there was no certainty of the origin of affected animals. Affected animals were in excellent condition, and there had been no disease problem in the lot prior to the onset of TEM. Treatment prior to shipment was unknown, but the cattle had been vaccinated against infectious bovine rhinotracheitis (IBR), virus diarrhea (VD) and parainfluenza-3 (PI3), injected with vitamin A and implanted with stilbesterol within one week after arrival. The lot had been wet and muddy throughout the Fall, the freezing weather occurred only after TEM had run its course.

CASE IV (5609-69): This outbreak occurred in north central Indiana in November 1969 in one drylot of 75 Hereford steers weighing 1000 lbs. All had been transported from a single ranch in North Dakota and placed on pasture in March 1969. Self feeding with corn in addition to pasture began in July, and the animals were placed on drylot in October where they received corn and hay.

Two animals were affected with TEM without premonitory signs. Within 2
days both were found recumbent with temperatures of 41 C (105 F). One was euthanized for necropsy. The other was treated with antibiotics and recovered. The animals affected with TEM were in excellent condition, and there had been no previous disease problems in the herd.

In March 1970, a group of 102 calves weighing 575 lbs each were placed in the same lot, and there had been no problems as of September 15, 1970.

CASE V (Accession 5774-69): This lot located in central Indiana contained 167 cattle in two pens with fence contact and a common water trough. One lot contained 51 market weight cattle which remained normal throughout. The affected lot contained 59 Angus steers weighing 400 lbs each from a farm in Kentucky, and 58 Angus-Hereford heifers of similar weight which had been purchased from several sources at a Kentucky sale.

Six steers and 2 heifers developed TEM in November 1969, 3 weeks after arrival. These animals were noted to be depressed, or were found down, 2 were coughing and dyspneic and all died within a few days. Medication of sluggish animals with antibiotics was instituted, and the losses stopped.

Since arrival on the farm the calves had been fed primarily haylage or corn silage and some corn. The affected animals were in excellent condition. The calves had been weaned long before shipping, but there was no information about further preconditioning. On the second day after arrival in the feedlot the animals had been vaccinated against IBR, VD, and PI3. During the outbreak the weather had been cold but not freezing.

CASE VI (Accession 141-70): This outbreak of TEM began in late December 1969 in a feedlot located in southern Indiana. Approximately 480 cattle were on feed in 4 open drylots and 240 were in 4 pens in a barn. The disease occurred in a nearby starting-lot containing a group of 80 steers weighing 400 lbs each. In this lot nervous signs and deaths occurred in 3 animals which had come from a single source in Louisiana. They had been receiving corn silage and corn since arrival 3 weeks earlier. They were small but clinically normal before the onset of TEM.

The affected cattle were anorectic, became ataxic and recumbent, and died within a few days. Treatment with antibiotics, fluids, cortisone and antihistamine was ineffective. All other animals, including the 77 animals in the pen, remained in good health. No prophylaxis was instituted.

The calves had not been preconditioned before shipment, and they had been tagged and wormed on the day of arrival in the feedlot. Again, the weather was cold but not freezing when the disease occurred.

Post Mortem Findings

The diagnostic lesions observed in each case were widely disseminated small foci of hemorrhagic necrosis in the brains and spinal cords. (Figures 1, 2 and 3) Other gross findings consisted of widespread minute foci of hemorrhage and necrosis. The generalized lesions were prominent only in Case V, which also had severe peracute fibrinonecrotic laryngitis, serofibrinous polyarthritis, hemorrhagic retinitis (Fig. 4) and multiple hemorrhagic ulcerations of the urinary bladder
mucosa. Small areas of lobular consolidation were found in the lungs of all animals necropsied.

The microscopic lesions consisted of suppurative thrombophlebitis of small veins with necrosis of the adjacent parenchyma. Venous thrombi were usually heavily laden with bacteria, and large numbers of neutrophils infiltrated the vein walls and the adjacent necrotic tissues. While the vascular lesions were most readily observed and diagnostic in the central nervous tissue (Figure 5) they were observed in all organs of the body. In the urinary bladder (Figure 6) and larynx (Figure 7) thrombophlebitis was accompanied by necrosis of the mucosa and underlying tissues.

**Bacteriology**

Significant numbers of bacteria were isolated from 2 steers submitted from Case V. Brains and visceral organs yielded heavy growths of an organism which was designated IN₁. It was characterized and used in pathogenicity studies in cattle and laboratory animals. The original isolation was obtained on sheep BA and in brain heart infusion broth enriched with 2% bovine serum (BHI+BS) incubated at 37°C in a candle jar. Colonies on solid media were small and reached full size of about 1 mm in 3 days. Hemolysis was not observed. Isolation from infected tissue, held at -10°C, was repeated several times during a 6 month period, but the quantity of bacterial growth lessened considerably during that time.

The organism was a small, gram-negative, non-motile, unencapsulated, pleomorphic coccobacillus, measuring 0.5 to 1.5 m. Excellent growth was obtained at 37°C on sheep BA, cow BA, horse BA, egg yolk agar, tryptose agar with yeast extract added, and in BHI+BS. Growth was enhanced by the CO₂ atmosphere of a candle jar and partially inhibited by anaerobic conditions. No growth took place on McConkey’s agar, and growth was poor on chocolate agar, tryptose agar, nutrient agar, tryptase soy broth and BHI without serum. *Staphylococcus* feeder colonies did not enhance growth on chocolate or nutrient agar or result in satellitism on BA. In the biochemical tests with 2% bovine serum added to the Difco basal phenol red media, neither acid nor gas was produced with mannitol, glucose, lactose, sucrose, maltose, trehalose, sorbitol or dulcitol. There was no growth on citrate or in gelatin, and neither indol nor H₂ was produced. Nitrate was reduced and the organism was catalase, urease, and oxidase positive. In comparison, cultures of *H. oakley* produced acid and gas in glucose, maltose, mannitol and trehalose, but no reaction with lactose, sucrose, or sorbitol. Idol and urease were not produced, nitrate was not reduced and there was no growth in motility medium.

Using Difco antibiotic sensitivity discs, growth of IN₁ was inhibited by all the commonly used antibiotics.

Cultures of brains from intact animals submitted in Cases I, II and III and from the head submitted in Case IV demonstrated the presence of small numbers of *Streptococcus*, *Escherichia coli*, *Proteus* and *Streptococcus*, respectively. All were considered to be contaminants. Brains from 2 animals in case 6 had been submitted in formalin. Nasal swabs, collected from 66 calves 59 days after the last death in
Case V, were frozen in BHI and later cultured. Significant bacteria were not isolated from these swabs.

*Pathogenicity studies*

Experiment 1 — Two 400 lb. steers were inoculated into the jugular vein (j.v.) with 4 ml. of a suspension of IN1 prepared as described in the materials and methods. Within 4 hrs. both calves had a halting respiration and by 12 hrs. after inoculation (p.i.) both were febrile, 41.2 C (106.2 F) and 40.6 C (105.1 F) and depressed. One was staggering, pressing into the walls, and apparently blind. At 18 hrs. this calf was dead and the other calf was prostrate. It was killed *in extremis* at 24 hr. p.i.

The gross and microscopic lesions observed in these animals were similar to those in the bladder mucosa were accentuated. Bacteria were reisolated from the brain, cerebral spinal fluid, liver, lung, spleen, lymph nodes and joint fluids of both calves.

Experiment 2 — Two calves were inoculated i.v. with 5 ml and 2 were inoculated by slow intranasal instillation (i.n.) with 15 ml of inoculum. One calf inoculated i.n. and one inoculated i.v. had transient fevers of 40.8 C (105.4 F) and 40.2 C (104.4 F) at 12 hours p.i. All calves remained otherwise normal until they were killed between 7 and 21 days p.i. Post mortem examination revealed no lesions referable to the inoculations, and significant organisms were not recovered from any of the tissues. It was later discovered that antibiotics had been added to the ration inadvertently. Mice inoculated intraperitoneally (i.p.) with 1 ml or i.v. with 0.5 ml, either in a normal cage environment, or having been exposed to 4 C for 48 hrs. before and after inoculation, or after being given cortisone acetate for 6 days, were unaffected. Guinea pigs and gerbils inoculated i.p. and rats and rabbits inoculated i.v. with 1 ml. of the inoculum were unaffected also. Chicken embryos inoculated into the yolk sac with 0.1 ml. of the bacterial suspension died in 24 to 48 hours, and the organism was recovered readily from the embryos.

*Serologic investigations*

Serum samples collected at random from 66 calves 59 days after the last death in Case V, were tested for antibodies to IN1 and *H. oakey* by the plate agglutination method. Twelve samples from heifers were tested; 6 agglutinated IN1, and 6 did not. Of the 54 samples tested from steers, 31 reacted with IN1. All samples which had reacted with IN1 except samples from one heifer and one steer, reacted with *H. oakey* as well. In addition, 13 samples from the steers and 1 sample from a heifer agglutinated *H. oakey* but not IN1.

Serum samples from twenty calves newly arrived at Purdue, with no history of disease from a ranch in Oklahoma were tested. One reacted with antigens of both IN1 and *H. oakey*. Serum samples collected before inoculation and at necropsy from 4 calves in experiment 2 did not react with IN1.

The antigen prepared from *H. oakey* was agglutinated by the serum of rabbits hyperimmunized against both *H. oakey* and IN1. However, the IN1 antigen reacted
only with the homologous hyperimmune rabbit serum.

DISCUSSION

The observations on TEM in Indiana feedlots agree in substance with those made in other parts of the United States\(^1,2,3,4,5,6,7,8\). The prominent and dramatic nervous signs, the rapid clinical course, the low morbidity, the high mortality in untreated animals, the seasonal incidence, and the occurrence usually in cattle in feedlots but occasionally on pasture, are evident in the cases reported here and are in agreement with the observations of others. In addition, the occurrence of fibrinonecrotic laryngitis as a lesion of TEM, as reported by Panciera, \textit{et al}\(^7\), was observed in Indiana cases, and the high incidence of inapparent infections, demonstrated by Kennedy, \textit{et al}\(^5\), is further substantiated by the serologic investigation of Case V.

The causative agent isolated in Case V has cultural characteristics similar to but differing from those of agents isolated by other workers\(^1,5,7\). The IN\(_1\) strain appears to be less fastidious than the California and Oklahoma agents in artificial media. But as judged by experiment 1, IN\(_1\) is at least as pathogenic for cattle since it readily reproduced the disease by intravenous inoculation of saline suspension while the other strains have required either intracarotid inoculation\(^7\) or the use of blood cultures as inocula.\(^5\) On the other hand IN\(_1\) appeared to be innocuous in laboratory animals. The general cultural characteristics of the organism are similar to those of \textit{Moraxella} \textit{spp.} However, the cultural work performed for this report is not adequate for a definitive classification of the organism.

While it is difficult and perhaps dangerous to draw conclusions from limited numbers of cases, several epidemiologic features are suggested by the histories included in this report. The seasonal incidence of the disease coincided with periods of variable, wet and moderately cold weather rather than periods of extreme cold. Also, while the disease occurred primarily in animals in feedlot operations, the feeding regimens differed markedly, including silage, pasture and highly concentrated diets. This variation and the lack of digestive disturbances in affected cattle suggests that diet plays a minor role in the pathogenesis of the disease. In a similar fashion, the correlation between the time of onset of disease and the times of shipment, changes in diet, or other stresses was too variable in this study to suggest that any one of these factors is regularly important in TEM.

Most cattle fed in Indiana have been shipped from southern and southwestern regions of the country. The detection of a serologic reactor to IN\(_1\) in the cattle brought to Purdue from Oklahoma suggests that the infection can be carried into the Indiana lots by these cattle. Kennedy, \textit{et al}\(^5\) described an epizootic in a large feedlot that lasted several months and involved spread of infection through 15 pens in a random fashion. In most instances, however, TEM appears to be limited to groups of cattle of a single origin\(^6,7\). This may suggest that the infection is brought to the lot by that group with little or no spread after mingling with animals from other sources. This would further suggest that infection had been widespread.
throughout that group for sometime prior to clinical manifestations. In that case it would be presumed that some unknown factor precipitates septicemic dissemination of infection from a nidus in the individual animals. However, in case 1, TEM occurred in a group of cattle, which likely had not been exposed to the infection previously, within a few days after exposure to a pasture in which diseased animals had been present 3 weeks earlier. This suggests that infection took place and manifested itself rapidly after exposure to infective fomites. Such a mode of transportation might be more likely in a pasture than a feedlot simply because of environmental requirements for maintenance of the organism. Further evidences of spread outside of a group of single origin were the serologic findings and the manifestations of disease concurrently in 2 groups known to be of different origins in Case V. It seems likely that apparent restriction of some outbreaks to a single group of animals may be a reflection of the practice in small establishments of acquiring and feeding cattle as a group, with little grading and regrouping during the feeding process because of limited facilities.

The pasture infection in case 1 suggests that seeding of the environment with organisms by infected animals must be massive. Since cases in new groups of cattle brought into previously effected drylots are not common, it is likely that the feedlot environment is not suitable for preservation of the organism. Massive shedding of the organism from an animal with an inapparent infection in close contact with his pen mates could explain the usual sudden appearance of TEM in several animals in a group. The less common prolonged epizootics can be explained by the same mechanism, especially when the animals involved are being mixed in the process of grading.

The manner by which the organism might be shed in massive numbers has not been determined in this study. The likely mechanisms would be by nasal or oral secretions and fecal or urinary excretions. Respiratory discharge and diarrhea are not prominent features of the disease. On the other hand, urinary bladders were involved by mucosal ulcerations caused by septic infarctions in several animals in this study. It is tempting to suggest this route as a mechanism of dissemination. However, cultures of urine were not made in any of these cases.

While much of the discussion has been speculative, it can be stated with certainty that TEM is becoming an important problem in feedlot cattle throughout the state of Indiana. It has been diagnosed at Perdue in numbers approaching those of polioencephalomalacia during the past 2 years. The increasing importance of TEM has been paralleled by a decline in cases of listeriosis in feedlot cattle. It appears that this reflects the changing nature of Indiana cattle feeding operations. Many small establishments, feeding primarily silage to homogeneous groups of cattle, often of local origin, are being replaced by fewer, larger operations feeding more highly concentrated diets to large heterogenous groups of varied origins which are liable to include animals with inapparent infections.
Figures 1, 2 and 3: Focal hemorrhagic necrosis randomly scattered through the brain and spinal cord of a steer with TEM.
Figure 4: Thrombotic dilation and hemorrhage involving the retinal veins as viewed through the vitreous of a hemisectioned eye from a steer with TEM.

Figure 5: Thrombophlebitis of meningeal veins associated with meningitis and parenchymal abscess formation.
Figure 6: A. Ulceration of the Urinary bladder mucosa associated with a septic lesion in the lamina propria. B. Bacterial colonies (anous) at the luminal surface of the ulcer in 6A.
Figure 7: Thrombophlebitis associated with degeneration of deep mucosal glands of the larynx.

REFERENCES

COMPARISON OF TECHNIQUES FOR THE DIAGNOSIS OF IBR VIRUS ABORTION

E. J. Bicknell and D. E. Reed

Substantial monetary losses occur each year in the beef cattle industry in South Dakota as a result of abortion. For the past three years an organized effort has been made at the Animal Disease Research and Diagnostic Laboratory to improve the diagnostic acumen as regards abortion problems. Infectious bovine rhinotracheitis (IBR) virus has been incriminated during this time as the greatest single cause of bovine abortion in South Dakota. The importance of IBR virus abortion prompted the present comparative study of techniques for its diagnosis.

MATERIALS AND METHODS

Four techniques for the diagnosis of IBR virus abortion were used on bovine abortion cases submitted to the laboratory. These methods were:

1. Frozen Tissue Section: Fetal liver and spleen tissue were collected at necropsy, mounted on tissue chucks and frozen by immersion in -70°C acetone. Sections were cut at 10 microns with a cytostat*, mounted on glass slides and stained by a modified Hematoxylin and Eosin (H&E) technique4.

2. Fluorescent Antibody Staining: Frozen sections of liver, spleen, kidney and/or lung taken as above were fixed in -20°C acetone and stained with fluorescein isothiocyanate conjugated calf anti-IBR serum**. Sections were observed for specific fluorescence with a darkfield fluorescence microscope using a UG-1 (blue) excitor filter and a BG-12 barrier filter.***

3. Paraffin Sections: In addition to liver and spleen; heart, lung, lymph nodes, adrenal, kidney, thymus, brain and muscle tissue were collected in 10% buffered neutral formalin, sectioned at 6 microns and stained by H & E technique1.

4. Virus Isolation: IBR virus isolation was accomplished by inoculation of fetal tissue homogenates (pool of lung, liver, spleen and kidney) or placental tissue homogenates onto second passage bovine embryonic kidney (BEK) cell monolayers. Inoculated BEK cell cultures were


**Courtesy of Dr. E. J. Carbrey, National Animal Disease Laboratory, Ames, Iowa.

***E. Leitz, Inc., 468 Park Avenue, South, New York, N.Y.

From the Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, S.D. 57006.

Approved for publication by the Director, Agriculture Experiment Station, South Dakota State University as Journal Series No. 998.
incubated at 37°C and observed daily for IBR cytopathic changes. If no cytopathic changes were observed by 7 days post-inoculation, cultures were harvested by freezing and thawing and passed to fresh BEK cell monolayers. Second passage was considered negative if no cytopathic changes were observed after 7 days incubation. IBR virus isolates were identified by tissue culture fluorescent antibody techniques.

RESULTS

Diagnoses of IBR virus abortion based on IBR-specific fluorescence, histopathologic lesions and/or virus isolation were made in 32 of 169 bovine abortion cases examined. In all but one IBR-positive case, IBR-specific fluorescence was observed in one or more tissues. Lesions of focal necrosis were present in 29 (91%) of the 32 cases. These lesions were observed in both paraffin and frozen sections. Virus isolation was successful in 15 cases (47%).

Lesions in fetal liver typical of IBR abortion are depicted in Figures 1 (frozen section) and 2 (paraffin section). Typical IBR fluorescence in a kidney section is seen in Figure 3.

DISCUSSION

It is important to assess the relative values of the various diagnostic tests for a particular disease entity. In few instances is each test diagnostic in itself without the use of additional procedures. This study indicates that each test described is important in the diagnosis of IBR virus abortion.

In this study fluorescent antibody staining of fetal tissue sections has proven to be the best single approach in the diagnosis of IBR abortion. It can be completed in a relatively short period of time, is not expensive and appears to give very specific results. Brilliant foci of fluorescence, particularly in liver, kidney and lungs of IBR positive cases are easily read and false positive results were not encountered. Cautions to be observed when interpreting results obtained from decomposed or autofluorescent tissues have been discussed elsewhere.

Diagnosis of IBR abortion based on histopathologic lesions of focal hepatic or splenic necrosis in the bovine fetus is evidently quite sound. False positive diagnoses apparently did not occur by this method but approximately 10% of the positive cases would have been missed if histopathologic examination, alone, had been employed. Correlation between results of H & E stained frozen liver section.

Diagnosis of IBR virus abortion based on isolation of the etiologic agent is unequivocal but was accomplished in less than 50% of the cases. In this study it was considered as the least reliable method of diagnosis as well as the most expensive and time consuming.
Figure 1 – Focal necrotic lesions in fetal bovine liver. Frozen section. H&E stain. X 250
Figure 2 – Focal necrotic lesions in fetal bovine liver. Paraffin section. H&E stain. 
X 100
Figure 3 – A focus of fluorescence in a section of fetal bovine kidney stained with fluorescence conjugated IBR antiserum. X 250
REFERENCES


PARAINFLUENZA 3 VIRUS INFECTION OF TRACHEAL ORGAN CULTURES

A. B. Angulo* and M. Savan
Veterinary Microbiology and Immunology
Ontario Veterinary College, Guelph, Ontario, Canada

INTRODUCTION
Parainfluenza 3 (PI3) virus is incriminated as one of the etiological agents of Shipping Fever (SF) disease. The virus infection in the uncomplicated case is generally mild or inapparent, however in association with secondary infections e.g. pasteurella, mycoplasma or other viruses a severe fatal respiratory condition results. The significance of PI3 virus in the disease is not well established but it has been suggested\(^1,2\) that the virus initiates a primary infection and this predisposes the animal to secondary infections resulting in the disease recognized as SF. The pathogenesis of PI3 infection is not fully understood because of the difficulty of reproducing the disease experimentally in susceptible cattle, and/or studying a natural uncomplicated case. In this connection the tracheal organ cultures could provide a model for the study of PI3 infection in an organized dedifferentiated tissue in the absence of complicating or modifying factors such as secondary infections. Although the organ cultures retain their physiological activity as \textit{in vivo}, the capacity of an inflammatory response and other defensive mechanisms of the fully intact animal is lacking. However in spite of the limitations of the system a better understanding of the pathogenesis of PI3 virus could be obtained.

MATERIALS AND METHODS

\textbf{Preparation of Tracheal Organ Cultures} – Bovine fetuses approximately 4-5 months old were obtained from a nearby slaughterhouse. The organ cultures were prepared following the technique described by Ho01m.\(^3\) Briefly this was done by aseptically dissecting the trachea and cutting it into roughly 3 mm square pieces. Four explants with the ciliated epithelium facing upward were carefully placed in a plastic petri dish previously prepared by making scalpel incised grids for tissue attachment. Buffered medium 199\(^4\) containing 15% fetal calf serum and antibiotic mixture (10,000 IU penicillin and 100 ugm Streptomycin/ml) was added until the medium was flush with the surface of the explants. The tracheal organ cultures were incubated in a mixture of 5\% CO\(_2\) and 95\% air at 37\(\text{°C}\). The ciliary activity of the explants was examined daily in a low power dissecting microscope with a reflected light source that was moved into 3, 12 and 9 o'clock position.

\(\hspace{1cm}\)

*The senior author is supported by a Colombo Plan Scholarship through the Canadian International Development Agency. The investigation was partially supported by a NRC Grant (A-1389).
Inoculation of Tracheal Organ Cultures — A SF4 strain of PI3 passage 6 (10^5 CCID_50 0.1 ml) was used to infect the explants. Originally this was obtained from Connaughy Medical Research Laboratories and was propagated in embryonic bovine spleen cell cultures (EBS) in this laboratory. Varying dilutions of the virus inoculum from undiluted to 10^{-6} were prepared and 0.4 ml of each dilution was inoculated onto the explants in separate petri dishes. The virus was allowed to absorb for 30 minutes before fresh medium was added. At daily intervals up to 20 days the infected fluid was collected and replaced with fresh medium. The ciliary activity of the infected and uninoculated explants were observed daily.

In another experiment eight petri dishes containing tracheal explants, were infected with 0.4 ml 100 CCID_50/0.1 ml SF4. Daily up to day 10 and thereafter every second day until day 50 the infected fluids were harvested and replaced with fresh medium. The ciliary activity of the infected and uninoculated explants were observed daily throughout the duration of the experiment. At various times tracheal explants were removed from one petri dish, fixed in Bouins, sectioned and stained with hematoxylin and eosin (H & E). Explants were also frozen in an alcohol-dry ice bath and were sectioned in a cryostat for fluorescent antibody staining.

Two sets of organ cultures similarly infected were treated with 2 ml of 1:600 anti PI3 serum 2 days post infection. At 2 day intervals the infected fluid was harvested and replaced with fresh medium containing anti-serum. In this manner the explants were exposed to the antibody up to day 8, and from then on remained free of antibody until day 18. From day 18 to 40 the explants were again treated with antisera and thereafter no antisera was added until day 50.

Medium from the controls of uninoculated tracheal explants and PI3 inoculated petri dish without explants were also collected.

Virus Titration — The culture fluids were titrated in EBS cell cultures. Ten fold dilution of the fluids from undiluted to 10^{-7} were made in phosphate buffered saline and 0.1 ml of each dilution was added into each of 3 cell culture tubes. The titer was determined by hemadsorption with 0.2 ml of 0.3% bovine red blood cells 3 days after infection.

Fluorescent Antibody Staining (FA) — The procedure for preparing the fluorescein labelled antibody and staining method has been previously described.5

RESULTS

Virus Production of Infected Tracheal Organ Cultures

The tracheal organ cultures were infected with various dilutions of the PI3 virus stock. The patterns of virus growth show that high virus titers were detected early in about one week in the explants infected with high virus dose (undiluted and 10^{-1} dilution). With low dose (10^{-5} and 10^{-6} dilutions) high virus titers were obtained in about two weeks. Eventually the highest titers of the different explants
were about the same (Table 1). The ciliary activity of the explants infected with undiluted and 10⁻¹ dilution remained for about 10 to 12 days respectively while those infected with 10⁻⁵ and 10⁻⁶ dilutions were observed for 20 days. In the uninfected control the ciliary movement lasted for 28 days.

A persistent infection (for 50 days) was established in the explants inoculated with 100 CCID₅₀/0.1 ml. The virus yield of the persistently infected explants is presented in figure 1.

Although there is much fluctuation in the virus production, the virus titers were generally high in the first 3 weeks and generally declined to a relatively steady level in about the 6th week. The ciliary activity of these organ cultures were observed for as long as 20 days.

The addition of high titered anti PI3 serum for periods of 8 and 22 days did not cure the infection of the organ cultures. During this time no virus was detected but when the antibody was removed, the infectious virus gradually increased to a peak titer of 10⁶ in about 4 days. The ciliary activity of the antibody treated explants remained for 30 days.

**Histopathology**

In the uninoculated control the histological structures were well maintained. At four weeks the epithelium was even and regular and the ciliated pseudo-stratified columnar cells were well preserved (figure 2). However the epithelium had been transformed into 1 or 2 layers of low columnar or low squamous cells at the termination of the experiment on the 7th week (figure 6).

No histopathological changes were observed in the sections of explants 10 days post infection. The histopathological structures were well maintained, and appeared similar to the uninfected controls (figure 3). Some times an eosinophilic inclusion body was observed. By 3 weeks the cilia of the infected organ culture was lost. Some areas of the epithelium were desquamated and reduced in thickness (figure 4). Cell orientation to form syncytium and inclusion bodies were also observed. At 4 weeks the pseudo-stratified columnar architecture has been replaced by low columnar cells. There were also areas of extensive desquamation of the epithelium.

At the termination of the experiment, sections of the infected explants revealed 1 or 2 layers of squamous epithelial cells and in other areas no epithelial cells were present (figure 5).

**Fluorescent Antibody Staining**

Specific fluorescence was observed in the epithelial cells of the explants as early as 24 hours. The relatively intact epithelial lining was brightly fluorescing at 48-72 hours (figure 7). The specific fluorescence was confined to the epithelium and the epithelial cells of the submucous glands. The single layer of flattened cells or squamous cells were positive at 7 weeks.
DISCUSSION

Results of the experiments indicate that PI3 virus infection of tracheal organ cultures lead to (a) virus localization in the epithelial cell lining, (b) impairment of ciliary activity, (c) production of cytopathic effects and (d) establishment of a persistent infection.

The tracheal organ cultures had been infected with various dilutions ranging from undiluted to $10^{-6}$ of PI3 virus stock. Since the inoculum has a titer of $10^5$ CCID$_{50}$/0.1 ml as determined by titration in EBS cell culture, the infection of the organ cultures with a dilution as high as $10^{-6}$ suggests that it is slightly more sensitive. In the infected explants the virus localizes in the epithelium as established by direct fluorescent antibody staining. In this connection no previous work have been reported regarding PI3 virus localization in the epithelial cell lining of the tracheal organ culture. Furthermore supporting this finding is the observation that the histopathological changes were confined only to the epithelium.

The persistence of ciliary activity varies from one batch of explants to another and maybe influenced by many factors such as age of the fetus or degree of injury during preparation. In some explants the activity may last for a few days or may remain for several weeks. In general however the loss of ciliary activity is observed earlier in the PI3 infected explants compared to the uninfected controls. This impairment of the ciliary movement by PI3 maybe very significant in the pathogenesis of the virus in vivo because the ciliary mechanism is a major factor in the elimination of foreign materials or irritants from the respiratory tract. Unlike the earlier observation of Shroyer and Easterday$^6$ no re-establishment of ciliary activity was observed in either the infected or uninfected organ cultures.

There is a conflict in the literature regarding the ability of PI3 virus to produce cytopathic changes in the tracheal epithelium of organ explants. Hoorn$^7$ reported that no CPE was observed in the epithelium in spite of the high virus yield and even after 4 serial passages of the virus in tracheal explants. On the other hand Campbell et al$^8$ and Reed$^9$ reported that in the infected explants the ciliated pseudo-stratified columnar epithelial cells were transformed to flat or squamous epithelial cells. Formation of syncytia and inclusion bodies were also observed. In the present study similar types of histopathological changes were observed. However the transformation from the ciliated pseudo-stratified columnar architecture to squamous epithelial cells was also observed in the un-inoculated controls that were maintained for 7 weeks. In addition the type of transformation was also observed in the respiratory tract of swine exposed for 6 weeks to sulfur dioxide and corn dust$^{10}$. It is suggested that these histopathological changes observed represents the reaction of the tracheal epithelium to irritation, infection or maybe a result of physiological degeneration process. Although these changes may not be virus specific their occurrence resulted in a decreased virus yield. The resultant decline in virus titer may be due to a reduced number of epithelial cells, rather than an increased resistance of the squamous cells. The establishment of persistent PI3 infection in tracheal organ cultures has been reported.$^8,9$ The present work is in
agreement with the earlier reports. However the role of interferon in the persistent infection was not undertaken in this study. The addition of specific antiserum for various periods of time did not cure the infection in the organ cultures. In this connection the question could be raised whether virus persistence in the presence of antibody implies the possibility of cellular survival through a direct cell to cell transfer or continuous virus production for long periods of time by the infected cells. The attempts to answer this question by fluorescent antibody staining however was not successfully carried out.

In addition whether we can relate the present findings in vivo to support the suggestion that PI3 virus infection is not a rapidly self limiting disease but rather that the virus is always harbored as a latent infection in the animal remains to be answered.
Fig. 1 Yield of Parainfluenza 3 Virus in organ cultures of fetal bovine trachea.

- - - - - virus titer of the inoculated control petri dish without explants,
- - - virus production of explants infected with 100 CCID$_{50}$ (10$^4$) PI 3 virus.

fast ciliary movement, slow ciliary movement, no ciliary movement.
TABLE 1
Virus Yield of Tracheal Organ Cultures Infected with Several Dilutions of Parainfluenza 3 Virus*

<table>
<thead>
<tr>
<th>Days Post Infection</th>
<th>Infecting Dose (0.4 ml/dilution)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>undiluted</td>
<td>$10^{-1}$</td>
<td>$10^{-5}$</td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td>1</td>
<td>$10^1$</td>
<td>$10^1$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>$10^4$</td>
<td>NT</td>
<td>$10^1$</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>NT</td>
<td>$10^3$</td>
<td>NT</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>$10^6$</td>
<td>$10^5$</td>
<td>$10^3$</td>
<td>$10^4$</td>
</tr>
<tr>
<td>5</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>6</td>
<td>NT</td>
<td>$10^6$</td>
<td>$10^4$</td>
<td>$10^3$</td>
</tr>
<tr>
<td>7</td>
<td>$10^5$</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>10</td>
<td>$10^3$</td>
<td>$10^4$</td>
<td>$10^6$</td>
<td>$10^4$</td>
</tr>
<tr>
<td>13</td>
<td>$10^4$</td>
<td>$10^5$</td>
<td>$10^6$</td>
<td>$10^6$</td>
</tr>
<tr>
<td>20</td>
<td>$10^1$</td>
<td>$10^1$</td>
<td>$10^2$</td>
<td>$10^3$</td>
</tr>
</tbody>
</table>

* $10^5$ CCID$_{50}$/0.1 ml Titrated in Embryonic bovine spleen cell cultures.
NT = Not tested.
Figure 2 – Uninoculated tracheal organ culture maintained for 4 weeks. The histological structures appear to be normal, the epithelium is regular and with well preserved cilia.

Figure 3 – P13 infected tracheal organ culture 10 days post inoculation. The ciliated epithelium appears normal.
Figure 4 – Three weeks post infection, there is loss of cilia and desquamation of the epithelium.
Figure 5 – Seven weeks post infection explant showing absence of epithelium.
Figure 6 - Uninoculated explant maintained for 7 weeks. There is loss of cilia and pseudo-stratified columnar epithelium is transformed to low columnar cells.
Figure 7 -- Fluorescent antibody staining of infected explant 2 days post infection. Epithelial lining showing specific fluorescence.
REFERENCES

DIAGNOSTIC METHODS FOR DETECTION OF PARATUBERCULOSIS (JOHNE'S DISEASE)

R. S. Merkal

Johne's disease is due to an infection within macrophages in the lamina propria of the intestines and the associated lymph nodes with the acid-fast organism, *Mycobacterium paratuberculosis*. The disease in domestic ruminants is of considerable economic importance. The exact distribution of the infection is not known, but in the United States, it occurs in all dairy and beef breeds and in all regions of the country; a particularly high prevalence has been found in some cattle herds into which foreign animals have been introduced. The disease exists in many sheep flocks, and large herds of goats.

The illness in cattle is protracted, with decreased milk production, emaciation, intermittent fever, inappetence, and diarrhea. Frequently the coat becomes rough and may be shed. Animals usually are infected while neonatants, but develop the disease later as adults. The organisms often are shed in the feces from 1 to 2½ years before signs of disease appear. In one large herd of dairy cows we examined, approximately one-third of the paratuberculous cattle shedding culturally demonstrable *M. paratuberculosis* developed clinically apparent Johne's disease.

The basic intestinal reaction in paratuberculous cattle is a chronic catarrhal inflammation with intense infiltration of the lamina propria with macrophages. Some of the macrophages may be distended with organisms, whereas other nearby macrophages may contain only a few organisms.

The diagnostic tests tried for early detection of paratuberculous animals have run the gamut of serology, hypersensitivity, and microscopic and cultural examination of feces. Complement fixation tests have employed whole cells, culture filtrates, and various extracts of the organisms as antigens. Johnin and johnin PPD prepared like tuberculin from the filtrates of steamed cultures have been used as antigens in hemagglutination tests. Agglutination tests with whole cells have been tried, as well as liquid and gel diffusion precipitin tests employing PPD, cell extracts, and protoplasm. The results with these serological tests varied, but they all failed to detect many of the infected animals, and detected antibodies in many other animals that may have been exposed, but were not currently infected. In addition, it has been difficult to distinguish between antibodies due to *M. paratuberculosis* and those due to sensitivity to other mycobacteria, particularly *Mycobacterium avium*.

Hypersensitivity tests, including intradermal tests for cutaneous hypersensitivity, subcutaneous and intravenous thermal tests, and macrophage migration inhibition tests had the same deficiencies as serologic tests. Microscopic examination of fecal specimens failed to detect many of the infected animals, and many

From the National Animal Disease Laboratory, Veterinary Sciences Research Division, Agriculture Research Service, U.S. Department of Agriculture, Ames, Iowa 50010

620
DIAGNOSTIC METHODS FOR DETECTION OF PARATUBERCULOSIS

of the saprophytic mycobacteria that occur naturally in ruminant feces are indistinguishable visibly from the Johne's bacillus.

Cultural examination of fecal specimens from paratuberculous animals can detect the organisms when substantial numbers of organisms are being shed. In practice, positive cultures usually are obtained when 50 to 100 organisms are being shed per gram of feces. It is at this stage of infection that such animals are dangerous to keep in the herd, even though they appear healthy.

For cultivation from fecal or intestinal tissue specimens, a decontaminant must be used which will destroy a broad spectrum of organisms, but be innocuous to the pathogenic mycobacteria. Benzalkonium chloride solution fits this description. It is bacteriocidal for aerobic bacteria commonly found in ruminant fecal specimens, but only bacteriostatic for mycobacteria. Hence, it can be used to treat such specimens, and then be washed away or neutralized. In practice, neutralization is much more convenient than washing because it can be accomplished by the culture medium. The most satisfactory media for purposes of neutralization are those containing egg yolk – in addition, egg yolk media are adequate for most pathogenic mycobacteria. \textit{M. paratuberculosis} has one medium requirement not shared by other cultivable mycobacteria, it needs mycobactin. Other species of mycobacteria each produce their own specific mycobactins.

Pure mycobactin \textit{P} (from \textit{Mycobacterium phlei}) is a white, amorphous compound, almost insoluble in water and hydrocarbons, but quite soluble in fat solvents. It chelates readily with metals such as aluminum and ferric ions. The ferric chelate of mycobactin is a reddish brown compound which can be crystallized, and is much more soluble in water than the nonchelated compound.

Each species of mycobacteria produces a different mycobactin, with the differences chiefly on the side chains. The quantity of mycobactin produced is related inversely to the amount of iron in the medium, therefore, in the production of mycobactin, the medium employed contains the smallest amount of iron consistent with good growth of the producing organism. Among the species I have tested, \textit{M. phlei} produces the greatest amount of readily purified mycobactin.

Mycobactin for use in media to cultivate the Johne's bacillus is now being produced by the Diagnostic Services, Animal Health Division of Agricultural Research Service. They provide the mycobactin to local diagnostic laboratories where personnel have had adequate training in the preparation of the medium, decontamination and inoculation of specimens, and isolation and identification of \textit{M. paratuberculosis}. Arrangements for the necessary training should be made through Dr. A. F. Ranney, Chief, Tuberculosis Eradication Section, Federal Center, Hyattsville, Maryland.

The best medium we have found for isolation of \textit{M. paratuberculosis} is an agar base medium containing mycobactin and raw egg yolk. (Table 1).

Fecal specimens are decontaminated by shaking the specimens in about 20 volumes of sterile water, allowing the heavy particles to settle for about an hour, and then adding an aliquot of the supernatant suspension to 5 to 10 volumes of 0.3% benzalkonium chloride solution. Each specimen is allowed to stand in the
decontaminant overnight at room temperature. During this period, soluble materials from the specimen precipitate and settle to the bottom of the tubes, carrying most of the mycobacteria to the bottom also. This sediment is then taken up with a pipette, without removing the decontaminant, and distributed in 0.2 ml amounts onto the surface of the egg-yolk medium. The tubes are incubated at 38°C in a slanted position with the caps loosened for 1 week to allow the surface of the medium to dry. The caps are then tightened and incubation is continued for 3 months.

Specimens of intestinal or lymph node tissues taken at necropsy (particularly ileocecal mucosa and mesenteric nodes) are ground in a blender, digested with trypsin centrifuged and the sediment suspended on 0.1% benzalkonium chloride. Inoculation and incubation are identical to procedures for fecal specimens.

In most cases, small, firm, glistening, whitish colonies can be detected by the end of 2 months' incubation, at least with a dissecting microscope, but some develop more slowly; therefore, 3 months' incubation is used routinely.

When typical colonies develop, they are subcultured on media with and without mycobactin, and a smear is stained by the Ziehl-Nielsen technique to determine acid-alcohol fastness. To be classified as *M. paratuberculosis*, the organisms must be acid-fast and mycobactin-dependent.

When fecal culture is used as a basis for culling infected animals from a herd, I recommend that specimens from all adult animals in the herd be cultured semi-annually. All animals found to be shedding the organisms and their recent progeny should be slaughtered. All newborn animals in the herds should be removed from their dams at birth and be raised separately from the adults. These practices reduce the contamination of pastures, lower the level of exposure to infection of replacement animals and result in reduced incidence of clinical paratuberculosis.
## TABLE 1

EGG YOLK MEDIUM FOR CULTIVATION OF MYCOBACTIN DEPENDENT M. PARATUBERCULOSIS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>peptone</td>
<td>9.0 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>4.5 g</td>
</tr>
<tr>
<td>agar</td>
<td>15.3 g</td>
</tr>
<tr>
<td>beef extract</td>
<td>2.7 g</td>
</tr>
<tr>
<td>glycerin</td>
<td>25.0 ml</td>
</tr>
<tr>
<td>ferric mycobactin — 2 mg dissolved in 4 ml ethanol</td>
<td></td>
</tr>
<tr>
<td>NaOH (4%) to adjust pH to 7.5</td>
<td></td>
</tr>
<tr>
<td>malachite green solution (2%)</td>
<td>5.1 ml</td>
</tr>
<tr>
<td>hot water</td>
<td>870.0 ml</td>
</tr>
</tbody>
</table>

The above ingredients are mixed, with the mycobactin solution added last and in drops while the hot solution is being stirred so that the mycobactin is in colloidal suspension. It is then autoclaved, and when it has cooled to about 65°C the yolks of 6 eggs are added. While still hot, it is dispensed in sterile screw-cap tubes and allowed to cool into slants.
HISTOLOGICAL TECHNIQUE FOR
DIAGNOSTIC LABORATORIES

David C. Tudor, V.M.D.

Diagnostic laboratories should routinely prepare stained histological sections of tissues of selected cases as an aid to diagnosis. The following is thus presented as a guide for new laboratory personnel who are required to prepare such sections. Most anyone who exhibits patience and is neat in their work can follow these procedures. Many laboratories are equipped with automated equipment which aid in the preparation of tissues and here the manufacturer's directions should be followed in the use of the equipment. Where such equipment is not available, the following guide which emphasizes special non-automated procedures, may be helpful

TISSUE COLLECTION

Tissue for histological preparations should be collected immediately when specimens are necropsied. If specimens die or if they are previously killed, autolytic or pathological changes may be expected. For satisfactory fixation, pieces of tissue should be no more than five to ten mm. in thickness. Thin pieces are usually fixed in a shorter period of time.

FIXATION

For routine work, tissue should be fixed or held for 24 to 48 hours in a 10-15% formalin buffered solution at a pH of 7.5 to 8.0 and at room temperature. There should always be at least ten times the amount of fluid as tissue. Frequent agitation of the tissue in the formalin improves the fixation. If the tissue is large enough to be examined by incision, a uniform color should be observed throughout the tissue and this generally indicates adequate fixation. Tissues with pink or discolored center sections should be fixed for longer periods. Remaining pieces of tissue which are not processed may be held in 10-15% formalin almost indefinitely, if the fluid is replaced periodically.

Where automatic equipment is not available, we suggest the use of special trays for processing tissue. (Fig. 1) These trays are made of perforated stainless steel and are 3-1/4" x 5 x 3/4". The interior of the tray is adjustable and is divided into 24 compartments by removable stainless steel dividers. A perforated top is held in place by side clips. Pieces of tissue may be identified by their location in the tray which is also identified by number or letter. Larger pieces may be accommodated by removal of one or more separators. One corner of the tray is marked and this coincides with the card for each tray. Separate numbers for each tissue identifies the tissue and can be related to data, as recorded in a card file or notebook. Tissue remains in this tray throughout fixation, dehydration, clearing and impregnation. (Fig. 2, 3 and 4)
WASHING & DEHYDRATION

Before the tissue is dehydrated, it should be washed overnight in running water. Properly fixed and washed tissues may be dehydrated in successive baths of ethyl or isopropyl alcohol for at least two hours in 70%, two hours in 95%, one hour in 100% or absolute alcohol, and another hour in fresh absolute. The alcohol solutions should be kept fresh and clean. All solutions should be stored in screw cap bottles.

CLEARING

Following dehydration, overnight clearing in methyl salicylate is convenient. It does not leave tissues brittle, and it has a pleasant odor. An old favorite, xylene, on the other hand, has tendency to leave the tissues brittle. Tissues should not be left in this agent for a prolonged period. Continued inhalation or excessive vapor levels of either product may cause mucous membrane irritation.

IMPREGNATION

Infiltration or impregnation may be accomplished by three 2-hour baths of freshly melted paraffin, (Fisher “Tissue Mat, or Aloe “Paraplast”) melting point 56-58° C. In this procedure the tissue trays are transferred from the commercial glass dehydration dishes to fabricated metal paraffin containing boxes which are just large enough to accommodate the trays. An alternative routine dehydration and clearing method which is desirable for most tissue, employs the use of p-Dioxane (Matheson Coleman & Bell). Three separate one-hour baths of p-Dioxane followed by three separate one-hour baths of paraffin permits embedding the same day. The produce should be used under a chemical hood since the vapors are flammable and may be harmful if inhaled. Prolonged contact with the skin should be avoided. Explosive peroxides may also form upon standing.

VACUUM IMPREGNATION

A vacuum oven should not be used with (p-Dioxane), but is a 400 watt vacuum oven is available, methyl salicylate cleared tissue may be impregnated more quickly. The oven temperature should be about 60 to 62° C. The time required for impregnation varies from one quarter to one-half of that required for the same objects at atmospheric pressure. At a vacuum gauge reading of 15 or 175 mm. of mercury, tissue 5 mm. in thickness may be left in each of two paraffin baths for a period of 15 minutes each. The third bath may have the vacuum raised to 25 or 290 mm. Care must be taken to raise the lower the vacuum slowly to avoid cell destruction and distortion. At least two minutes should be allowed for each of these procedures.
EMBEDDING

Tissues may then be embedded in fresh paraffin at a melting point temperature of 56-58°C. Forceps are used to remove the paraffin impregnated tissue from the tray which is kept in the melted paraffin on a variable temperature hotplate. We locate and orient the paraffin-coated tissue on a metal tray surface in partially, paraffin-filled lead angle molds. Freshly melted paraffin is then added to fill the block. We always cut the surface of the block which faces the try. The blocks are identified with their respective serial numbers by small numbered pieces of paper which are fixed on the side of each block. We use a table knife or spatula heated in the flame of a bunsen burner to slightly melt the paraffin on the side of the block. The paper should not be close to the cutting surface of the block to avoid dulling the knife when the blocks are cut.

As an alternate, tissue may be placed in molds for “Tissue-Tek Universal Embedding Rings” after the molds have been sprayed with “Tissue Tek Mold Release Spray” (Arthur H. Thomas). Additional paraffin is added after the rings have been mounted on the mold. These blocks may be identified by merely writing on the flange of the white plastic ring.

MOUNTING BLOCKS

Prior to sectioning the tissue, the blocks must be trimmed to an octagon shape and mounted on an object disc. Mounting is done by warming the disc in a bunsen flame and pressing the base of the block on the face of the disc. These blocks are then chilled with ice cubes or are held briefly in a freezer for a few minutes.

SECTIONING AND SLIDE MOUNTING

We use an 820 Rotary Microtome for sectioning tissues and our routine sections are cut 6 to 10 microns in thickness. Ice cubes may help to cool the mounted blocks and also the knife after they are secured in the microtome. We float our tissue sections in a large petri dish suspended in an “Autotechnicon” water bath using a water glass solution of sodium silicate in the petri dish and plain water below the dish to transfer the heat which is at a temperature of about 60°C. The silicate serves as a tissue adhesive. The formulae for the stock solution is:

- Distilled water.............................................................................................. 2000 cc.
- Ammonium hydroxide.................................................................................. 200 cc.
- Sodium silicate............................................................................................ 200 cc.

This solution is diluted using 30 cc. of stock in 1000 cc. of distilled water for floating the tissues. A very thin layer of Mayer’s albumin may be used as an adhesive in place of silicate if plain tap water is used to float the tissues. The slides are then air dried in a bacterial incubator or on a controlled temperature warming plate at a temperature of 37-38°C.
STAINING

After the slides are well dried they are placed in staining trays. There should be sufficient separation between the slides to facilitate fluid movement. The following tissue staining procedure is used:

- **Xylene**: 10 minutes
- **Xylene**: 5 minutes
- **Absolute Alcohol**: 2 minutes
- **Absolute Alcohol**: 2 minutes
- **95% Alcohol**: 2 minutes
- **95% Alcohol**: 2 minutes
- **70% Alcohol**: 2 minutes
- **Distilled Water**: 1 minute
- **Hematoxylin**: 4-7 minutes (Paragon)
- **Tap Water**: 2-3 dips
- **Acid Water**: 2 dips (1 drop HCL in 400 cc.)
- **Distilled Water**: 3 dips
- **Ammonia**: 2 minutes or until blue (4 cc. NH4OH in 400 cc. distilled water)
- **Distilled Water**: 1 dip
- **95% Alcohol**: 1 minute
- **Eosin Y**: 10 to 15 minutes (6.25 gm. in 500 cc. 95% ethyl Alcohol)
- **95% Alcohol**: 2 minutes
- **Absolute Alcohol**: 2 minutes
- **Carbol-Xylol**: 1 dip (75 cc. Xylene, 25 cc. Phenol)
- **Xylene**: 2 minutes
- **Xylene**: 2 minutes
- **Xylene**: 2 minutes

We have used staining dishes which are 4-3/4” x 4” x 2-1/2” deep to accommodate our tissue trays. The Hematoxylin Stain-Paragon™ may be obtained in 500 cc. quantities from the Paragon C. & C. Co., Inc., 190 Willow Ave., Bronx, N.Y. 10454. Eosin Y, water and alcohol soluble, may be purchased as a dry powder from the Hartman-Leddon Co., in Philadelphia, Pa.

MOUNTING COVERSLEIPS

After the tissues have been stained, they may be held in xylene indefinitely until coverslips are mounted. We use non-corrosive slides, one end etched, and cover glasses, 22 x 50 mm; 22 x 40 mm; and 22 x 22 mm. The stained slide is removed from the staining tray and placed on a paper towel. A coverslip is then immersed in xylene. One small drop of “Permount” or Balsam is placed on the center of the coverslip and inverted and inclined on the slide. Air bubbles are gradually expressed.
by gentle pressure and the slides are then again air dried in a bacterial incubator or on a controlled temperature warming plate at a temperature of 37-38° C.

**KNIFE CARE**

We use plane-edge knives which we clean with xylene on soft cleansing tissue. The edges are coated with vaseline when not in use. The knife edges are maintained in a sharp condition with a glass plate and a leather strop. The angle of the cutting edge is kept constant by a sharpening guide. Levigated alumina, cerium oxide white rouge, jeweler’s rouge and emery have been used to sharpen the blades. We prefer cerium oxide. About a 5% suspension is made with castile soap for the glass plate, whereas dry powder is used on the strop. Blades may be checked under a dissecting scope for serrations. Deep nicks may require the assistance of a professional sharpener such as Dr. Lorimer Rutty, Niagara Falls, N.Y. Where accuracy in tissue thickness is not essential, a razor blade microtome holder may be helpful.

**DECALCIFYING BONES**

Win 3000 (Winthrope Stearns) is used for decalcifying bone. Two grams of resin is added to 80 ml. of 15% formic acid. Tissue is left in this solution until decalcified as indicated by the ability to force a pin through the tissue.

**ACKNOWLEDGMENT**

Acknowledgement is hereby made of the active support of our histological technician, Mr. Charles Rodriguez.
Figure 1 - Stainless steel tray for holding tissues for dehydration, clearing and impregnation.
Figure 2 – The trays are adapted for available glass dehydration dishes. Wooden covers prevent excessive evaporation loss.
Figure 3 – Fabricated stainless steel boxes accommodate the trays for paraffin infiltration. Wooden lids may cover the paraffin box when not in use.
Figure 4 – The fabricated steel box with a tray of tissues is held on a variable temperature hot plate to prevent the paraffin from solidifying while the tissues are embedded.
THE DIAGNOSIS AND CONTROL OF ANAPLASMOSIS

H. D. Anthony
College of Veterinary Medicine
Manhattan, Kansas

INTRODUCTION
The role of the diagnostic laboratory in the diagnosis and control of anaplasmosis may become of increasing importance in the next decade. Today, many nations are requiring that imported animals be free of anaplasmosis. Canada has recently asked that cattle moving from the United States into that country be accompanied by a health certificate stating that the cattle are negative to the anaplasmosis complement-fixation test and that the cattle originate from a herd that has not experienced anaplasmosis during the preceding two years. Two states, New York and Hawaii, require imported cattle to be serologically negative to anaplasmosis. On occasion, production herd owners will ask that replacement stock be free of anaplasmosis. The requests for diagnostic procedures on healthy salable cattle may increase as additional health requirements are initiated either officially or by demand by the buyer. Diagnosticians need to be aware of the modes of transmission, the diagnostic methods, treatment procedures and the use of vaccines. It is necessary to recognize the acutely sick animal and to realize the problem of asymptomatic anaplasmosis carrier animal. Research workers have published data on anaplasmosis that has enabled a good understanding of the pathogenesis. The economic importance of anaplasmosis can no longer be measured by mortality, medical cost, and loss of production. The cost of interference to commerce by the loss of sale of breeding stock, either locally or internationally, must also be recognized.

Anaplasmosis is not a new disease to the cattle industry as it was diagnosed and reported nearly 50 years ago. Actually, it is now known that the disease was present back in the days of tick fever. Anaplasmosis is not static, and neither is the bovine population. There are endemic areas in the South and East and anaplasmosis-free areas in the Northeast and Midwest. It might be considered that the occurrence of anaplasmosis is more of a herd than an area problem. It is not reasonable to consider eradication of this disease, but anaplasmosis-free herds can be recognized and maintained.

DIAGNOSIS

The clinical signs of acute anaplasmosis are rather definite and may support sufficient information for diagnosis. As the organism, Anaplasma marginale, enters the erythrocyte and multiplies the infected erythrocytes are removed from circulation; acute progressive anemia results, and the animal demonstrates many vivid clinical signs. The muzzle becomes pale when the circulating erythrocyte value has been diminished by one half, an increased respiratory rate is present and, due to cerebral anoxia, irrational behavior may be observed in some animals. Elevated
temperatures are common and may persist until the acute infection has passed. Icterus may appear and become quite vivid in the older animal. Rumenn atony and constipation are common signs. Acutely infected animals demonstrating anemia and icterus are subject to cardiac failure due to myocardial anoxemia. Anaplasmosis in cattle has a definite age susceptibility. Animals less than one year of age have subclinical infections, those one to two years may be acutely infected but rarely fatal, while animals three years or older may have acute or peracute infections with fatalities occurring. Animals that are infected and survive are carriers of the infectious agent. This carrier state of anaplasmosis may persist for the life of the animal.

At necrospy, common findings are anemia and icterus. The spleen is enlarged and the gall bladder greatly distended. The liver is swollen and yellow-brown in color. Hemorrhages are usually found on the epicardium and pericardium.

A tentative diagnosis of anaplasmosis can usually be made by observing the clinical signs and reviewing the history of the herd. Microscopic examination of a stained blood smear can confirm the diagnosis. Giemsa stain is commonly used; however, several other stains are adequate. Serological diagnosis of anaplasmosis is quite reliable. Two serological procedures are available. The capillary agglutination (CA)* test is a quick test and should be available in most laboratories. This test can be read in ten minutes although a longer period of time is recommended for final interpretation. The complement-fixation (CF) test is more laborious, requiring a number of reagents and some experience in using the test procedures. Both the CA and the CF tests are capable of diagnosing both the acute and the carrier infections.

Anaplasmosis is transmitted from the infected carrier animal to the susceptible animal. Minute quantities of carrier infected blood is sufficient to produce infection. Insect vectors such as mosquitoes, flies and ticks are known to transmit the disease from animal to animal. Man can also transmit anaplasmosis from animal to animal through poor husbandry practices at the time of dehorning, castrating, or collecting blood samples. One infected carrier animal can be a hazard in any herd of cattle as a possible source of infection.

CONTROL

Personnel in diagnostic laboratories are often asked for suggestions for the control of anaplasmosis once it has been diagnosed in a herd. In the acute infection, suppression of the etiologic agent, *Anaplasma marginale*, can be affected by the use of the tetracyclines at a dosage of 3 to 5 milligrams per pound of body weight. This treatment should be repeated once within 48 hours along with other supportive procedures. On occasion, when a herd outbreak appears evident, low level feeding of antibiotics have been attempted to suppress acute infections. These procedures have varying results due to the inability to control proper consumption and to establish antibiotic blood levels that are sufficient to be effective.

*Diamond Laboratories Anatest, Diamond Laboratories, Des Moines, Iowa.
Effective control measures may be initiated in herds located in nonendemic areas. After the vector season has passed, a serological test of the herd will identify the carrier infected animals. Those animals may be identified and (1) isolated from the herd during the vector season, (2) sold to slaughter or (3) treated for the eradication of carrier infection. Recommendations for treatments of infected carrier animals have been investigated and reported. A second blood test is recommended to eliminate the possibility that an infected carrier was not detected with the first test. Anaplasmosis can be eliminated from a herd of cattle and the herd again be free of the disease. Such procedures may become essential in purebred herds.

Some five years ago an inactivated vaccine for anaplasmosis was licensed. This vaccine has proven to be quite efficacious in the control of anaplasmosis in endemic areas. Diagnosticians should be aware of an isoimmunohemolytic condition that has occurred in calves born to cows that were vaccinated with this Anaplasma vaccine. The disease occurs in calves soon after the first nursing of colostrum. Both peracute and acute forms are reported and are well described. This condition has been reported in a small percent of calves born to vaccinated cattle when one considers the number of doses of vaccine that has been used. However, in one herd an alarming number of calves were reported to have died of isoerythrolysis.

SUMMARY

Diagnostic laboratory personnel can aid in the diagnosis and control of anaplasmosis. Rapid diagnoses can be expected with microscopic examination of stained blood smears and capillary agglutination serology tests. The complement-fixation test, though it is more laborious, is extremely reliable. Control measures by serological herd tests can be of great value in identifying infected carriers and by treatment or removal of these animals from the herd. Anaplasmosis can be controlled and even eradicated from many herds of cattle that are not located in nonendemic areas.
REFERENCES

THE PASSIVE HEMAGGLUTINATION TEST IN AVIAN VIROLOGY

E. G. Trewick and G. Lang
Department of Veterinary Microbiology
Ontario Veterinary College
University of Guelph, Guelph, Canada

INTRODUCTION

The routine diagnosis of virus infections in birds uses mostly the chicken embryo for isolation of viral pathogens. Embryo mortality, retardation of development or tissue lesions are the main signs of the presence of a disease agent. The serological identification of the isolate is relatively easy with hemagglutinating viruses, but is very cumbersome with non-hemagglutinating agents. Serum neutralization tests in embryonated eggs are the classical method for virus identification, but they are so costly in money and effort that they cannot be applied routinely. Besides, many isolates must be adapted to the embryonated egg before an acceptable titration pattern is established, a process which requires several weeks of work with the agent.

Simpler and faster means of virus typing are needed. Immunofluorescence has been recommended for this purpose, but it certainly lacks simplicity. The equipment required is costly, the manipulations of specimens is delicate and time-consuming, and great expertise is required for reading and interpreting the results. The immune-precipitation in gels is another approach for virus identification. It has merits when high-titering reagents of antibody and antigen are available, otherwise it lacks sensitivity and is difficult to read.

In this study, the passive hemagglutination (PHA) test has been explored. Other investigators have studies PHA with infectious bronchitis virus, fowl pox, Marek's disease, but essentially for detecting antibody in convalescent bird sera. The principal goal of our work was to detect the virus-specific antigen by the inhibition of PHA. Secondly, fixed erythrocytes were to be used to obtain a durable reagent for routine work. Glutaraldehyde was selected as the fixing agent.

MATERIALS AND METHODS

1) Red Blood cells (RBC) -- Blood was collected to equal parts of Alsever's solution. The washed RBC were fixed within 3 days after bleeding time. Initial fixation attempts of turkey and chicken RBC yielded frequently preparations which agglutinated spontaneously. Sheep RBC, however, were easily fixed and sedimented into good buttons; thus, they were used throughout the investigation.

2) Fixation of RBC -- The method of Bing et al. was followed. Sheep RBC, washed 3 times in saline, were resuspended to 2% in cold (4°C) glutaraldehyde in phosphate buffer pH 8.2. The fixing solution was made up of 1 volume 0.15 M PO₄ buffer, 9 volumes 0.15 M NaCl and 5 volumes distilled water. The commercial (25%) glutaraldehyde (Baker technical grade) was diluted 1:25 in the buffer to obtain a 1% solution. The glutaraldehyde - RBC suspension was left for 30 minutes.
at 4°C. The fixed cells were collected by centrifugation (500 g for 10 minutes at room temperature) and were washed 5 times in saline and 5 times in distilled water. The cells were stored at 4°C as a 30% suspension in distilled water with 1:10,000 methiolate.

3) Tanning of fixed RBC — This was carried out just before coating of the cells with the sensitizing antigen. The fixed cells were washed in 0.15 M phosphate buffer pH 7.2 and suspended to 3% in the same buffer. An equal volume of tannic acid (1:20,000 in buffer pH 7.2) was added, and the mixture was left for 10 minutes in an incubator at 37°C. Tannic acid was then removed by 2 washes in 0.15 M phosphate buffer pH 6.4, and the cells were resuspended in this buffer at a 30% concentration.

4) Antigen coating of tanned RBC — Virus concentrates have a tendency to cause spontaneous agglutinability of tanned cells, and it is necessary to establish the optimum dilution of antigen which is devoid of this defect. The optimal dilution was determined by a preliminary titration of the virus antigen. Doubling dilutions of antigen were prepared from 1:1 to 1:64 in buffer pH 6.4 and 0.25 ml of these dilutions were mixed with 1.0 ml buffer pH 6.4 and 0.25 ml tanned RBC (30% concentration). One tube receiving 0.25 ml of buffer instead of antigen, but all the other ingredients was processed as a RBC control. After 20-30 minutes incubation 37°C, the RBC were washed at least twice in buffer pH 6.4 to which 2% normal rabbit serum (Inactivated and absorbed for 1 hour at 4°C with 25% fixed SRBC) had been added. A 1 or 2% cell suspension in the latter diluent was used in the agglutination test, carried out as a box titration of the cells coated with the various antigen concentrations and the control RBC against 2-fold serial dilutions of antiserum. Each antigen — cell preparation was also tested without specific antiserum for spontaneous agglutinability. The optimum concentration was the one which showed a clear sedimentation button in the latter controls and gave the highest agglutination pattern with the antiserum. Larger batches of coated RBC were then prepared with the optimum antigen dilution under conditions identical to the sensitisation of cells for the preliminary antigen titration. Methiolate was added at a concentration of 1:10,000 to these reagents to reduce the risk of contamination.

5) Antigen coupling with carbodiimide — An alternative mode of sensitisation was by a modification of the method of Johnson et al.9: The coupling agent was 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide hydrochloride (ECDI) supplied by the Ott Chemical Co., Muskegon, Michigan. The overall disposition of the preliminary antigen titration or stock preparation was the same as for the tanned cell procedure, except that 0.2 ml of antigen dilutions in phosphate buffer pH 7.2, 0.20 ml 30% fixed RBC and 0.5 ml of 15% ECDI in distilled water were mixed, incubated at room temperature for 1 hour, and were washed twice and suspended to 2% in 1% normal rabbit serum - saline.

6) Antigen preparation — Avian adenovirus type 1 (CELO Phelps strain and Ontario field isolates 4199/69 and 4401/69) infectious bronchitis (IB) virus (Beaudette strain and Bronchitis vaccine MM, Massachusetts type from Salsbury
Laboratories, Charles City, Iowa) and infectious bursal agent (IBA), Winterfield strain, obtained from the Animal Disease Research Laboratories, Hull, Quebec) were used. The viruses were passed by the allantoic route in 9 day chicken embryos. Allantoic fluids and chorio-allantoic membranes (CAM) were harvested when the viruses reached peak titers: 24 hours after infection for infectious bronchitis virus, at 3 days for infectious bursal agent and the adenoviruses. Allantoic fluids were processed immediately after harvesting, by a preliminary clarifying centrifugation (2,000 g for 10 minutes) and then sedimentation of the virus at 100,000 g for 2 hours. The virus pellet was resuspended in saline to 1/30 of the original volume. This antigen concentrate was used for sensitizing the RBC. For the inhibition tests, CAM extracts were also used; these were prepared by grinding the membranes in a Ten Broek grinder without added fluid, and centrifugation at 30,000 g for 15 minutes. The supernatant was collected for testing.

7) Antisera - SPAFAS inc., Norwich, Connecticut, supplied the following specific antisera: anti IBV Massachusetts (N.I.3.0) anti IBV Connecticut (N.I.3.4) anti CELO (N.I. 4:25) and anti IBA (N.I. 2.5) as well as a normal chicken serum negative for common poultry viruses. Another IBA antiserum was supplied by the Animal Diseases Research Institute, Hull, Quebec. Hyperimmune sera to the 3 Adenoviruses were prepared in one turkey (CELO) and in chickens (isolates 4199/69 and 4401/69) by intraperitoneal injection of 10 ml virus allantoic fluids. All sera were stored frozed. Few sera needed absorption with fixed SRBC because of non-specific agglutinins, but all sera were inactivated at 56°C for 30 minutes.

8) Purification of Chicken Globulins - In a limited experimented series, RBC were coated with turkey immune globulins to CELO virus. The native serum was clarified by centrifugation at 30,000 g for 20 minutes, and the globulins were precipitated by sloww addition of Na₂SO₄ to 34% final concentration and incubation at room temperature for 30 minutes. The precipitate collected by centrifugation (30,000 g for 10 minutes), was washed once in 17% Na₂SO₄, redissolved in m/15 Tris-HCl buffer pH 8.0, and reprecipitated with 15% Na₂SO₄. The precipitate was then redissolved to the initial volume of the serum in Tris-HCl buffer pH 8.0 and dialyzed overnight against saline.

9) Hemagglutination (PHA) and Inhibition (PHI) Tests - These were carried out in perspex plates by a micromethod using special loops and droppers (Flow Laboratories Inc., Rockville, Maryland). PO₄ buffered saline pH 7.2 with 2% normal rabbit serum was used as diluant. In the PHA test 0.05 ml of serum dilutions, 0.05 ml of diluent and 0.025 ml of a 1% suspension of sensitized RBC were mixed in this order, and incubated at room temperature. In the PHI test antigen dilutions in 0.05 ml volumes and 4 to 16 hemagglutinating units of antiserum contained in 0.05 ml were incubated at 37°C C for 30 minutes before 0.025 ml of the senistized RBC suspension was added. Readings were made after 2 to 3 and after 18-20 hours.
RESULTS:

I. Studies on Avian Adenoviruses

CELO virus propagates easily in the chicken embryo and reaches high infectivity titers in allantoic fluids; thus it was not difficult to obtain virus concentrates which sensitized well tanned SRBC, both fresh cells and cells previously fixed with glutaraldehyde. The potency of such antigen preparations is illustrated in Table 1. It can be seen that the serum agglutination titer increases with increasing concentrations of the antigen during the coating process, the upper limit being at the point where cells become auto-agglutinating. When native and GA-fixed cells of the same batch were sensitized with the same antigen preparation in a parallel experiment, native cells gave slightly higher titers than fixed cells. Table 2 lists antibody titers of some sera. Titer endpoints were similar with a given batch of virus-coated cells, even after prolonged storage of the coated cells at 4°C. The longest storage time so far was 4 months. However, much variation of serum titers was seen with cell batches coated at different times, even when the same antigen preparation and the same stock of fixed RBC were used. The exact cause of this variability is not known, but it must be related to some technical aspect of the coating process. Nevertheless, the specificity of the PHA test clearly appears from the data. This is further testified by inhibition results, illustrated in Tables 3, 4 and 5. Table 3 shows the inhibition pattern of an antigen solution or suspension with increasing amounts of antibody. With 2 or 4 antibody units the inhibition is maximum, then declines two-fold with 8 to 16 units, and inhibition decreases steadily until it is no longer evident when about 100 units or more are used. No inhibition is seen with normal allantoic fluid at any level of antibody. The specificity is also demonstrated by the inhibition test given in Table 4. Neither normal allantoic fluids no allantoic fluids from embryos infected with IB or IBA viruses produced inhibition whereas the homologous antigen (CELO) and fluids from 2 field isolates from chickens were clearly inhibitory. The relationship of the 2 field isolates with CELO virus was also verified in cross-SN tests in ovo, and they produced a cytopathic effect in chicken kidney tissue culture similar to CELO virus, but distinct from that of IBA. Both field isolates are resistant to chloroform. GA-TA-SRBC could be coated with antigen concentrates from the 2 field isolates with the same ease as CELO virus, and their reactivity in the PHA and the inhibition tests were of similar magnitude.

Table 5 shows the titers and distribution of the PHA antigen in CELO-infected chicken embryos taken from the incubator at different intervals after inoculation. The specific antigen was detectable from 48 hours onwards, greater amounts being present in the CAM than in the allantoic fluid during the early stages of infection but equal titers being reached during the terminal phase.

A limited experimental series was carried out with GA cells sensitized by carbodiimide (ECDI), a divalent bonding agent recommended by Johnson et al. for coupling antigens to red blood cells. Using the same reagents and an identical technical arrangement as in the studies whit tannic acid sensitized cells, similar
results were obtained in PHA and inhibition tests carried out with TA or ECDI sensitized cells coated with CELO antigen. No data, however, are available on the durability of the ECDI sensitized cells during prolonged storage.

Finally, globulins precipitated from a hyperimmune anti-CELO turkey serum were successfully coated unto GA-TA-SRBC. Unlike viral antigens which had a low auto-agglutinating potential for the RBC, the globulins had to be diluted to 1:64 to obtain coated cells settling into a normal button. Nevertheless, sensitized cells were obtained which agglutinated specifically with CELO antigens but not with similar preparations devoid of the antigen. Globulin-coated cells revealed the antigen to slightly higher titers than were detected by the inhibition of the PHA with antigen coated cells.

II. Studies with IB and IBA Viruses

Antigen concentrates prepared with IB (Beaudette and Vaccine strains) and IBA (Winterfield) were coated onto GA-TA-SRBC by the method established for the adenoviruses. Such coated cells agglutinated in the presence of their respective antisera, but the agglutination titers were very low, the highest titer ever observed being 1:160. Furthermore the agglutination was inhibited not only by the specific antigen but also by allantoic fluids from normal embryos and embryos infected by unrelated viruses. Using carbodiimide as coupling agents, preparations were obtained of similar reactivity. In no instance was it possible to sensitize cells in such a manner that they would permit specific inhibition by the homologous antigen but not by paralled preparations free of the virus.
DISCUSSION

Without doubt, the PHA test is a versatile method of immunological analysis. This is well demonstrated in our studies with the avian adenoviruses. With these viruses we have obtained our objective, that is stable and specific reagents could be prepared with which the presence of the viruses was readily detected in infected chicken embryos. Both tannic acid and carbodiimide were effective in coupling the virus antigens onto fixed RBC. Furthermore, the inverse arrangement where the anti-virus serum globulin was attached to the cells was successfully applied. The usefulness of the method is obvious. The two field isolates were among a large array of unidentified chloroform-resistant agents obtained from diseased birds during routine virological examinations. The PHA permitted their identification at little cost and in a short time.

Several serotypes of avian adenoviruses are known, and it will be necessary to determine whether the PHA test reveals group- or strain-specific antigens.

Although versatile, the PHA test is not of universal applicability by one standard method. Thus, the method which gave good results with adenoviruses, failed to sensitize specifically RBC with the infectious bursal agent and IB viruses, despite strenuous efforts. This failure is particularly perplexing in regard to IB virus, since others have reported successful application of the method with it. Our method was modelled after theirs, and we obtained, to a certain degree, parallel results. IB antisera agglutinated IB coated cells, and the agglutinating titers were related to the neutralization indices of the sera. But the agglutination was inhibited by preparations containing the homologous virus antigen as well as by reference preparations where it was absent. Thus, the inhibition test was non-specific. The claim made in one publication that inhibition tests proved specificity of the IB PHA test is questionable, since Newcastle disease virus material was used as reference antigen. This was a rather unhappy choice, for the virus is hamagglutinating in itself, and no indication was given if and how the hemagglutinin was removed. Furthermore, the published table indicates results which are inconsistent with the principle of the inhibition test. The agglutination pattern with the Newcastle virus antigens ceases within the dilution series, though the IB-coated cells and the antibody, both present in the reaction mixtures, are expected to produce agglutination.

It may be, that the difference in behaviour between the adenoviruses on the one hand, and IBA and IB virus on the other hand is a simple matter of virus concentration, for infectivity titers usually obtained in embryo fluids with the former viruses are more than 1000-fold higher than with the latter viruses. If so, more refined concentration and purification methods might lead to success. It is also possible, however, that we are hunting a chimaera, because the antigens of the two recalcitrant viruses is of a type that is not properly fixed by tanned RBC. A study of the literature shows that viruses to which Boyden’s tanned cell method was successfully applied are mostly deoxy-viruses, more specifically adeno- and herpesviruses. Thus, we find here herpes simplex virus, IBR virus.
Marek's disease virus\textsuperscript{7}, equine rhinopneumonitis virus\textsuperscript{14}, human adenoviruses\textsuperscript{15,16}, canine hepatitis virus\textsuperscript{17}, avian adenoviruses and fowlpox virus\textsuperscript{6}; among the riboviruses only poliomyelitis virus\textsuperscript{18} and respiratory syncytial virus\textsuperscript{19} have been found. Reports on rhinoviruses\textsuperscript{20} and the influenza A nucleoprotein\textsuperscript{21} use coupling methods quite unlike Boyden's standard method. There are many other protein coupling agents available acting on different functional groups of the molecule. We tried carbodiimide on a limited scale. Whereas Boyden's method requires two preparatory steps (tanning and coating with multiple washings) carbodiimide coupling is carried out in one step; thus it is simpler. Its efficiency was good with the adenoviruses, but it failed also with IBA and IB virus.

Finally, if virus natigens cannot be coated onto RBC, there is always hope to prepare agglutinating reagents by attaching the anti-virus globulins to the cells.

ACKNOWLEDGEMENT

This study was supported by Operating Grant No. 0038 from the Canada Department of Agriculture.
## TABLE 1

### Preliminary Antigen Titration

SRBC coated with CELO Virus Concentrate

<table>
<thead>
<tr>
<th>AG DIL</th>
<th>GA-TA- SRBC coated with CELO concentrate from allantoic fluid</th>
<th>Serum Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1:2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:64</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SERUM DILUTIONS** in PBS + 1% NRS

<table>
<thead>
<tr>
<th>Dilution</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>80</th>
<th>160</th>
<th>320</th>
<th>640</th>
<th>1280</th>
<th>2560</th>
<th>5120</th>
<th>10240</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<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>Optimum</td>
</tr>
<tr>
<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>
</tr>
<tr>
<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>
</tr>
<tr>
<td>4</td>
<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>5</td>
<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>6</td>
<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>7</td>
<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>8</td>
<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>9</td>
<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>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>
</tr>
<tr>
<td>11</td>
<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>12</td>
<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>

**Distribution:**

- Serial Dilutions of serum 0.050
- Diluent (PBS/NRS) 0.050
- Coated cell suspension 1% (1:1 to 1:64) 0.025

**Incubation at room temperature**

**Reading after 18 hours**

**Symbols:**

- + agglutination
- - normal sedimentation button
### Table 2: PHA Test with CELO Coated SRBC Against Various Antisera.

<table>
<thead>
<tr>
<th>ANTIserA</th>
<th>CELLS:</th>
<th>Serum Dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 40 80 160 320 640 1280 2560 5120 10240 20480 40960 81920</td>
</tr>
<tr>
<td>Anti CELO Turkey Hyperim.</td>
<td>1 2</td>
<td>3 4 5 6 7 8 9 10 11 12 13 14 15</td>
</tr>
<tr>
<td>Anti CELO SPAFAS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti 4199 Chicken #2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti 4401 Chicken #5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti IBA Hull</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti IBA SPAFAS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti IB (Mass) SPAFAS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti IB (Conn) SPAFAS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norm. Chicken SPAFAS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Turkey Serum #1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Turkey Serum #2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Distribution:**
- Serum Dilutions in PBS/NRS 0.050
- Diluent PBS/NRS 0.050
- CELO coated SRBC (GA-TA) 0.025

**Incubation:** 4 hours at room temperature
**Symbols:**
- + agglutination
- - normal sedimentation button
Table 3: Inhibition Pattern of the PHA-CELO System by CELO Allantoic fluid and Normal Allantoic fluid.

<table>
<thead>
<tr>
<th>INHIBITORY ANTIGEN</th>
<th>CELO ALLANTOIC FLUID</th>
<th>Normal 15 day Embryo Allantoic fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 4 8 16 32 64 128 256</td>
<td>2 4 8 16 32 64 128 256</td>
</tr>
<tr>
<td>Antigen DILUTIONS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti CELO 1:640 2 u.</td>
<td>- - - - + + + +</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>Serum SPAFAS 1:320 4 u.</td>
<td>- - - - + + + +</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>Serum 1:160 8 u.</td>
<td>- - - + + + + +</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>Serum 1:80 16 u.</td>
<td>- - - + + + + +</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>Serum Dilutions 1:40 32 u.</td>
<td>- - - + + + + +</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>1:20 64 u.</td>
<td>- - + + + + + +</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>1:10 128 u.</td>
<td>+ + + + + + + +</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>1:5 256 u.</td>
<td>+ + + + + + + +</td>
<td>+ + + + + + + +</td>
</tr>
</tbody>
</table>

Distribution: Soluble antigen dilutions in PBS/NRS 0.050
Antiserum dilutions in PBS/NRS 0.050
Incubation 30 minutes/room temp.
CELO coated SRBC (GA/TA) 0.025
Incubation 18 hours/room temp.

Symbols: + Agglutination
- Normal sedimentation button
**Table 4: Specificity of Inhibition of the PHA-CELO System.**

<table>
<thead>
<tr>
<th>INHIBITING ANTIGENS (Allantoic fluids)</th>
<th>ANTIGEN DILUTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>CELO (Phelps strains)</td>
<td>-</td>
</tr>
<tr>
<td>IBV (Beaudette)</td>
<td>+</td>
</tr>
<tr>
<td>IBA (Winterfield)</td>
<td>+</td>
</tr>
<tr>
<td>Isolate Chicken 4199/69</td>
<td>-</td>
</tr>
<tr>
<td>Isolate Chicken 4401/69</td>
<td>-</td>
</tr>
<tr>
<td>Isolate Chicken 5025/69</td>
<td>+</td>
</tr>
<tr>
<td>Isolate Chicken 5271/66</td>
<td>+</td>
</tr>
<tr>
<td>Normal All. fluid 15 days #1</td>
<td>+</td>
</tr>
<tr>
<td>Normal All. fluid 15 days #2</td>
<td>+</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; &quot; &quot; #3</td>
<td>+</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; &quot; &quot; #4</td>
<td>+</td>
</tr>
<tr>
<td>CONTROL CELO SYSTEM</td>
<td>+</td>
</tr>
</tbody>
</table>

**Distribution:**

- Soluble antigen dilutions in PBS/NRS: 0.050
- CELO Antiserum SPAFAS 8 PHA units: 0.050
- Incubation 30 minutes/room temp.
- CELO coated SRBC (GA/TA): 0.025
- Incubation 18 hours/room temp.

**Symbols:**

- +: Agglutination
- -: Normal sedimentation button
Table 5: Development of PHI-Antigen in Allantoic Fluid and Membranes of Chicken Embryos Infected with CELO virus.

<table>
<thead>
<tr>
<th>Time of Harvest Following Inoculation</th>
<th>PHI Titer Allantoic Fluid</th>
<th>CELO System CAM Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours Egg # A</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>24 hours Egg # B</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>48 hours Egg # C</td>
<td>8 (+)</td>
<td>64</td>
</tr>
<tr>
<td>48 hours Egg # D</td>
<td>2 (+)</td>
<td>32</td>
</tr>
<tr>
<td>72 hours Egg # E</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>72 hours Egg # F (dead)</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>96 hours Egg # G</td>
<td>32</td>
<td>8</td>
</tr>
</tbody>
</table>

Test Disposition: 10-day chicken embryos infected with $10^3$ EID$_{50}$ of CELO virus by allantoic inoculation.

Inhibition System: CELO Coated SRBC (GA/TA)

CELO Antiserum SPAFAS 8 PHA units

Titers expressed in Reciprocals.
REFERENCES

TECHNIQUES FOR DETECTION OF INFECTION OF CHICKENS WITH THE HERPESVIRUS OF MAREK’S DISEASE

S. H. Kleven, C. S. Eidson and D. P. Anderson
Poultry Disease Research Center
University of Georgia, Athens

INTRODUCTION

After the isolation of the herpesvirus of Marek’s disease (MD) by Churchill and Biggs7 and Solomon et al.20, procedures for the detection of MD virus, antigen and antibody have been rapidly developed. This paper is intended to provide a review of the available techniques and to point out some advantages and disadvantages of these procedures.

DETECTION OF MD VIRUS AND VIRAL ANTIGENS

In Vivo Assay.

Until the isolation of the herpesvirus of MD in cell culture7,21, the only available procedure for detecting MD virus was by chick inoculation. Early studies were hampered by the lack of RIF-free chicks for use in virus assay.

Sevoian et al.20 were able to demonstrate visceral and neural lesions at 2 to 3 weeks of age in chicks inoculated at one day of age with blood or tissue suspensions from naturally infected chickens. The JM isolate of MD was easily propagated by chick to chick passage.

In vivo assay of the GA isolate of MD was reported by Eidson and Schmittle10, who were able to demonstrate visceral and neural lesions at 2 to 3 weeks of age in chicks inoculated at one day of age with blood or tissue suspensions from naturally infected chickens. The JM isolate of MD was easily propagated by chick to chick passage.

In vivo assay of the GA isolate of MD was reported by Eidson and Schmittle10, who were able to demonstrate visceral and neural lesions at 2 to 3 weeks of age in chicks inoculated at one day of age with blood or tissue suspensions from naturally infected chickens. The JM isolate of MD was easily propagated by chick to chick passage.

Cell Culture Assay.

Primary isolations of MD herpesvirus have been made by direct culture of kidneys from MD-infected birds7,9,24 and by inoculation of infective tissue suspensions, plasma, or blood onto normal chick kidney monolayers7,9,24 or duck embryo fibroblast monolayers19,21,24.

Cytopathic areas in chick kidney (CK) cell culture may appear within 3-6 days. These foci contained rounded, highly refractile cells which have a tendency to pile up into dense, grape-like clusters. The rounded cells in areas showing CPE may be
DETECTION OF INFECTION WITH HERPESVIRUS

mono-, bi-, or multinucleated, have basophilic staining characteristics, and may contain Cowdry type A intranuclear inclusion bodies. Plaques rarely exceed 0.5 mm in diameter7,9,24.

Cytopathic effects in duck embryo fibroblast (DEF) monolayers are quite similar, but plaques rarely appear before 6-9 days. Plaques are somewhat larger than in CK cells, often reaching a diameter of greater than 1 mm. In addition to the rounded, refractile cells, areas of CPE often contain shrunken spindle cells with long protrusions19,21,24.

Witter et al.23 evaluated direct and indirect CK and DEF cell culture techniques for the primary isolation of MD herpesviruses. Virus was isolated from 73.7% of 133 MD-exposed birds by direct kidney culture, 54.9% on indirect CK culture, and 36.1% on DEF. Assay on CK cells gave 87% agreement with in vivo assays, and 80% agreement was obtained with DEF. They found chick inoculation assays somewhat more sensitive than cell culture assays.

Witter et al.24 also compared isolation rates from various tissues. Highest plaque counts were obtained from kidney, tumor, spleen, and buffy-coat inoculums.

Chick Embryo Assay.

A method for the isolation and propagation of MD-herpesvirus in embryo-nating chicken eggs has been developed by von Bulow2. Four-day embryos inoculated via the yolk sac with infective blood, spleen, thymus, or tumor cells developed pock-like foci on the chorioallantoic membrane (CAM) from the 11th day after inoculation. Inoculation of the CAM of 11 day embryos resulted in generalized CAM foci on the 8th day after inoculation.

However, blood of both infected and uninfected chicks (immunologically competent cells), when inoculated onto the CAM, produced pocks (graft vs. host reaction) indistinguishable from those produced by the MD virus. It was also found that specific antibodies present in the yolk can influence the results significantly. Thus, eggs to be used for this technique should be derived from MD-free source flocks.

Immunofluorescent Techniques for the Detection of MD-Herpesvirus Antigen.

Fluorescent antibody (FA) tests for the detection of MD virus antigens have been used by several investigators, utilizing both the direct4,12,17,22 and indirect1,16,22 techniques.

Specific fluorescence of foci in MD-infected monolayers may be detected as early as 1 day post-inoculation3,16, before morphological foci can be recognized. After 3 days post inoculation, fluorescent foci correspond to areas showing visible CPE. Both cytoplasmic and nuclear staining may be observed1,3,16,22.

Purchase15, using the FA test to determine antigenic relationships among 8 isolates of MD virus, found that antigens prepared from these isolates were indistinguishable from one another, indicating serological similarities among these isolates.
This technique has also been used in the detection of viral antigens in chickens infected with MD\textsuperscript{3,4,12,17,22}. Kottaridis and Luginbuhl\textsuperscript{12} detected specific fluorescence in bone marrow smears from Md-infected birds. Specific fluorescence has also been detected in feather follicle epithelium\textsuperscript{4,17}, and is commonly found in the bursa of Fabricius\textsuperscript{3,4,17,22}. Other tissues which may show specific fluorescence include spleen, thymus, nerve tissue, cecal, tonsil, lungs, and kidneys\textsuperscript{17,22}. von Bulow and Payne\textsuperscript{3} found spleen, nerves, and tumors to be either negative or show indistinct staining properties. Spencer and Calnek\textsuperscript{22} found fluorescence limited to parenchyma and not to tumor cells, while Purchase\textsuperscript{17} did not find fluorescence in tumors of any origin.

Immunofluorescence has been used to attempt to differentiate tumors caused by various agents\textsuperscript{14,23}. Tekeli and Olson found specific fluorescence in birds infected with the JM isolate of MD when using chicken antiserum prepared against the transmissible lymphoid tumor of Olson\textsuperscript{23}. However, it seems possible that the birds used to prepare the sera may have been naturally infected with MD. Page et al\textsuperscript{14} conjugated antiserum prepared by inoculating chickens with either tumors of MD or lymphoid leukosis (LL). They also found that MD tumors had relatively few cells showing fluorescence, those cells fluorescing showing both nuclear and cytoplasmic fluorescence. However, the fluorescence obtained with LL tumors was cytoplasmic, giving a halo effect to the cell.

**Detection of MD Virus Antigens by Immunodiffusion.**

The agar gel precipitin (AGP) technique has been utilized in the detection of viral antigens in the tissues of birds infected with MD\textsuperscript{4,17}. Antigen has been detected regularly in material from feather follicle epithelium. It has also been detected in lung, bursa of Fabricius, thymus, spleen, leukotic gonad, and nerve plexuses. Good correlation has been found between the presence of specific fluorescence with MD antisera and antigens detectable by the AGP test. The immunofluorescence test, however, appears to be somewhat more sensitive than the AGP test\textsuperscript{17}.

**DETECTION OF ANTIBODY**

The agar gel precipitin and indirect fluorescent antibody tests have been the most commonly used procedures for the detection of antibodies against MD-herpesvirus. However, the hemagglutination and passive hemagglutination tests have also been used.

**Agar Gel Precipitin Tests:**

The use of the agar gel precipitin (AGP) test for the detection of MD antibodies was reported by Chubb and Churchill\textsuperscript{6} in 1968. Since then, this procedure has been used by several workers\textsuperscript{13,18,22}.

Antigen for the AGP test may be obtained from DEF of CK cultures of MD herpesvirus showing extensive cytopathology. Cells are harvested by scraping or
DETECTION OF INFECTION WITH HERPESVIRUS

trypsinizing, centrifuged, and resuspended. They are then ruptured by alternate freeze-thaw cycles or by sonication\textsuperscript{6,7,13,18,22}.

Several species of specific antigen (A or BC antigens) may be detected in preparations made as described above\textsuperscript{8}. The A antigens (which often give the strongest precipitin lines) are associated with the supernatant fluids, whereas, BC antigens are cell-associated. Thus, A antigens may be prepared by concentrating supernatant fluids with 80% ammonium sulfate\textsuperscript{a}. Since high passage strains of MD virus may lose the A antigen\textsuperscript{8}, BC antigens may be prepared by using cells infected with these viruses\textsuperscript{a}.

MD specific antigens have been located in the skin using the AGP test\textsuperscript{4,17}. Thus, AGP antigen can be prepared by making a 10% extract of skin from feather tracts or feather tips from birds 3-6 weeks of age after inoculation with virulent virus at day of age. The material is ground, disrupted by freeze-thawing or sonication, filtered through cheesecloth, and clarified by centrifugation\textsuperscript{a}. Precipitin lines obtained with skin antigens probably contain the A antigen\textsuperscript{9}.

Okazaki \textit{et al.}\textsuperscript{13} investigated selected variables for their importance in formation of precipitin lines. They found optimum conditions for the AGP test to be 1% special agar – Noble, 8% NaCl, NaK phosphate buffer, pH 7.4, and incubation at 37°C for 72 hours.

\textit{Indirect Fluorescent Antibody Tests.}

The indirect FA test for the presence of MD antibody had been utilized by several workers\textsuperscript{18,19,22}. Antigen for the test was prepared by growing MD virus on CK or DEF growing on coverslips. The coverslips were fixed, coated with serum to be tested, and incubated. They are then washed and incubated with conjugated anti-chicken globulin, washed, and mounted for examination. Preparations treated with positive sera show specific fluorescence in foci showing cytopathic effects, whereas, treatment with negative sera yields no specific fluorescence.

Purchase and Burgoyne\textsuperscript{18} found 92% agreement between the results of the FA and AGP tests on sera from 418 chickens. However, the FA test detected antibodies in several sera which were negative on the AGP test. In addition, comparative titrations indicated that the FA test is 10 to 320 times more sensitive than the AGP test.

\textit{Passive Hemagglutination Test.}

Eidson and Schmittle\textsuperscript{11} developed an indirect hemagglutination test for the detection of MD antibodies. Antigens were prepared from DEF showing extensive cytopathology after inoculation with MD virus. Antigens were attached to tanned sheep RBC’s, which were then washed and added to serial dilutions of sera which had been previously heat inactivated and absorbed with untagged RBC’s. They found this test to be very useful in the detection and quantitation of antibodies

against MD virus.

*Hemagglutination Test.*

The presence of hemagglutinins for sheep red blood cells has been detected in 42 chickens infected with the JM strain of MD virus, whereas agglutinins were not detected in 25 isolated, unexposed birds. However, Payne and Rennie, while attempting to confirm those results, found no correlation between presence of hemagglutinins and infection with MD. They suggested the agglutinins detected were natural hemagglutinins, and that absence of these factors in isolated, unexposed chickens could be due to absence of some other exogenous antigen (possibly bacterial), which cross reacted with a sheep blood-group substance.
REFERENCES


23. Tekeli, S., and C. Olson. Distribution of an antigen in avian leukemia demonstrable by im-


THE OPERATION OF A
MASTITIS DIAGNOSTIC LABORATORY

Harriet D. Emmette* and D. S. Postle, D.V.M., M.S.**

Why do we need mastitis diagnostic laboratories?

There are field workers who believe that screening tests, teat dipping and dry
treatment will control mastitis. There are many other vital factors that should be
studied in endeavoring to control or eliminate mastitis infections. The existing
physiological conditions, herd management and a bacteriological examination of
the mammary gland secretion are needed for a complete herd udder health picture.
The diagnostic laboratory’s identification of any pathogens present is the apex of
the diagnosis of the existing mastitis in a herd. However, there is always a
percentage of animals that will not respond regardless of all accepted treatments for
prevention or elimination of mastitis infections.

The purpose of establishing a diagnostic laboratory is threefold, a service to
public health, the veterinarian and the farmer. The function of the laboratory must
first be evaluated before the organization and its support can be determined.

A state mastitis control laboratory should be strategically located, and have a
director who has a keen interest in mastitis control and research, with a far-reaching
capacity to closely cooperate with veterinary practitioners. There must always be
an awareness of the development of new and better techniques and an organization
of well-trained, interested personnel who are salaried comparable to their ability.
Only with such an organization is one justified in expecting the maximum of state
financial support.

The commercial laboratory’s location is most important for accessibility of
practicing veterinarians and shipping facilities. The director and staff should have
the same qualifications as a state control laboratory staff. Constant awareness of
new methods and techniques will keep cost of tests nominal.

A laboratory facility for culturing milk samples within a veterinary practice is
valuable. This speedy diagnosis permits the veterinarian to apply more effective
treatment, and apply it earlier. A tow-or-more-man practice can easily support a
secretary-lab technician who would be capable of: identifying common mastitis
pathogens — such as streptococci, staphylococci, coliforms and yeasts, use of
selective media, conducting antibiotic sensitivity tests, and microscopic examina-
tion of questionable cultures. Problem or exotic cultures would be referred to state
or commercial laboratory for extensive study and confirmation.

For the past five years the New York State Veterinary College at Cornell
requires the senior student in mastitis clinic to spend time in the diagnostic control
laboratory. During this clinic time each student is exposed to a complete diagnostic

*Research Technician, New York State Mastitis Control Program.
**Associate Professor, Large Animal Medicine, Obstetrics and Surgery, New York State Veterinary College, Cornell University, Ithaca, New York 14850.

657
study of a herd or herds he has visited and examined. Application of this type of bacteriological culturing to other than just bovine work and all new laboratory techniques and tools are discussed. Hopefully this exposure to the diagnostic laboratory during clinic time will give each future veterinarian valuable mastitis control information that will prove useful in his own or an established practice.

The New York State Mastitis Research and Control Program was established in April, 1946 with a $75,000 grant made by the State Legislature for the study of bovine mastitis. The money was assigned to the Department of Large Animal Medicine and Obstetrics of the New York State Veterinary College. The responsibility of organizing such a program of service and research was assigned to Dr. M. G. Fincher, then Head of Department of Large Animal Medicine. Dr. Harry G. Hodges was appointed Supervising Veterinarian to aid in the development of an organized approach to this long overdue study.

Research herds were initially selected in various areas of the state with the aid of veterinary practitioners and county agents. Progressive herd owners were willing and anxious to cooperate with the program. Thus much valuable information was obtained which aided greatly in setting up a pattern of procedure for future work.

The laboratory at Cornell was and still is the Central Laboratory. As time progressed regional laboratories were established at strategic locations across the state. Today there are six laboratories, each staffed by a full time field veterinarian who is responsible for the operation. One or more field technicians assist him in conducting herd surveys. However, at the Central Laboratory it is the senior veterinary student in mastitis clinic who assists the field veterinarians during field surveys. The milk samples are cultured by one or more laboratory technicians in order to identify those quarters that are shedding mastitis pathogens, and classify the pathogens involved. A secretary completes the individual laboratory team.

The Central Laboratory at Ithaca investigates and institutes procedures concerned with quality control. The staff is made up of a supervising veterinarian, two veterinarians in the field, a research technician, two regular technicians, a secretary-technician and a secretary-stenographer.

The research technician's position was created April, 1963 to standardize techniques in all regional laboratories and assist them with any problems. The duties of this position have developed far beyond the basis of the original work program. Some of the duties today are: organization of two-week training periods with each new technician, semi-annual tours of each regional laboratory to check techniques and discuss problems, supervision of the Central Laboratory, instruction of veterinary students in mastitis clinic, and developing and instituting protocol for investigational studies.

One of the regular technicians handles most of the problem cultures under the supervision of a researcher. This technician is trained to do bio-chemical tests, culturing on selective media, serological examination, phage typing, microscopy and all mastitis screening tests.

The other technician is responsible for all samples from regular herd surveys and milk samples collected by ambulatory clinicians. Her part of the laboratory
teamwork is to culture and identify pathogens present on primary media, confirming any questionable organisms with selective mediums, and microscopic study.

Individual quarter samples comprise the majority of our routine culturing. Composite samples of all four quarters are collected occasionally during a *Streptococcus agalactiae* elimination program, after the initial survey, until a particular herd is free of this infection. Bulk tank samples are routinely collected when milk is available. These samples are cultured on esculin blood agar, and/or thallium chrysal violet (TCV) media. A Modified Whiteside test is conducted and a milk film is prepared for Direct Microscopic Somatic Cell Count. The selective TCV media enables an early identification of all streptococci present in a heavy or mixed culture.

Milk samples are routinely cultured on quadrants or halves of esculin blood agar plates. A 0.01 ml. inoculum of well-mixed sample is plated, using a Breed loop with a two-inch shank. One quadrant of the plate is used for each quarter sample, but when composite or bulk tank samples are cultured one-half of a plate is used. A uniform streaking pattern that does not overlap itself is very essential. (Figure I) The milk should be drawn out on the medium so that discrete isolates are available for identification. Inoculated plates are incubated at 37.0° C. and examined at 24 and 48 hours plus or minus four hours.

Blood agar plates prepared from Difco Extract enriched with 5% selected bovine blood were the primary culture media until December, 1969. At that time, the primary culturing medium was changed to esculin blood agar as a result of a research project conducted jointly with the Animal Science Department and the Veterinary College. The basic design for this project was developed by the mastitis team at the National Institute of Research in Dairying (NIRD), Reading, England. The project at Cornell was conducted simultaneously with that at NIRD using as nearly as possible identical techniques.

**Figure 1**

STREAKING PATTERN
There was a great need in routine diagnostic work for a faster, accurate identification of *S. agalactiae*. A study to determine the advisability of using esculin blood agar for routine culturing was made. The results indicated that this medium was very advantageous for identifying most streptococci without further tests. The results indicated that 90% of the time which had previously been used for setting CAMP Tests, could be saved. Other pathogens also grew well and were easily identified. As a result of this work the decision was made to change the primary medium to esculin blood agar.

This medium enables a technician to identify *S. agalactiae* from non-agalactiae streptococci following 18-24 hours incubation. Identification is based on colony morphology and esculin reaction. Hemolytic staphylococci, corynebacterium, enteric organisms and other bacteria are identified. Cultures are examined with reflected and transmitted light to reveal cultural and morphological characteristics. A trained technician can readily interpret the various types of growth and determine if hemolysis is present. Re-examination of cultures at 48 hours is important to identify any slow growing bacteria and to confirm hemolytic reaction. Any isolates which remain unidentified at 48 hours are subjected to biochemical tests, serological tests, cultured on selective media and examined microscopically.

The microbiological procedures are shown in Figure II.

During the investigational work some studies were made using 5% and 3% blood enrichment in esculin extract agar. The colony morphology was good with both concentrations of blood, however, the bluish color exhibited by colonies *S. agalactiae* was a little more pronounced and the hemolyses more obvious on the media enriched with 3% blood.

The esculin blood agar formula presently used in the New York State Mastitis Laboratories for primary culturing is:

* Extract agar 35.0 grams
* Esculin 1.0 grams
* Distilled H₂O 1,000 ml.

Tempered to 47-40° C. after sterilization. Enriched with 5% selected bovine blood.

Approximately 10 ml. poured per (15x100 mm.) plastic plate.

Blood donor selection is based on ability to support *alpha* and *beta* hemolysis. Blood is acceptable if it will support a 4 to 6 millimeter grey zone of hemolysis around a standard stock *beta* staphylococcus which is used in the CAMP Test. Each bottle of blood is checked before using in routine work.

The cost of culturing the milk from one cow (4 quarters) using single service vials for milk samples and a disposable plastic petrie dish is approximately 26 cents (25.78 cents). Labor makes up three-fifths of this cost. This cost figure does not include salaries for professional and field personnel, sustenance and transportation, or materials used in field work.

* - Difco Extract Agar and Esculin, Difco Laboratories, Detroit, Michigan.
In the New York State Mastitis Control Program all services to farmers were free from 1946 to 1960. At that time a fee of 25 cents per cow was charged and in 1965 the fee increased to 35 cents, in 1969 to 50 cents and today it is 60 cents per cow plus a $5.00 service fee for each farm visit. Support from the State has increased, but not in proportion to the size of the operational budget. In 1969, the New York State Mastitis Control Program budget was $407,000. Our earned income sustained about one-fourth of this amount. Continued rising costs necessitated an increase in the fees and most farmers have not rebelled against such nominal charges.

The average base salary for a laboratory technician in the New York State Mastitis Control Program is $5,800 per annum with five years of increments, to a maximum of $7,200. Fringe benefits include three weeks vacation after one year of service, free hospitalization, generous sick leave, educational leave and free retirement benefits.

Today's changing economic conditions are causing all dairy industries and agencies to consider laboratory relocation and organization. Combination of pathology and diagnostic laboratories, using lay technicians for collecting samples and professional personnel for consultant and advisory work would help control high operational costs.

There is a great need to develop inexpensive practical single service laboratory sampling containers, inexpensive, lightweight, insulated shipping cases and less time-consuming, accurate laboratory tests. Sources of screw top plastic single service, sterile vials are extremely limited when the size, volume and cost are essential factors. Only one supplier manufactures a vial we can use without replacing all of our other equipment such as vial racks and insulated carrying cases. Standardization of sampling containers for diagnostic as well as quality control work would certainly develop a competition among manufacturers and might produce nominally priced single service, sterile, plastic, screw top vials.

Some of the New York State practitioners have found the use of a "Cul-Pak" very advantageous for collecting and sending a milk sample to the mastitis diagnostic laboratory for culture. This sampling method was devised for human throat cultures. It consists of a rectangle 3/4 x 1-1/2 inches of sterile filter paper enclosed in a laminated wrapper within an envelope. The milk sample is collected by carefully opening the wrapper and saturating the filter paper with milk drawn from one or more quarters, carefully draining off excess and refolding the saturated rectangle into the paper covering. The original envelope is labeled and mailed to the laboratory in a stamped envelope.

Data of an investigation indicate that reliable results were obtained in the

---

1 Manufactured by Falcon Plastics, Division of Becton, Dickinson and Company, (B-D), Los Angeles, California.

2 Simple and Efficient Sampling Procedure for Laboratory Culture and Identification. Dr. Lincoln Field, New York State Mastitis Program. New York State Veterinary News, December 1968.
laboratory from milk samples taken on Cul-Pak Kits. The Cul-Pak is probably most useful for identifying *S. agalactiae*. It was hoped that the practicability of this sampling method would be accepted by many practitioners; however, response has been limited probably due to lack of publicity. Further work in this field would certainly be desirable.

Durable, reasonably priced insulated cases are another expensive item. Most well-insulated cases that will maintain cool samples in transit are cumbersome to handle. Cases of lighter construction and insulation are efficient, but not adequate for maintaining cool samples for longer period than 5 or 6 hours.

Barn equipment such as work tables are mostly custom made and usually not very maneuverable in the barn area. Development of a practical, lightweight table, which would be useful in barns, should be encouraged.

The New York State Mastitis Control Program developed a single, comprehensive report form that lists vital information for the practitioner and contains a statistical summary which simplifies the IBM analysis. Changing of the IBM programmed analysis is costly; probably other mechanical analyzers would be less expensive. Simplification of reports and quicker analysis of data would permit the veterinary practitioner to use more effective treatments.

There should be a standardization of mastitis culturing procedures acceptable to all federal, state and commercial laboratories. An effort in this direction has been published by the National Mastitis Council. This would develop reports that would contain more valuable data and information.

If I organized a State Mastitis Central Laboratory, the director would be a graduate D.V.M. with an advanced degree in microbiology. The staff would be made up of well qualified personnel interested in their work, not just high salaried clock watchers. The director should periodically do a job analysis on staff members and recommend merit salary increases for deserving individuals.

A research technician would be his key person for supervision and research studies.

Laboratory technicians would be well qualified. A qualified technician should have a microbiology background and/or at least 2 years experience in a medical or milk quality control laboratory. A good technician should possess initiative, inquisitiveness, ingenuity and intelligence enough to seek advice with problems; I could train a high school graduate for routine work who possessed these qualifications, and has a keen interest in sciences. Experience of milk producing animals and knowledge of general farming broadens the understanding of this type of laboratory work.

New laboratory technicians would be taken on herd surveys periodically to understand how environmental conditions can affect cultural results.

The secretary should also be a dual purpose secretary-technician. This type of person is being graduated today from two year colleges with an associate degree in

---

medical technology.

There would be semi-annual practical workshops for all personnel in all phases of laboratory work. Ample time would be allowed for discussion during workshop for exchange of ideas, techniques and problems.

**SUMMARY**

My final wishful hope is the development of a technician school within our veterinary school such as in European veterinary colleges. A three-year (12-month) curriculum including laboratory specialization and the choice of hiring these graduates for a laboratory contingent, would be ideal for a laboratory director.

**FIGURE II.**

**MICROBIOLOGICAL PROCEDURES**

<table>
<thead>
<tr>
<th>COLONY MORPHOLOGY</th>
<th>PRIMARY CHARACTERISTICS</th>
<th>CONFIRMATORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>STREPTOCOCCI</td>
<td>Esculin</td>
<td>Coagulase</td>
</tr>
<tr>
<td></td>
<td>Splitting</td>
<td>Mannitol</td>
</tr>
<tr>
<td></td>
<td>Color</td>
<td>DNA Agar</td>
</tr>
<tr>
<td>STAPHYLOCOCCI</td>
<td>Hemolysis</td>
<td>Tellurite Glycine Agar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Catalase Test</td>
</tr>
<tr>
<td>COLIFORMS</td>
<td>Odor</td>
<td>Russells Double Sugar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Triple Sugar Iron</td>
</tr>
<tr>
<td>PSEUDOMONADS</td>
<td>Hemolysis</td>
<td>Biochemicals</td>
</tr>
<tr>
<td></td>
<td>Odor</td>
<td></td>
</tr>
<tr>
<td>YEAST</td>
<td>Non-esculin</td>
<td>Microscopic</td>
</tr>
<tr>
<td></td>
<td>Splitting</td>
<td>Sabouraud Agar</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>Hemolysis</td>
<td>Urea Slant</td>
</tr>
<tr>
<td></td>
<td>48-hr. growth</td>
<td>Microscopic</td>
</tr>
<tr>
<td>MicrococcI</td>
<td>Density</td>
<td>Coagulase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mannitol Agar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Catalase</td>
</tr>
</tbody>
</table>
EXAMINATION OF SPECIMENS FROM SUSPECTED HOG CHOLERA CASES BY THE FLUORESCENT ANTIBODY TISSUE SECTION AND CELL CULTURE TECHNIQUES

Ellen J. Henry, B.A., M.S. and H. A. McDaniel, D.V.M., Ph.D.

SUMMARY

A total of 787 swine tissue submissions from 25 states were examined by the fluorescent antibody cell culture technique (FACCT)* and fluorescent antibody tissue section technique (FATST) for hog cholera (HC) between January and December 1969. Tonsil, spleen and lymph node were routinely examined. Tonsil was most often positive on FATST examination. Results of the two techniques were in agreement on 746 submissions (95 percent). Nineteen submissions were positive on FATST and negative on FACCT and 22 submissions were positive on FACCT and negative on FATST. The procedure as published by scientists at the University of Nebraska for conducting FATST was modified by use of thicker tissue sections, alkaline buffer, and dry darkfield examination. False negative findings, due to unsatisfactory equipment or reagents, were eliminated by use of positive control sections cut from infected tissue. False positive findings were eliminated by antiserum inhibition controls.

INTRODUCTION

Typical clinical signs and postmortem lesions are not always found in the HC infections currently observed by veterinarians. Fluorescent antibody tests (FAT) are an important aid in the diagnosis of HC.

Immunofluorescence was first successfully used to detect HC viral antigen in 1963 with the development of the FACCT8, using tissue homogenates from HC suspicious swine to inoculate cell cultures, and the FATST14, employing frozen sections of tissues. Both methods were effective in detecting HC viral antigen in experimentally infected animals1,7,9,12,13. Several workers have compared results of the two FAT on field submission^. Salivary gland was the tissue recommended by Stair as the most consistently positive tissue employing FATST15, but the tonsil was recommended in most recent reports2,9,11,12. In contrast, Torlone16 considered pancreas the organ most suitable for FATST; however, in his studies with experimentally infected pigs, viral antigen was detected in the tonsil three days earlier than in the pancreas. Results of another study indicated HC viral antigen tended to be limited to tonsils, salivary glands, ileal mucosa and kidneys in subacute and chronic HC.4

The purpose of this paper is to compare laboratory findings from 787 field submissions examined by both FAT and describe modifications made in the FATST.

MATERIALS AND METHODS

Submissions — During 1969, veterinarians in 25 states and Puerto Rico submitted tissue samples from 787 hog cholera investigations for cell culture and
tissue section examinations. Tissues for each examination were usually packed separately by the submitting veterinarian, and preserved during shipment by freezing with dry ice. Most submissions consisted of tonsil, spleen and lymph node from one or more pigs.

**Fluorescent Antibody Cell Culture Technique (FACCT)** — This examination was conducted in the virology section of Diagnostic Services, NADL, by a method previously described by Carbrey, et al.³.

**Fluorescent Antibody Tissue Sections Technique (FATST)** — The modifications in the FATST¹,⁴,¹⁵ were: (1) tissue sections were cut to a thickness of 8 microns rather than 4 microns; (2) alkaline carbonate-bicarbonate buffer rather than a neutral phosphate buffer was used; (3) microscopic examination was conducted by using a high-dry objective and a dry darkfield condenser rather than an oil immersion objective and condenser; (4) an antiserum inhibition control test was conducted on each submission.

Triplicate sections of tonsil were processed: 1 test section, 1 normal serum control section, and 1 antiserum inhibition control section. Only test sections were prepared from spleen and lymph node. Each test section was cut 8 microns thick, mounted on a slide and treated as follows: (1) Fixed in acetone for 10 minutes, (2) flooded with conjugate and incubated at 37⁰c in moist environment for 30 minutes, (3) washed vigorously in carbonate-bicarbonate buffered saline, pH 9.0, for 10 minutes, (4) rinsed quickly in distilled water, and (5) a cover slip was mounted over the section with buffered glycerine mounting medium. Each normal serum control section was flooded 1:1 mixture of conjugate and normal porcine serum and each antiserum control section was flooded 1:1 mixture of conjugate and anti-hog-cholera serum. Each time field submissions were processed, a section of tissue from a specific pathogen free pig infected with HC virus was prepared as a positive control.

Tissue sections were examined with a Leitz Ortholux or Othoplan fluorescent antibody microscope equipped with a HOB 200 bulb, BG38 heat filter, BG12 exciter filter, OG01 barrier filter, and a high-dry objective and dry darkfield condenser. Sections were examined microscopically for fluorescing cells at magnifications of x100 or x130. When fluorescence was detected, cellular detail was observed at x250 to determine if the fluorescence was limited to the cytoplasm, the nucleus remained dark, and the infected cell appeared structurally intact. The cells were also viewed microscopically with a tungsten light source to detect nonspecific fluorescence. Usually nonspecifically fluorescing material appeared bright and light refractive when examined with tungsten light. If the fluorescence observed was specific, the positive cells appeared no different than the negative cells.

A field submission was reported as positive for HC when fluorescing cells were present on the test section of one or more tissues of any pig of the submission and fluorescing cells were also present on the normal serum control section but absent or markedly reduced in brilliance on the antiserum control section.

**Conjugate** — The anti-HC conjugate was prepared in the virology section of
Diagnostic Services, NADL, as previously described by Carbrey^6.

RESULTS
Results obtained by FACCT and FATST on 787 submission from 26 states were compared (Table 1). The FATST and FACCT findings were in agreement in 302 positive cases and 444 negative cases. The findings differed in 41 cases. The FATST was positive and FACCT negative in 19 cases, and FATST was negative and FACCT positive in 22 cases. It should be emphasized that this comparison was based on field submissions rather than individual pigs or tissues. The percentage of agreement for FACCT and FATST results was 94.79. A 95 percent confidence interval is 92.74 to 96.83.

Tonsil was confirmed as the tissue of choice for the FATST as it was consistently positive when another tissue from the same pig was positive and was occasionally positive when spleen and/or lymph node were negative.

DISCUSSIONS
A high degree of agreement between FATST and FACCT findings was obtained on submissions from suspected cases of hog cholera (Table 1). Some of the differences may have been due to variations in sampling techniques, such as the veterinarian submitting tissues from one pig for FATST and from another pig for FACCT. The degree of correlation indicated that either procedure would have been adequate for laboratory confirmation of hog cholera.

It was not necessary to use antiserum inhibition controls on all tissues examined because the examination was considered positive if any tissue was positive. Since tonsil was reported to be the most consistently positive tissue, it was selected for inhibition control testing. If fluorescing cells had been absent in tonsil and present in other tissues, antiserum controls would have been conducted and interpreted as in the tonsil.

The antiserum control system was based on the one-step blocking technique described by Moody^10. The preparation and examination of control sections required very little time and made it easier to interpret the results.

TABLE 1
Comparison of FATST and FACCT on 787 field submissions examined during 1969.

<table>
<thead>
<tr>
<th>Cases with FATST and FACCT Findings in Agreement</th>
<th>746</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>302</td>
</tr>
<tr>
<td>Negative</td>
<td>444</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cases with FATST and FACCT Findings in Disagreement</th>
<th>41</th>
</tr>
</thead>
<tbody>
<tr>
<td>FATST - Pos., FACCT - Neg.</td>
<td>19</td>
</tr>
<tr>
<td>FATST - Neg., FACCT - Pos.</td>
<td>22</td>
</tr>
</tbody>
</table>
REFERENCES


6. Diagnostic Services, National Animal Disease Laboratory, Coverslip Tissue Culture, Fluorescent Antibody Technique for the Detection of Hog Cholera Virus (Mimeograph).


The clinical and pathological features of an isoimmunohemolytic disease in calves in Texas herds were recently described by Dennis, et al. The present report concerns a similar disease in a beef herd in Screven County, Georgia.

Clinical History
The death of newborn calves in a crossbred beef herd of 210 cows and 7 bulls was investigated. These deaths totaled 29 in 1968 and 39 in 1969. They amounted to about 20 per cent of the calves born each year. Calves sired by each of the 7 herd bulls were affected. Charolais, Angus and Hereford breeds were represented.

The calves appeared normal at birth, but died 1 to 5 days later. The calves became weak and unable to nurse before death. Rapid, shallow breathing was also observed.

Laboratory Examinations
Necropsy findings in calves which lived 1 or 2 days were pulmonary edema and hydrothorax. Calves which lived longer also had anemia, icterus, hepatic congestion, hemoglobinuria, and splenomegaly. Microbiologic and histopathologic findings were unremarkable. Tests on serum from dams whose calves died demonstrated no antibodies against *Leptospira* species.

Serum and erythrocytes were collected from bulls, cows and calves in the herd and tested for evidence of an immunohemolytic disease using hemagglutination and direct Coombs technics. Results for 2 affected calves, numbers 53A and 37A, are given in Table 1. Antibodies in the dam's serum against erythrocytes of both the calf and sire were demonstrated. Serum from a rabbit immunized against bovine gamma globulin also agglutinated calf erythrocytes. Results of hemagglutination tests using serum from 8 cows and the 7 bulls are presented in Table 2. The latter tests were done to determine if matings which would avoid affected calves could be predicted. Cows would be bred only by bulls for which tests demonstrated nonagglutination of erythrocytes.

Discussion
The natural occurrence of isoemolotylic diseases is well-known in man and horses but not in cattle. The disease has also been observed in swine following vaccination with blood-origin vaccines. Such a vaccine for protection against
anaplasmosis had been used in the affected herd for approximately 4 years. This vaccine is prepared from inactivated and dessicated blood from cattle infected with anaplasmosis. Affected calves came from dams which were recently vaccinated as well as those having been vaccinated annually for 1 to 4 years. It was theorized that, similar to the disease in swine vaccinated with an inactivated blood vaccine, these cows were immunized against blood types inherited by their calves. A placental barrier protected the calf from these antibodies during gestation. After birth, the calf absorbed these antibodies from the colostrum and developed the hemolytic disease.

Reports of a similar clinical disease in two other Georgia herds which had been vaccinated against anaplasmosis have been received. About 20 per cent of the calves were affected and died.

Acknowledgements

This is to acknowledge Dr. W. H. Gibson, Sylvania, Georgia for his clinical investigation of the case, and Mrs. Merrill Lago, Medical Technologist, Department of Pathology and Parasitology, College of Veterinary Medicine, University of Georgia, for her participation in the laboratory immunologic tests.
Table 1. Results of Immunologic Tests on Affected Calves, 53A and 37A.

<table>
<thead>
<tr>
<th>Test</th>
<th>Red Blood Cells</th>
<th>Rabbit Serum&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Calf Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Coombs</td>
<td>Calf</td>
<td>Yes</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>. . .</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test</th>
<th>Serum</th>
<th>Red Blood Cells</th>
<th>Rabbit Serum&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Calf Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-Match</td>
<td>Calf</td>
<td>Dam</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Calf</td>
<td>Sire</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dam</td>
<td>Calf</td>
<td>No</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dam</td>
<td>Sire</td>
<td>No</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Sire</td>
<td>Calf</td>
<td>No</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>A</sup>Antibovine gamma globulin
TABLE 2. Results of Hamagglutination Tests Using Serum from 8 Cows and Erythrocytes from the Herd Bulls.

<table>
<thead>
<tr>
<th>Cow no.</th>
<th>Bull Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>4+</td>
</tr>
<tr>
<td>37</td>
<td>3+</td>
</tr>
<tr>
<td>81</td>
<td>4+</td>
</tr>
<tr>
<td>91</td>
<td>4+</td>
</tr>
<tr>
<td>55</td>
<td>-</td>
</tr>
<tr>
<td>36</td>
<td>-</td>
</tr>
<tr>
<td>95</td>
<td>-</td>
</tr>
<tr>
<td>63</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: Breeding records indicate calves from Bull 1 and Cows 53 and 37 were affected and died; a calf from Bull 2 and Cow 81 survived; a calf from either Bull 3 or 4 and Cow 91 survived; calves from Bull 6 and Cows 55, 36 and 63 survived; and, a calf from either Bull 4 or 5 and Cow 95 survived.
REFERENCES

75th ANNUAL MEETING
October 24-29, 1971
SKIRVIN HOTEL
Oklahoma City, Oklahoma

76th ANNUAL MEETING
November 5-10, 1972
AMERICANA OF BAL HARBOUR
Miami Beach, Florida

77th ANNUAL MEETING
October 6-12, 1973
RADISSON HOTEL
Wichita, Kansas