PROCEEDINGS

SEVENTY-EIGHTH

ANNUAL MEETING

of the

UNITED STATES ANIMAL HEALTH ASSOCIATION

and

SEVENTEENTH ANNUAL CONFERENCE OF AMERICAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS

HOTEL ROANOKE
Roanoke, Virginia
October 13, 14, 15, 16, 17, 18, 1974
PROCEEDINGS

SEVENTY-EIGHTH
ANNUAL MEETING

of the
UNITED STATES
ANIMAL HEALTH
ASSOCIATION

Hotel Roanoke
Roanoke, Virginia

October 13, 14, 15, 16, 17, 18, 1974
CONTENTS

Page

Report of the Committee on Ad Hoc Epizootic Attack Plans —
L. E. Bartelt, et al .................................................. 1
The Minnesota Disease Reporting System for Food Producing Animals —
S. L. Diesch, et al .................................................. 3
Report of the Committee on Meat and Poultry Hygiene — E. D. Baker,
et al .................................................. 28
Report of the Committee on State—Federal Relations — J. F. Andrews,
et al .................................................. 31
Report of the Committee on Livestock Commerce — J. F. Hudelson,
et al .................................................. 34
Report of the Committee on Rabies — R. Keith Sikes, et al ............ 41
Report of the Committee on Import—Export — C. L. Campbell, et al ... 45
Communications Problems — S. B. Guss ................................ 47
Report of the Committee on Professional Relations — R. C. Hammond,
et al .................................................. 51
Report of the Committee on Animal Welfare — J. C. MacFarlane, et al .. 52
Report of the Committee on Parasitic Diseases and Parasiticides —
J. L. Hourigan, et al .................................................. 54
Report of the Committee on Evaluation and Development of State—
Federal Programs — J. L. O'Harra, et al ................................ 56
Report of the Committee on Public Health and Environmental Quality —
R. L. Parker, et al .................................................. 58
Report of the Committee on Nominations, Resolutions and Internal
Affairs — W. C. Tobin, et al .................................................. xxxvii

BIOLOGICS

Host Animal Efficacy and Laboratory Potency Tests for Non Specific
Bacterins, Mixed Bacterins, and Bacterial Antiserums — M. H. Bairey
and N. K. Jungk .......................................................... 60

CATTLE

Infectious Diseases
Lead Toxicosis in Cattle — C. M. Hibbs ................................ 72
Report of the Committee on Infectious Diseases of Cattle — N. B. Swanson,
et al .................................................. 75

Anaplasmosis
Culture of Anaplasma marginale in Lymph Node Cells — R. J. Hidalgo .. 77

Brucellosis
45/20 Vaccination in a Brucella Infected Herd — J. B. Hendricks and
W. C. Ray .......................................................... 88
Progress of the State—Federal Brucellosis Eradication — G. J. Fichtner ... 104
Award Given to Dr. Carroll K. Mingle — J. H. Steele ......................... 114
Leptospirosis
Isolation of Serotype Szwajizak from Dairy Cattle in Oregon — 119
J. W. Glosser, et al
The Control of Outbreaks of Leptospirosis in Beef Cattle by Simultaneous
Vaccination and Treatment with Dihydrostreptomycin — P. J. South
and H. G. Stoenner 126
Host Animal Efficacy Studies Using a Multivalent Leptospira Bacterin —
H. L. Strother 131
Report of the Committee on Leptospirosis — L. E. Hanson, et al 136

Mastitis
Mobilizing Extension for Mastitis Control — R. P. Natzke 140
The Role of Delayed-Type Hypersensitivity and Cell-Mediated Immunity
in Bovine Staphylococcal Mastitis — R. L. Jones 143

LIVESTOCK IDENTIFICATION
The Impact of Electronic Identification and Disease Detection on the Roll
of the Veterinarian — J. C. Hensley, et al 152

FOREIGN ANIMAL DISEASES
Foot-and-Mouth Diseases in White-tailed Deer; Clinical Signs and Trans-
mission in the Laboratory — J. W. McVicar, et al 169
Application of Immuno-Peroxidase Techniques for the Detection of Foot-
and-Mouth Disease Virus Antigens — P. Sutmoller and K. M. Cowan 181
Clinical and Serological Response of the Nine-Banded Armadillo (Dasypus
novemcinctus) to Viruses of African Swine Fever, Hog Cholera,
Rinderpest, Vesicular Exanthema of Swine, Vesicular Stomatitis
and Foot-and-Mouth Disease — F. W. Wilder, et al 188
Susceptibility of One-Toes Pigs to Certain Diseases Exotic to the United
States — F. W. Wilder, et al 195
Thermal Inactivation Data for Swine Vesicular Disease Virus — H. R. Cun-
liffe 200
Antigenic Variation of Venezuela Strains of Foot and Mouth Disease
Viruses — G. Gomez, et al 205
Swine Vesicular Disease: Virus Survival in Pork Products — P. D. McKercher,
et al 213-a
Report of the Committee on Foreign Animal Diseases — T. G. Murnane,
et al 214
Research on Vesicular Diseases (Bibliography Compiled by R. J. Uskavitch) 228-a

DISEASES OF HORSES
Florida’s Program Against E. I. A. — C. L. Campbell 229
Report of the Committee on Infectious Diseases of Horses — R. C. Knowles,et al 244
PHARMACEUTICALS

Proper Drug Usage and the Public Health — R. E. McKinley .................. 250
Antibacterial Drugs in Food Animals — Their Environmental Impact —
I. A. Solomons ............................................................................. 257

DISEASES OF POULTRY

Detection of Newcastle Disease Virus by Complement-fixation — W. K.
Butterfield and J. H. Graves ......................................................... 271
Report of the Subcommittee on Avian Influenza to the Committee on
Transmissible Diseases of Poultry — W. K. Butterfield ..................... 278
Report of the Committee on Transmissible Diseases of Poultry — R. A.
Bamkowski, et al ........................................................................ 283

SALMONELLOSIS

Salmonellosis — An Environmental Problem Affecting Animals and Man —
E. V. Morse and Margo A. Duncan ............................................ 288
Report on the Dillon Beach Project Cooperative Agreement on Specific
Pathogen Free Turkeys — J. W. Walker, et al ................................. 300

SWINE

Transmission of Hog Cholera Virus by Flies — L. D. Miller, et al ............ 324
Status of the State-Federal Hog Cholera Eradication Program — R. E.
Thompson .................................................................................. 331
Report of the Committee on the Nationwide Eradication of Hog Cholera —
D. L. Smith, et al ..................................................................... 336
Congential Tremors in Swine — D. P. Gustafson, et al .......................... 338
Pseudorabies in Indiana: Current Status, Laboratory Confirmation, and
Epizootiologic Considerations — C. L. Kanitz, et al ........................ 346
Recovery and Characterization of a Coronavirus from Military Dogs with
Diarrhea — L. N. Binn, et al ......................................................... 359
Report of the Committee on Transmissible Diseases of Swine — E. A.
Butler, et al ............................................................................. 367

SHEEP AND GOATS

Current Studies on Hydatid Disease in Utah — F. L. Andersen, et al ....... 370
Ovine Foot Rot: A Review — J. A. Schmitz and J. L. Gradin .................. 385
Report of the Committee on Diseases of Sheep and Goats — F. J. Schoen-
feld, et al ............................................................................. 393
# TUBERCULOSIS

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Status of the State—Federal Tuberculosis Eradication Program —</td>
<td>404</td>
</tr>
<tr>
<td>R. W. Bennett</td>
<td></td>
</tr>
<tr>
<td>Report of the Committee on Tuberculosis and Johne's Disease — P. L.</td>
<td>415</td>
</tr>
<tr>
<td>Smith, <em>et al</em></td>
<td></td>
</tr>
</tbody>
</table>

# ANIMAL VIRUS CHARACTERIZATION

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Papovavirus Group — S. McConnell</td>
<td>420</td>
</tr>
<tr>
<td>U.S.A.H.A. Committee on Animal Virus Characterization — F. A. Murphy</td>
<td>423</td>
</tr>
</tbody>
</table>
OFFICERS AND COMMITTEES FOR 1975

PRESIDENT
J. F. Andrews .................................................... Atlanta, Georgia

PRESIDENT ELECT
H. E. Goldstein .................................................... Columbus, Ohio

FIRST VICE-PRESIDENT
A. E. Janawicz .................................................... Montpelier, Vermont

SECOND VICE-PRESIDENT
L. E. Bartelt .................................................... Sacramento, California

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

TREASURER
J. C. Shook ..................................................... Mechanicsburg, Pennsylvania

COMMITTEE
Committee on Ad Hoc Epizootic Attack Plans—1975
L. E. Bartelt, Chairman, Sacramento, Calif.
R. Bankowski, Davis, Calif.
W. K. Butterfield, Southold, N. Y.
C. L. Campbell, Tallahassee, Fla.
Joe Finley, Encinal, Texas
H. E. Goldstein, Columbus, Ohio
R. P. Hanson, Madison, Wis.

Committee on Animal Virus Characterization—1975
S. McConnell, Chairman, College Station, Tex.
C. J. York, Co-Chairman, LaJolla, Calif.
R. A. Bankowski, Davis, Calif.
E. H. Bohl, Wooster, Ohio
V. J. Cabasso, Berkeley, Calif.
E. A. Carbrey, Ames, Iowa
R. A. Crandell, Urbana, Ill.
J. H. Gillespie, Ithaca, N. Y.
A. S. Greig, Hull, Que, Canada
L. E. Hanson, Urbana, Ill.

Committee on Animal Welfare—1975
A. E. Docoteau, Chairman, Waltham, Mass.
J. C. Macfarlane, Co-Chairman, Pembroke, Mass.
H. P. Schneider, Philadelphia, Pa.
Christine Stevens, Washington, D.C.
A. F. Moreland, Gainesville, Fla.
Oscar Clabaugh, Hyattsville, Md.
L. A. Griner, San Diego, Calif.
L. F. Jennings, Greenport, L.I.N.Y.

John Smiley, Augusta, Maine
Committee on Anaplasmosis—1975

B. W. Hawkins, Chairman, Ontario, Ore.
W. E. Brock, Co-Chairman, Stillwater, Okla.

F. C. Neal, Gainesville, Fla.
J. A. Howarth, Davis, Calif.
T. O. Roby, Clarksville, Md.
M. Ristic, Urbana, Ill.
D. G. Luther, Plaquemine, La.
J. L. O'Harr, Reno, Nev.
F. E. Henderson, Baton Rouge, La.
J. C. Trace, Ft. Dodge, Iowa
Dryfus Froe, Terre Haute, Ind.
Bob Laramore, Gillette, Wyo.
J. Lee Alley, Auburn, Ala.
R. F. Hall, Caldwell, Idaho
A. A. Cuthbertson, Elko, Nev.
R. J. Hidalgo, College Station, Tex.
Harvey McCrory, Jackson, Miss.
B. R. McCallon, Adelphi, Md.
Clayton Paddock, Harrison, Ark.
Doyle McAdams, Jr., Huntsville, Tex.
E. E. Kerr, San Francisco, Calif.
G. I. Roberts, R. T. Park, N.C.
Floyd Frank, Moscow, Idaho
Robert Gadd, Highmore, S.D.
Bill Gallagher, Highmore, S.D.
T. B. Ryan, Cary, N.C.
Joe Pearce, Jr., Okeechobee, Fla.
K. J. Peterson, Coral City, Fla.
Bruce Walker, Jacksonville, Tex.
Jamie White, Beaumont, Tex.
Charles York, LaJolla, Calif.

Committee on Biologics—1975

R. E. Hall, Chairman, Caldwell, Idaho
J. W. Glosser, Co-Chairman, Helena, Mont.

R. B. Bushnell, Davis, Calif.
W. L. Anderson, Dallas, Tex.
J. Ralph Bishop, Tipton, Ind.
Tom Cook, Denver, Colo.
H. R. Cunliffe, Southold, N.Y.
E. L. Drake, Reno, Nev.
D. A. Fuller, Indianola, Iowa
M. T. Goff, Ames, Iowa
Peter Langer, Ottawa, Ont., Can.
Lavon Koger, Pullman, Wash.

B. B. Hancock, Fort Dodge, Iowa
L. E. Hanson, Urbana, Ill.
N. Bruce Haynes, Ithaca, N.Y.
Majon Huff, Denver, Colo.
G. V. Peacock, Hyattsville, Md.
M. L. Sutton, St. Joseph, Mo.
Skip Thayer, Ontario, Ore.
I. M. Paton, Overland Park, Kan.
Alex Canales, Jacksonville, Ill.
J. F. Frank, Ottawa, Ont., Can.

James Jackson, Oakland, Calif.

Committee on Brucellosis

Bob Laramore, Chairman, Gillette, Wyo.
G. J. Fichtner, Hyattsville, Md.

D. E. Flagg, Bismarch, N.D.
Billy L. Deyoe, Ames, Iowa
Jack Dahl, Cackle, N.D.
W. D. Knox, Ft. Atkinson, Wis.
Robert Gadd, Highmore, S.D.
Fred Phillips, Keating, Ore.
Cornelius McIntre, Sewell, N.J.
Joe Finley, Austin, Tex.

Larry Schaffer, O'Neill, Neb.
Clint Booth, Dallas, Tex.
Skip Thayer, Ontario, Ore.
Don Brothers, Paducah, Tex.
A. A. Erdmann, Madison, Wis.
A. E. Lewis, Ottawa, Ont., Can.
A. J. Roth, Richmond, Va.
W. C. Tobin, Denver, Colo.
John Holcombe, Okla. City, Okla.
F. W. Haenel, Alabany, N.Y.

J. Ralph Bishop, Tipton, Ind.
L. E. Bartelt, Sacramento, Calif.
A. E. Janawicz, Montpelier, Vt.
Forrest Lee, Brownlee, Neb.
J. O. Pearce, Jr., Okeechobee, Fla.
Harvey F. McCrory, Jackson, Miss.
J. D. Branscome, Grenada, Miss.
Bill Gallagher, Stephan, S.D.
Clayton Paddock, Harrison, Ark.
A. Clyde Wilson, Cedar Rapids, Iowa
E. A. Schif, Hyattsville, Md.
T. A. Kincaid, Jr., San Antonio, Tex.
W. C. Ray, Beltsville, Md.
P. B. Doby, Springfield, Ill.
G. C. Halver, Helena, Mont.
Paul Zillman, Hinsdale, Ill.
Floyd Jones, Houston, Tex.
B. W. Hawkins, Ontario, Ore.

Neal Black, St. Paul, Minn.
Committee on Infectious Diseases of Cattle—1975

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

E. A. Butler, Des Moines, Iowa         F. W. Hansen, Jr., Springfield, Va.
George Lambert, Ames, Iowa             Charles Koontz, Austin, Tex.
Rolland McClymont, Holdrege, Neb.      R. C. Searl, N. Fort Dodge, Iowa
Paul Spencer, Springfield, Ill.        N. R. Swanson, Cheyenne, Wyo.
J. L. Wilbur, Austin, Tex.             T. H. Woods, Jefferson City, Mo.
T. F. Zweigart, Raleigh, N.C.

Committee on Evaluation and Development of State-Federal Programs

J. L. O'Harra, Chairman, Reno, Nev.
J. G. Milligan, Co-Chairman, Montgomery, Ala.

W. L. Bendix, Richmond, Va.            J. R. Bishop, Tipton, Ind.
Tom Cook, Denver, Colo.                D. E. Flagg, Bismarck, N.D.
H. E. Goldstein, Columbus, Ohio        J. L. Hourigan, Hyattsville, Md.
E. A. Janawicz, Montpelier, Vt.        T. A. Ladson, Annapolis, Md.
E. A. Schilf, Hyattsville, Md.         H. Q. Sibley, Austin, Tex.
Paul Zillman, Hinsdale, Ill.

Committee on Food Animal Hygiene & Inspection

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

E. Baker, Cross Plains, Wis.            James A. Bell, Raleigh, N.C.
D. C. Kelley, Manhattan, Kan.           R. J. Lee, College Park, Md.
L. J. Rafoth, Alameda, Calif.           Lyle Scott, Denver, Colo.
M. A. Simmons, Denton, Tex.
Committee on Foreign Animal Diseases—1975

Col. T. G. Murnane, Chairman, Washington, D.C.
H. Q. Sibley, Co-Chairman, Austin, Texas

A. H. Dardiri, Southold, N.Y.
L. A. Griner, San Diego, Calif.
E. C. Sharman, Bowie, Md.
F. D. Maurer, College Station, Tex.
S. McConnell, College Station, Tex.
R. E. Omohundro, Hyattsville, Md.
J. H. Graves, Greenport, N.Y.
R. J. Yedloutschnig, Southold, N.Y.
J. L. Hyde, Southold, N.Y.
Keith Sharman, Ames, Iowa
G. B. Rea, Salem, Ore.
Larry Schaffer, O'Neill, Neb.
John McVicar, Greenport, N.Y.
J. F. Frank, Hull, Que. Canada
Joe Finley, Jr., Encinal, Tex.
Fritz Gluckstein, Bethesda, Md.

Tom Cook, Denver, Colo.
P. D. McKercher, Southold, N.Y.
R. O. Spertzel, Frederick, Md.
C. J. Mare', Ames, Iowa
J. J. Callis, Southold, N.Y.
E. W. Jenney, Ames, Iowa
A. Gonzales Origel, Mexico, D.F.

Committee on Infectious Diseases of Horses—1975

C. L. Campbell, Chairman, Tallahassee, Fla.
W. O. Kester, Co-Chairman, Golden Colo.

J. Castaneda G., Maracay, Venezuela
W. L. Anderson, Dallas, Tex.
C. B. Dearborn, Jr., Concord, N. Hampshire
Charles S. Duncan, Albany, N.Y.
Jack M. Gaskin, Gainesville, Fla.
J. B. Healy, Sacramento, Calif.
C. K. Jewell, Trenton, N.J.
H. C. King, Hyattsville, Md.
R. C. Knowles, Hyattsville, Md.
T. S. Maddox, Frankfort, Ky.
Sidney R. Nusbaum, Ithaca, N.Y.
J. A. Smiley, Augusta, Maine

W. B. Smith, Saipan, Marina Islands
John Briggs, Austin, Texas
Paul B. Doby, Springfield, Ill.
Dr. R. Keith Farrell, Pullman, Wash.
E. F. Hackett, Columbus, Ohio
E. H. Honnen, Denver, Colo.
Floyd Jones, Austin, Tex.
Wayne Kirkham, Lafayette, Ind.
Mrs. Rosalie MacWilliam, Tyron, N.C.
Fred D. Maurer, College Station, Tex.
W. W. Powell, Tallahassee, Fla.
M. E. Teigland, Miami, Fla.

Charles D. Vail, Littleton, Colo.

Committee on Import-Export—1975

Glenn B. Rea, Chairman, Salem, Oregon
C. K. Jewell, Co-Chairman, Trenton, New Jersey

Clint Booth, Dallas, Texas
R. W. Gerdin, Lincoln, Neb.
Bert Hawkins, Ontario, Ore.
D. E. Herrick, Bowie, Md.
M. B. Hynes, Dublin, Ireland
J. R. Langridge, Greenbelt, Md.
Clarence H. Pals, Washington, D.C.
D. H. Spangler, Lacey, Wash.
Pierre A. Chalaux, Columbia, Md.
J. M. Tufts, St. Louis, Mo.

R. L. Evinger, Salem, Oregon
Frank Harding, Geneva, Ill.
F. E. Henderson, Baton Rouge, La.
J. L. Hyde, Southold, N.Y.
T. A. Kincaid, San Antonio, Tex.
Claude A. Smith, Hyattsville, Md.
H. M. Steinmetz, Washington, D.C.
J. E. Thomas, Roseville, Minn.
R. H. Rumler, Brattleboro, Vt.
B. C. Swindle, Hyattsville, Md.
A. R. Miller, Falls Church, Va.
Committee on Leptospirosis—1975

H. G. Stoenner, Chairman, Hamilton, Mont.
J. W. Glosser, Co-Chairman, Helena, Mont.

H. Stewart Powell, Nashville, Tenn.
S. L. Diesch, St. Paul, Minn.
W. E. Lyle, Madison, Wis.
L. A. Rosner, Jefferson City, Mo.
G. B. Smith, Kansas City, Mo.
R. L. Larson, Frutdale, S.D.
O. H. Stalheim, Ames, Iowa
Larry Schaffer, O'Neill, Neb.

H. C. Ellinghausen, Ames, Iowa
R. L. Morter, Lafayette, Ind.
Charles S. Roberts, Auburn, Ala.
R. E. Smith, Amherst, Mass.
H. R. Smith, Cincinnati, Ohio
Lewis P. Thomas, Charleston, W. Va.
Bruce Walker, Jacksonville, Texas

Committee on Livestock Commerce—1975

John F. Hudelson, Chairman, Denver, Colorado
L. N. Butler, Co-Chairman, Phoenix, Ariz.

Lee Abraham, Memphis, Tenn.
John C. Augustine, Brighton, Colo.
L. G. Berg, Hyattsville, Md.
G. R. Gurss, Topeka, Kan.
M. D. Mitchell, Pierre, S.D.
Raymond Schnell, Dickinson, N.D.
D. L. Smith, Rushville, Ind.
Paul Spencer, Springfield, Ill.
Mark Trask, Elm Springs, S.D.
O. J. Barron, Spur, Tex.
Ingvard Svarre, Sidney, Mont.
W. W. Bird, Miami, Fla.
Allen Christiansen, Omaha, Neb.
David Daniels, Kansas City, Mo.
Forrest Lee, Brownlee, Neb.
Andrew Sabensten, Madison, Wis.
F. J. Schoenfeld, Salt Lake City, Utah
T. B. Snodgrass, Dallas, Tex.
Mickey Stewart, Alliance, Neb.
Harry L. Williams, Springfield, Va.
Stanley Schoelman, Spencer, Iowa
Y. Spence, Okla. City, Okla.

Committee on Livestock Identification—1975

S. H. Flora, Chairman, Brownsville, Texas
J. Ralph Bishop, Chairman, Tipton, Indiana

L. R. Barnes, Kingston, Jamaica
Bernard Ebbing, Waterloo, Iowa
Norman Powers, Lake Luzerne, N.Y.
Elmer Haas, Newport, Ky.
Tom Cook, Denver, Colo.
Jerry Houck, Ft. Pierce, S. Dak.
Mark Trask, Wasta, S. Dak.
Raymond Schnell, Dickinson, N.Dak.
Norman R. Swanson, Cheyenne, Wyo.
Keith Farrell, Pullman, Wash.
J. H. Baldwin, Greene, N.Y.
Floyd Jones, Austin, Tex.
R. H. Henderson, Austin, Tex.
Dr. J. B. Young, Austin, Tex.
J. B. Herrick, Ames, Iowa
G. R. Snyder, Washington, D.C.
Richard L. Evinger, Salem, Ore.
Bill Gallagher, Stephan, S. Dak.
E. F. Sterner, Denver, Colo.
Don Brothers, Paducah, Tex.
E. C. Roukema, Hyattsville, Md.
Cliff Crago, Belle Fourche, S.Dak.
Charles S. Duncan, Albany, N.Y.
J. R. Taylor, Austin, Tex.
Harold Minderman, W. Des Moines, Iowa
J. Coleman Hensley, Los Alamos, N.Mex.
Bill Jones, Omaha, Neb.
### Committee on Mastitis—1975

L. F. Williams, Chairman, Storrs, Conn.
R. B. Bushnell, Co-Chairman, Davis, Calif.

<table>
<thead>
<tr>
<th>Member</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. R. McCallon</td>
<td>Adelphi, Md.</td>
</tr>
<tr>
<td>Rufus Weidner</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td>Douglas N. Stern</td>
<td>Amherst, Mass.</td>
</tr>
<tr>
<td>C. A. Kirkbride</td>
<td>Brookings, S. Dak.</td>
</tr>
<tr>
<td>E. L. Henkel</td>
<td>Silverton, Ore.</td>
</tr>
<tr>
<td>N. B. Haynes</td>
<td>Ithaca, N.Y.</td>
</tr>
<tr>
<td>K. J. Peterson</td>
<td>Corvallis, Ore.</td>
</tr>
<tr>
<td>J. S. McDonald</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>K. M. Weinland</td>
<td>Lafayette, Ind.</td>
</tr>
<tr>
<td>J. D. Kornder</td>
<td>Atlanta, Ga.</td>
</tr>
<tr>
<td>Nelson Philpot</td>
<td>Baton Rouge, La.</td>
</tr>
<tr>
<td>Donald Postle</td>
<td>Ithaca, N.Y.</td>
</tr>
<tr>
<td>A. R. Smith</td>
<td>Madison, Wis.</td>
</tr>
</tbody>
</table>

### Committee on Nationwide Eradication of Hog Cholera—1975

D. L. Smith, Chairman, Rushville, Ind.
J. B. Taylor, Co-Chairman, Montgomery, Ala.

<table>
<thead>
<tr>
<th>Member</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>W. L. Bendix</td>
<td>Richmond, Va.</td>
</tr>
<tr>
<td>P. B. Doby</td>
<td>Springfield, Ill.</td>
</tr>
<tr>
<td>H. Q. Sibley</td>
<td>Austin, Tex.</td>
</tr>
<tr>
<td>John Villari</td>
<td>Wemonsh, N.J.</td>
</tr>
<tr>
<td>L. D. Mark</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>R. S. Pyles</td>
<td>Wichita, Kan.</td>
</tr>
<tr>
<td>I. C. Pan</td>
<td>Greenport, L.I., N.Y.</td>
</tr>
<tr>
<td>Ron Thompson</td>
<td>Hyattsville, Md.</td>
</tr>
<tr>
<td>E. A. Butler</td>
<td>Des Moines, Iowa</td>
</tr>
<tr>
<td>J. M. Garner</td>
<td>Des Moines, Iowa</td>
</tr>
<tr>
<td>M. J. Tillery</td>
<td>Hyattsville, Md.</td>
</tr>
<tr>
<td>R. E. Hall</td>
<td>Madison, Wis.</td>
</tr>
<tr>
<td>L. N. Miller</td>
<td>Okla. City, Okla.</td>
</tr>
<tr>
<td>Don Brothers</td>
<td>Paducah, Tex.</td>
</tr>
<tr>
<td>Joe Hayden</td>
<td>Cedar Rapids, Iowa</td>
</tr>
<tr>
<td>R. A. Hendershott</td>
<td>Trenton, N.J.</td>
</tr>
</tbody>
</table>

### Committee on Nominations, Resolutions and Internal Affairs—1975

O. H. Timm, Chairman, Dixon, California

<table>
<thead>
<tr>
<th>Member</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>W. C. Tobin</td>
<td>Denver, Colo.</td>
</tr>
<tr>
<td>M. D. Mitchell</td>
<td>Pierre, S. Dak.</td>
</tr>
<tr>
<td>J. C. Shook</td>
<td>Mechanicsburg, Pa.</td>
</tr>
<tr>
<td>C. L. Campbell</td>
<td>Tallahassee, Fla.</td>
</tr>
</tbody>
</table>

### Committee on Parasitic Diseases and Parasiticides—1975

J. L. Hourigan, Chairman, Hyattsville, Md.
R. L. Pyles, Co-Chairman, Albuquerque, New Mexico

<table>
<thead>
<tr>
<th>Member</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. W. Baker</td>
<td>Albuquerque, New Mex.</td>
</tr>
<tr>
<td>A. C. Newman</td>
<td>Opelika, Ala.</td>
</tr>
<tr>
<td>D. E. Zinter</td>
<td>Bowie, Md.</td>
</tr>
<tr>
<td>W. C. Tobin</td>
<td>Denver, Colo.</td>
</tr>
<tr>
<td>John Holcombe</td>
<td>Okla. City, Okla.</td>
</tr>
<tr>
<td>R. H. Singer</td>
<td>Lexington, Ky.</td>
</tr>
<tr>
<td>Glen O. Schubert</td>
<td>Hyattsville, Md.</td>
</tr>
<tr>
<td>S. J. Couger</td>
<td>Austin, Tex.</td>
</tr>
<tr>
<td>Irvin Elliott</td>
<td>Littleton, Colo.</td>
</tr>
<tr>
<td>H. R. Smith</td>
<td>Cincinnati, Ohio</td>
</tr>
<tr>
<td>I. H. Roberts</td>
<td>Albuquerque, N. Nex.</td>
</tr>
<tr>
<td>John Poole</td>
<td>Beltsville, Md.</td>
</tr>
<tr>
<td>J. C. Augustine</td>
<td>Brighton, Colo.</td>
</tr>
<tr>
<td>Frank Hamilton</td>
<td>Austin, Tex.</td>
</tr>
<tr>
<td>R. K. Strickland</td>
<td>Beltsville, Md.</td>
</tr>
<tr>
<td>J. H. Womack</td>
<td>Hyattsville, Md.</td>
</tr>
<tr>
<td>J. B. Young</td>
<td>Austin, Tex.</td>
</tr>
<tr>
<td>W. W. Utterback</td>
<td>Austin, Tex.</td>
</tr>
</tbody>
</table>
Committee on Pharmaceuticals and Toxicology—1975.

R. A. Gessert, Chairman, East Orange, N.J.
Fred Kingma, Co-Chairman, Washington, D.C.

D. J. Anderson, Ft. Worth, Tex.
W. B. Buck, Ames, Iowa
E. E. Delinger, Harrisburg, Pa.
James Fox, Ashland, Ohio
H. E. Gouge, St. Joseph, Mo.
William Knapp, Rockville, Md.
G. A. Mitchell, St. Louis, Mo.
W. A. Rader, Washington, D.C.
S. F. Scheidy, Bryn Mawr, Pa.
C. C. Beck, Manchester, Mich.
W. C. Burnett, Kansas City, Mo.
G. T. Edds, Gainesville, Fla.
R. Fogleman, Princeton, N.J.
Arthur Freeman, Chicago, Ill.
James Hanson, St. Paul, Minn.
G. D. Lindsey, Carmel, Inc.
Fred Oehme, Manhattan, Kan.
Jane Robens, Rockville, Md.
Norman Tufts, Boston, Mass.

Clyde Wilson, Cedar Rapids, Iowa

Committee on Professional Relations—1975

John B. Herrick, Chairman, Ames, Iowa
N. B. Haynes, Co-Chairman, Ithaca, N.Y.

C. N. Dobbins, Athens, Ga.
Richard Bristol, Madison, Wis.
R. F. Hall, Caldwell, Idaho
J. L. O'Harra, Reno, Nev.
Fred Kingma, Washington, D.C.
Neal Black, St. Paul, Minn.
K. M. Weinland, Lafayette, Ind.
Al Kolte, Chicago, Ill.
Carlos Cooper, Bonner Springs, Kan.
W. R. Streiber, Hyattsville, Md.
G. E. Reynolds, Corvallis, Ore.
J. F. Quinn, Lansing, Mich.

R. C. Hammond, College Park, Md.

Committee on Transmissible Diseases of Poultry—1975

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

E. S. Bryant, Storrs, Conn.
A. H. Dardiri, Greenport, L.I.N.Y.
F. Golan, College Station, Tex.
J. E. Hanley, Dade City, Fla.
A. E. Janawicz, Montpelier, Vt.
W. E. Merritt, Washington, D.C.
W. C. Patterson, Athens, Ga.
C. J. Pfow, Hyattsville, Md.
James B. Roberts, Muldrow, Okla.
T. B. Ryan, Cary, N.C.
John A. Smiley, Augusta, Maine
H. W. Towers, Dover, Del.
F. G. Buzzell, Augusta, Maine
A. E. Decoteau, Waltham, Mass.
L. C. Grumbles, College Station, Tex.
R. L. Hogue, Lafayette, Ind.
T. L. Landers, Hot Springs, Ark.
H. E. Nadler, Albany, N.Y.
I. L. Peterson, Beltsville, Md.
B. S. Pomeroy, St. Paul, Minn.
A. S. Rosenwald, Davis, Calif.
W. C. Schofield, St. Louis, Mo.
J. B. Thomas, Columbia, S.C.
Porter Halbert, San Augustine, Tex.
Committee on Public Health and Environmental Quality—1975

R. L. Parker, Chairman, Atlanta, Georgia
R. H. Singer, Winchester, Ky.

Lewis Locke, Laurel, Md.
J. E. Spaulding, Beltsville, Md.
C. W. Schwabe, Davis, Calif.
E. E. Wedman, Corvallis, Ore.
J. F. Stara, Cincinnati, Ohio
M. J. Tweihaus, Lincoln, Neb.
L. P. Thomas, Charleston, W. Va.
Mark Trask, Wasta, S. Dak.
J. D. Kornder, Atlanta, Ga.
Charlie Jungmichel, Austin, Tex.
D. D. Juranek, Atlanta, Ga.
W. C. Patterson, Athens, Ga.
V. C. McKaughan, Lynchburg, Va.
H. G. Geyer, Washington, D.C.
W. B. Buck, Ames, Iowa
Raymond Fagan, Richmond, Va.
W. E. Jennings, Santa Rose Beach, Fla.
J. H. Steele, Houston, Tex.
D. E. Flack, Greeley, Colo.
R. A. Lilly, Austin, Tex.
William Hubbert, Baton Rouge, La.
T. B. Snodgrass, Dallas, Tex.
L. W. Schnurrenberger, Beltsville, Md.
J. L. Wilbur, Austin, Tex.
Forrest Ireland, Belvidere, S.D.
Sharon Corey, Charleston, W. Va.
H. M. Trabash, Washington, D.C.

L. W. Hinchman, Indianapolis, Ind.

Committee on Rabies—1975

R. K. Sikes, Chairman, Atlanta, Georgia
E. A. Carbrey, Co-Chairman, Ames, Iowa

L. N. Butler, Phoenix, Ariz.
E. H. Willers, Honolulu, Hawaii
John L. Brown, Washington, D.C.
Victor Cabasso, Berkeley, Calif.
Alfred Strating, Ames, Iowa
M. T. Goff, Ames, Iowa
J. F. Frank, Hull, Que, Canada
Bruce Kaplan, Louisville, Ky.

J. W. Glosser, Helena, Mont

Committee on Diseases of Sheep and Goats—1975

F. James Schoenfeld, Chairman, Salt Lake City, Utah
C. C. Beck, Co-Chairman, Manchester, Michigan

H. A. Hancock, Laramie, Wyo.
Ward Van Horn, Buffalo, S. Dak.
W. W. Hawkins, Jr., Bozeman, Mont.
J. E. Pearson, Ames, Iowa
R. F. Hall, Caldwell, Idaho
W. A. Hickman, Pierre, S. Dak.
Howard Whitford, College Station, Tex.
John Neimu, Buffalo, S. Dak.
T. B. Snodgrass, Dallas, Tex.
A. L. Klingsporn, Bowie, Md.
H. E. Metcalf, Lakewood, Colo.
C. E. Terrill, Silver Spring, Md.
M E. Macheak, Ames, Iowa
R. E. Simmons, Boise, Idaho

Committee on State-Federal Relations—1975

H. E. Goldstein, Chairman, Columbus, Ohio

J. F. Andrews, Atlanta, Ga.
L. E. Bartelt, Sacramento, Calif.
J. C. Shook, Mechanicsburg, Pa.
H. Q. Sibley, Austin, Tex.
A. E. Janawicz, Montpelier, Vt.
W. L. Bendix, Richmond, Va.
T. A. Ladson, Annapolis, Md.
D. H. Spangler, Olympia, Wash.
### Committee on Transmissible Diseases of Swine—1975

E. A. Butler, Chairman, Des Moines, Iowa  
T. F. Zweigart, Co-Chairman, Raleigh, N. Carolina

<table>
<thead>
<tr>
<th>Name</th>
<th>City, State</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. H. Bohl</td>
<td>Wooster, Ohio</td>
</tr>
<tr>
<td>James B. Nance</td>
<td>Alamo, Tenn.</td>
</tr>
<tr>
<td>John R. Ragan</td>
<td>Nashville, Tenn.</td>
</tr>
<tr>
<td>W. C. Stewart</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>Norman Kruse</td>
<td>Lincoln, Neb.</td>
</tr>
<tr>
<td>D. P. Gustafson</td>
<td>Lafayette, Ind.</td>
</tr>
<tr>
<td>R. E. Hall</td>
<td>Madison, Wis.</td>
</tr>
<tr>
<td>R. E. Thompson</td>
<td>Hyattsville, Md.</td>
</tr>
<tr>
<td>J. E. Fox</td>
<td>Ashland, Ohio</td>
</tr>
<tr>
<td>M. Ristic</td>
<td>Urbana, Ill.</td>
</tr>
<tr>
<td>Don Brothers</td>
<td>Paducah, Tex.</td>
</tr>
<tr>
<td>E. O. Haelterman</td>
<td>Lafayette, Ind.</td>
</tr>
<tr>
<td>Taylor Woods</td>
<td>Jefferson City, Mo.</td>
</tr>
<tr>
<td>Vaughan Seaton</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>Robert Giock</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>John P. Kluge</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>M. G. Hynes</td>
<td>Dublin, Ireland</td>
</tr>
</tbody>
</table>

### Committee on Salmonella—1975

H. G. Geyer, Chairman, Washington, D.C.  
J. W. Walker, Co-Chairman, Hyattsville, Md.

<table>
<thead>
<tr>
<th>Name</th>
<th>City, State</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. R. Ames</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td>Ralph Johnson</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>Rube Harrington, Jr.</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>E. V. Morse</td>
<td>Lafayette, Ind.</td>
</tr>
<tr>
<td>Walt Sadler</td>
<td>Davis, Calif.</td>
</tr>
<tr>
<td>Stanley A. Vezey</td>
<td>Athens, Ga.</td>
</tr>
<tr>
<td>Rufus Weidner</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td>William B. Bixler</td>
<td>Rockville, Md.</td>
</tr>
<tr>
<td>Marshall Fox</td>
<td>Atlanta, Ga.</td>
</tr>
<tr>
<td>C. W. Johnson</td>
<td>Des Plaines, Ill.</td>
</tr>
<tr>
<td>D. M. Wenger</td>
<td>Decatur, Ind.</td>
</tr>
<tr>
<td>Raymond Schar</td>
<td>Beltsville, Md.</td>
</tr>
<tr>
<td>R. C. Hammond</td>
<td>College Park, Md.</td>
</tr>
</tbody>
</table>

### Committee on Tuberculosis and Paratuberculosis—1975

P. L. Smith, Chairman, Sacramento, California  
A. R. McLaughlin, Co-Chairman, Madison, Wis.

<table>
<thead>
<tr>
<th>Name</th>
<th>City, State</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. W. Bennett</td>
<td>Hyattsville, Md.</td>
</tr>
<tr>
<td>J. L. Blair</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>A. M. Carey</td>
<td>Beltsville, Md.</td>
</tr>
<tr>
<td>C. S. Duncan</td>
<td>Albany, N.Y.</td>
</tr>
<tr>
<td>G. H. Frye</td>
<td>Hyattsville, Md.</td>
</tr>
<tr>
<td>A. F. Kaufmann</td>
<td>Atlanta, Ga.</td>
</tr>
<tr>
<td>A. B. Larsen</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>W. L. Mallman</td>
<td>E. Lansing, Mich.</td>
</tr>
<tr>
<td>H. E. Nadler</td>
<td>Albany, N.Y.</td>
</tr>
<tr>
<td>A. P. Schneider</td>
<td>Boise, Idaho</td>
</tr>
<tr>
<td>G. W. Spangler</td>
<td>Des Moines, Iowa</td>
</tr>
<tr>
<td>Charles Thoen</td>
<td>Ames, Iowa</td>
</tr>
<tr>
<td>J. T. White</td>
<td>Stowell, Tex.</td>
</tr>
<tr>
<td>H. Q. Sibley</td>
<td>Austin, Tex.</td>
</tr>
<tr>
<td>Neal Black</td>
<td>St. Paul, Minn.</td>
</tr>
<tr>
<td>Carl E. Boyd</td>
<td>Columbia, S.C.</td>
</tr>
<tr>
<td>John Dick</td>
<td>Harrisburg, Pa.</td>
</tr>
<tr>
<td>J. G. Flint</td>
<td>St. Paul, Minn.</td>
</tr>
<tr>
<td>D. W. Johnson</td>
<td>Roseville, Minn.</td>
</tr>
<tr>
<td>Victor LaBranch</td>
<td>Boston, Mass.</td>
</tr>
<tr>
<td>Rodney Larson</td>
<td>Fruitdale, S. Dak.</td>
</tr>
<tr>
<td>J. L. McMillan</td>
<td>Wheatland, Calif.</td>
</tr>
<tr>
<td>Bruce Pipkin, Sr.</td>
<td>Hamshire, Tex.</td>
</tr>
<tr>
<td>G. R. Snyder</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>R. J. Stadler</td>
<td>Hartford, Conn.</td>
</tr>
<tr>
<td>K. M. Weinland</td>
<td>Lafayette, Ind.</td>
</tr>
<tr>
<td>Lindsey Horn</td>
<td>Chicago, Ill.</td>
</tr>
<tr>
<td>Paul L. Spencer</td>
<td>Springfield, Ill.</td>
</tr>
<tr>
<td>J. B. Young</td>
<td>Austin, Tex.</td>
</tr>
</tbody>
</table>

### Committee on Wild & Marine Life Diseases—1975

Frank A. Hayes, Chairman, Athens, Georgia

<table>
<thead>
<tr>
<th>Name</th>
<th>City, State</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. E. Cooperrider</td>
<td>Kissimmee, Fla.</td>
</tr>
<tr>
<td>Joe T. Finley, Jr.</td>
<td>Encinal, Tex.</td>
</tr>
<tr>
<td>L. A. Griner</td>
<td>San Diego, Calif.</td>
</tr>
<tr>
<td>T. P. Kistner</td>
<td>Corvallis, Ore.</td>
</tr>
<tr>
<td>B. S. Pomeroy</td>
<td>St. Paul, Minn.</td>
</tr>
<tr>
<td>J. R. Ragan</td>
<td>Nashville, Tenn.</td>
</tr>
<tr>
<td>Col. G. S. Trevino</td>
<td>Hyattsville, Md.</td>
</tr>
<tr>
<td>L. L. Williamson</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>A. H. Dardiri</td>
<td>Greenport, L.I.N.Y.</td>
</tr>
<tr>
<td>H. G. Geyer</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>A. H. Hulsey</td>
<td>Little Rock, Ark.</td>
</tr>
<tr>
<td>S. H. Madin</td>
<td>Berkeley, Calif.</td>
</tr>
<tr>
<td>R. E. Putz</td>
<td>Washington, D.C.</td>
</tr>
<tr>
<td>James S. Smith</td>
<td>Hyattsville, Md.</td>
</tr>
<tr>
<td>James B. White</td>
<td>Cheyenne, Wyo.</td>
</tr>
<tr>
<td>W. G. Winkler</td>
<td>Lawrenceville, Ga.</td>
</tr>
</tbody>
</table>

---
<table>
<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 27-28, 1897†</td>
<td>Fort Worth, Tex.</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. D. O. Lively, Fort Worth, Tex.</td>
</tr>
<tr>
<td>Oct. 11-12, 1898</td>
<td>Omaha, Neb.</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. Taylor Riddle, Kan.</td>
</tr>
<tr>
<td>Oct. 8-9, 1901</td>
<td>Buffalo, N.Y.</td>
<td>*Dr. E. P. Niles, Va.</td>
<td>*Dr. F. T. Eisenman, Louisville, Ky.</td>
</tr>
<tr>
<td>Aug. 16-17, 1907</td>
<td>Springfield, Ill.</td>
<td>*Mr. M. M. Hankins, Quanah, Tex.</td>
<td>*Dr. S. H. Ward, St. Paul, Minn.</td>
</tr>
<tr>
<td>Dec. 5-6, 1911</td>
<td>Chicago, Ill.</td>
<td>*Dr. John F. Devine, Goshen, N.Y.</td>
<td>*Mr. J. J. Ferguson, Chicago, Ill.</td>
</tr>
<tr>
<td>Nov. 28-30, 1921</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. F. Crew, Bismarck, N.D.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
</tr>
<tr>
<td>Dec. 6-8, 1922</td>
<td>Chicago, Ill.</td>
<td>*Dr. T. E. Munce, Harrisburg, Pa.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
</tr>
<tr>
<td>Dec. 5-7, 1923</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. J. Butler, Helena, Mont.</td>
<td>*Dr. O. E. Dyson, Kansas City, Mo.</td>
</tr>
<tr>
<td>No.</td>
<td>Date</td>
<td>City, State</td>
<td>Name, City, State</td>
</tr>
<tr>
<td>-----</td>
<td>------------</td>
<td>----------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>29</td>
<td>Dec. 2-4, 1925</td>
<td>Chicago, Ill.</td>
<td>Dr. J. H. McNeil, Trenton, N.J.</td>
</tr>
<tr>
<td>30</td>
<td>Dec. 1-3, 1926</td>
<td>Chicago, Ill.</td>
<td>Dr. John R. Mohler, Wash., D.C.</td>
</tr>
<tr>
<td>31</td>
<td>Nov. 30-Dec. 1-2, 1927</td>
<td>Chicago, Ill.</td>
<td>Dr. L. Van Es, Lincoln, Neb.</td>
</tr>
<tr>
<td>32</td>
<td>Dec. 5-7, 1928</td>
<td>Chicago, Ill.</td>
<td>Dr. C. A. Cary, Auburn, Ala.</td>
</tr>
<tr>
<td>33</td>
<td>Dec. 6-8, 1929</td>
<td>Chicago, Ill.</td>
<td>Dr. Chas. G. Lamb, Denver, Colo.</td>
</tr>
<tr>
<td>34</td>
<td>Dec. 3-5, 1930</td>
<td>Chicago, Ill.</td>
<td>Dr. A. E. Wight, Wash., D.C.</td>
</tr>
<tr>
<td>35</td>
<td>Dec. 2-4, 1931</td>
<td>Chicago, Ill.</td>
<td>Dr. J. W. Connaway, Columbia, Md.</td>
</tr>
<tr>
<td>36</td>
<td>Nov. 30-Dec. 1-2, 1932</td>
<td>Chicago, Ill.</td>
<td>Dr. Peter Malcolm, Des Moines, Iowa</td>
</tr>
<tr>
<td>37</td>
<td>Dec. 6-8, 1933</td>
<td>Chicago, Ill.</td>
<td>Dr. E. T. Faulder, Albany, N.Y.</td>
</tr>
<tr>
<td>38</td>
<td>Dec. 5-7, 1934</td>
<td>Chicago, Ill.</td>
<td>Dr. T. E. Robinson, Providence, R.I.</td>
</tr>
<tr>
<td>39</td>
<td>Dec. 4-6, 1935</td>
<td>Chicago, Ill.</td>
<td>Dr. Edward Records, Reno, Nev.</td>
</tr>
<tr>
<td>40</td>
<td>Dec. 24, 1936</td>
<td>Chicago, Ill.</td>
<td>Dr. Walter Wisnicky, Madison, Wis.</td>
</tr>
<tr>
<td>41</td>
<td>Dec. 1-3, 1937</td>
<td>Chicago, Ill.</td>
<td>Dr. R. W. Smith, Concord, N.H.</td>
</tr>
<tr>
<td>42</td>
<td>Nov. 30-Dec. 1-2, 1938</td>
<td>Chicago, Ill.</td>
<td>Dr. D.E. Westmoreland, Frankfort, Ky.</td>
</tr>
<tr>
<td>43</td>
<td>Dec. 6-8, 1939</td>
<td>Chicago, Ill.</td>
<td>Dr. J. L. Axby, Indianapolis, Ind.</td>
</tr>
<tr>
<td>44</td>
<td>Dec. 4-6, 1940</td>
<td>Chicago, Ill.</td>
<td>Dr. H. D. Port, Cheyenne, Wyo.</td>
</tr>
<tr>
<td>45</td>
<td>Dec. 3-5, 1941</td>
<td>Chicago, Ill.</td>
<td>Dr. E. A. Crossman, Boston, Mass.</td>
</tr>
<tr>
<td>46</td>
<td>Dec. 2-4, 1942</td>
<td>Chicago, Ill.</td>
<td>Dr. I. S. McAdory, Auburn, Ala.</td>
</tr>
<tr>
<td>47</td>
<td>Dec. 1-3, 1943</td>
<td>Chicago, Ill.</td>
<td>Dr. W. H. Hendricks, Salt Lake City, Utah</td>
</tr>
<tr>
<td>48</td>
<td>Dec. 6-8, 1944</td>
<td>Chicago, Ill.</td>
<td>Dr. J. M. Sutton, Atlanta, Ga.</td>
</tr>
<tr>
<td>49</td>
<td>Dec. 5-7, 1945</td>
<td>Chicago, Ill.</td>
<td>Dr. C. U. Duckworth, Sacramento, Calif.</td>
</tr>
<tr>
<td>50</td>
<td>Dec. 4-6, 1946</td>
<td>Chicago, Ill.</td>
<td>Dr. William Moore, Raleigh, N.C.</td>
</tr>
<tr>
<td>51</td>
<td>Dec. 3-5, 1947</td>
<td>Chicago, Ill.</td>
<td>Mr. Will J. Miller, Topeka, Kan.</td>
</tr>
<tr>
<td>53</td>
<td>Oct. 12-14, 1949</td>
<td>Columbus, Ohio</td>
<td>Dr. T. O. Brandenburg, Bismarck, N.D.</td>
</tr>
<tr>
<td>55</td>
<td>Nov. 14-16, 1951</td>
<td>Kansas City, Kan.</td>
<td>Mr. F. E. Mollin, Denver, Colo.</td>
</tr>
<tr>
<td>56</td>
<td>Okt. 29-31, 1952</td>
<td>Louisville, Ky.</td>
<td>Dr. Ralph L. West, St. Paul, Minn.</td>
</tr>
<tr>
<td>57</td>
<td>Sept. 23-25, 1953</td>
<td>Atlantic City, N.J.</td>
<td>Dr. T. Childs, Ottawa, Canada</td>
</tr>
<tr>
<td>58</td>
<td>Nov. 10-12, 1954</td>
<td>Omaha, Neb.</td>
<td>Dr. T. C. Green, Charleston, W.Va.</td>
</tr>
<tr>
<td>59</td>
<td>Nov. 16-18, 1955</td>
<td>New Orleans, La.</td>
<td>Dr. H. F. Wilkins, Helena, Mont.</td>
</tr>
<tr>
<td>60</td>
<td>Nov. 28-30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. A. L. Brueckner, Baltimore, Md.</td>
</tr>
<tr>
<td>61</td>
<td>Nov. 13-15, 1957</td>
<td>St. Louis, Mo.</td>
<td>Dr. G. H. Good, Cheyenne, Wyo.</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>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>Dec. 15-18, 1959</td>
<td>San Francisco, Calif.</td>
<td>Mr. F. G. Buzzell, Augusta, Me.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>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>Oct. 10-14, 1966</td>
<td>Buffalo, N.Y.</td>
<td>Dr. C. L. Campbell, Tallahassee, Fla.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>Nov. 5-10, 1972</td>
<td>Miami Beach, Fla.</td>
<td>J. C. Shook, Mechanicsburg, Pa.</td>
<td>Dr. W. L. Bendix, Richmond, Va.</td>
</tr>
</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
T. F. Zweigart, D. V. M.

Our Father, as we transact the business of this seventy-eighth convention of our Association, with all its cares, decisions, and problems, help us to serve Thee that we may rightly serve all mankind.

Help us to make the most of our time by putting ourselves into Thy hands. Thou knowest the way is difficult for us and that we are often perplexed.

Help us to find the path to practice that which we know to be Thy will and give us guidance that will fill these days with good action and accomplishment.

Amen
MEMORIAL

T. F. Zweigart, D. V. M.,
Raleigh, North Carolina

President Timm, Distinguished Guests, Ladies and Gentlemen—

Each year the United States Animal Health Association takes time to pay tribute to those of our members who have passed on since our last meeting. This past month we lost a highly esteemed colleague, Dr. Howard Walter Dunne of Penn State University, died September 10, 1974.

Will everyone please rise and remain standing to participate in a moment of silent prayer?

SILENT PRAYER

Amen, Thank you for your participation.
REPORT OF THE SECRETARY

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

The Secretary wishes to advise the membership that for the year 1974 we had 610 paid individual members, and we had 56 paid official and allied organization memberships. We currently have 42 applications for new individual memberships, and we also have applications from three organizations for allied organization membership. These, I assume, will be acted upon by the Executive Committee at this meeting.

The Association at its 1973 meeting in St. Louis, and in accordance with our Constitution, separated the offices of Secretary and Treasurer. I would like at this time to commend Doctor Shook for his diligence in performing the duties of Treasurer and thank him for the assistance he has given the Association's office during the year in the transacting of our business affairs. Doctor Shook's report as Treasurer will follow this one on the program, and I will therefore leave our financial affairs to him and his report.

The Secretary was notified early in the spring that we would have to vacate our offices, as the building was to be demolished to make way for the construction of a branch bank building. As of June 1, 1974, we found suitable quarters in an office building actually within the city of Richmond, for which we negotiated a lease of 12 months. This lease now in effect will run through next May. We have reasonably adequate quarters, although they are all in one room, and the owners do provide one parking space. The rent we pay is $100 and a few cents per month. I have been informed recently that some of our neighbors in the same building whose leases are currently expiring are being presented with new leases for the same space with greatly increased rent being demanded. Regardless of where our offices are located, I feel that we must accept the fact that we will be faced with the same thing when our lease expires next year.

Our Association is faced with some rather hard decisions, and it is my opinion that we can no longer turn our back on them or attempt to solve them by merely talking about them. We are no less the victims of inflation than every other segment of our national life. It is here now, it is serious, it will probably continue for a considerable length of time, and it hurts. Our present income will not permit us to continue in our present method of operation. We have an agreement that will be implemented following this meeting that provides for a substantial payment to the American Association of Veterinary Laboratory Diagnosticians, and at the same time we will discontinue publishing their Proceedings in the same volume with ours. This will reduce the size of the Proceedings, which certainly is desirable, and it will somewhat reduce the cost; but, unfortunately, the reduction in cost will not be in an amount that will offset our payment to that distinguished group. In the matter of printing, we have only two local printers in Richmond who can handle our Proceedings, and therefore competition does not really get into the business of cost. One of them, who had the job originally, submits an estimate that is a great deal more than the other, and the quality of the work between the one and the other is not significantly different. Mistakes are frequent, mostly impossible to justify, and represent a careless approach to the whole job, even after the submission of approved proofs. We must find a higher degree of professionalism and a higher degree of
competitiveness if our printing is to stay anywhere near what we expect in the line of accuracy and quality and still be in the realm of the affordable. We have received a suggestion from one of the members that serious thought should be given to the future of our Association, perhaps a new setting of objectives and reorientation of our thinking with regard to the direction in which we wish the Association to go. Improved service to the membership certainly is a must; but in looking at it from a standpoint of the complexities faced in today's world, it is difficult for me to see how much, if any, of this can be accomplished with only one full-time office employee and one or two part-time employees who have full-time jobs elsewhere. The time is at hand for the United States Animal Health Association to either use its Internal Affairs Committee or to establish an ad hoc committee to study the direction in which the Association should move for the future and to recommend ways and means for getting there. To continue as we are going at present will assure that at the close of the next meeting in 1975 we will be an insolvent organization.

Doctor Campbell and I surveyed the hotels in the south of Florida early this spring and have again selected the Americana at Bal Harbour for the 1976 meeting of the United States Animal Health Association. At the time we met with the various hotels, there were not too many acceptable dates. Being aware of the difficulty of getting preferential dates in the southeastern Florida area, owing to its popularity, the Executive Committee last year authorized some extension of the previously required dates that it had established some years ago. We finally reached an agreement with the Americana Hotel for the last week in November and running over into the first couple of days of December, 1976—actually November 28 through December 3. We have since learned that this conflicts with one or two other important meeting dates that had already been established, one in particular affecting the research groups. We are at present negotiating with the Americana for new dates and are assured that before too long we can work out an agreeable change. The dates in question are the week beginning November 7 and the week beginning November 21. I believe the November 7 date will be most desirable. The hotel assures us that they will work it out to our satisfaction.

A final comment, about our Newsletter. This sort of venture is entirely new for the Secretary and his "office slave." Our plea for contents and publishable material, trivia, and whatnot have not gone unheeded, although most of what has been submitted has been in the area of the controversial, and we have not felt it desirable to publish it. It was decided that the Newsletter would be issued quarterly, and we have managed to put out three during this year. Hopefully there will be one following this meeting, which will report on significant details of what happened here. The Newsletter certainly is not a finished professional job as yet, but at least we have made a beginning. We are still sending it out to a mailing list of some 1,200 persons, about 100 extra to people who have requested it, other associations, and here and there as we felt might be helpful. This represents under current conditions a mailing list almost double our current paid membership list. The Secretary's office needs all the help it can get in the way of suggestions with regard to the Newsletter.
The Secretary hopes that you enjoy yourself while in Roanoke. He hopes that your accommodations, if not of the very best, are at least acceptable. And he hopes that you find your relatively short stay with us both pleasurable and profitable.

Respectfully submitted,

W. L. Bendix, D.V.M.
Secretary
STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR
PERIOD OCTOBER 1, 1973 THROUGH SEPTEMBER 30, 1974

CASH BALANCE—October 1, 1973:

Cash on Hand - October 1, 1973 $1,028.00
Southern Bank and Trust Company
Richmond, Virginia
Checking Account 188.51
Savings Account 7,947.04
Local Arrangements 143.23
Trevose Savings and Loan Association
Morrisville, Pennsylvania 1.00
Sandia Savings and Loan Association
Albuquerque, New Mexico 1.00

$ 9,308.78

INCREASED BY CASH RECEIPTS:

Individual Dues $11,780.00
Official Dues 8,300.00
Proceedings and Foreign Animal Books 5,706.02
Reprints 4,482.26
Registration Fees 11,700.00
Interest Income 767.04
Miscellaneous .85
Checks issues but which were never cashed—
added back to bank balance 115.41

42,851.58

TOTAL BEGINNING BALANCE AND RECEIPTS $52,160.36
UNITED STATES ANIMAL HEALTH ASSOCIATION
1910 BYRD AVENUE, ROOM 118
RICHMOND, VIRGINIA 23230

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR
PERIOD OCTOBER 1, 1973 THROUGH SEPTEMBER 30, 1974

INCREASED BY EXPENDITURES:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual Meeting</td>
<td>$3,089.72</td>
</tr>
<tr>
<td>Printing</td>
<td>25,362.03</td>
</tr>
<tr>
<td>Office Supplies</td>
<td>1,027.00</td>
</tr>
<tr>
<td>Salaries</td>
<td>11,741.74</td>
</tr>
<tr>
<td>Social Security Tax</td>
<td>687.12</td>
</tr>
<tr>
<td>Communication</td>
<td>1,848.60</td>
</tr>
<tr>
<td>Travel:</td>
<td></td>
</tr>
<tr>
<td>Dr. Harry E. Goldstein</td>
<td>121.02</td>
</tr>
<tr>
<td>Dr. J. C. Shook</td>
<td>30.81</td>
</tr>
<tr>
<td>F. G. Buzzell</td>
<td>106.00</td>
</tr>
<tr>
<td>Dr. W. L. Bendix</td>
<td>172.42</td>
</tr>
<tr>
<td>Olin H. Timm</td>
<td>1,696.73</td>
</tr>
<tr>
<td>Ella R. Blanton</td>
<td>298.80</td>
</tr>
<tr>
<td>Furniture and Fixtures</td>
<td>111.40</td>
</tr>
<tr>
<td>Rent—Office Space</td>
<td>1,168.32</td>
</tr>
<tr>
<td>American Association of Veterinary</td>
<td></td>
</tr>
<tr>
<td>Livestock Diagnosticians</td>
<td>600.00</td>
</tr>
<tr>
<td>Virginia Unemployment Insurance</td>
<td>36.06</td>
</tr>
<tr>
<td>Surety Bond—Treasurer</td>
<td>50.00</td>
</tr>
<tr>
<td>Moving Office</td>
<td>67.50</td>
</tr>
<tr>
<td>Miscellaneous Expense</td>
<td>637.76</td>
</tr>
<tr>
<td>Bank Service Charge</td>
<td>13.32</td>
</tr>
</tbody>
</table>

$48,866.35

CASH BALANCE—SEPTEMBER 30, 1974:

<table>
<thead>
<tr>
<th>Institution</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern Bank and Trust Company</td>
<td></td>
</tr>
<tr>
<td>Checking Account</td>
<td>328.42</td>
</tr>
<tr>
<td>Savings Account</td>
<td>2,811.64</td>
</tr>
<tr>
<td>Local Arrangements</td>
<td>151.95</td>
</tr>
<tr>
<td></td>
<td>$3,292.01</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>

3,294.01
UNITED STATES ANIMAL HEALTH ASSOCIATION
1910 BYRD AVENUE, ROOM 118
RICHMOND, VIRGINIA 23230

SUMMARY OF OPERATIONS
FOR PERIOD OCTOBER 1, 1973 THROUGH SEPTEMBER 30, 1974

REVENUE:

Total Cash Receipts                    $42,851.58
Less — Expenditures                  $48,866.35

Excess of Expenditures                $ 6,014.77

NET WORTH—SEPTEMBER 30, 1974

Accounts Receivable                  $ 8,450.00

Balance:
Southern Bank and Trust Company, Richmond, Virginia
  Checking Account                     328.42
  Savings Account                      2,811.64
  Local Arrangements                   151.95

Balance:
Trevose Savings and Loan Association
  Morrisville, Pennsylvania             1.00

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

Petty Cash Fund                       25.00
Deposit—C. & P. Telephone Company
  Richmond, Virginia                    100.00
Inventory—Supplies and Proceedings    7,000.00
U. S. Treasury Bond                   10,000.00
Furniture and Fixtures                1,405.92

NET WORTH—SEPTEMBER 30, 1974          $30,274.93
**ANALYSIS OF CHANGE IN NET WORTH:**

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net Worth—September 30, 1973</td>
<td>$33,718.30</td>
</tr>
<tr>
<td>Increased by:</td>
<td></td>
</tr>
<tr>
<td>Purchase of additional furniture and fixtures</td>
<td>$111.40</td>
</tr>
<tr>
<td>Inventory—Supplies and Proceedings</td>
<td>1,500.00</td>
</tr>
<tr>
<td>Accounts Receivable</td>
<td>960.00</td>
</tr>
<tr>
<td><strong>Total Increased</strong></td>
<td><strong>2,571.40</strong></td>
</tr>
<tr>
<td>Decreased by:</td>
<td></td>
</tr>
<tr>
<td>Excess of Expenditures of Receipts</td>
<td>6,014.77</td>
</tr>
<tr>
<td><strong>NET WORTH, SEPTEMBER 30, 1974</strong></td>
<td><strong>$30,274.93</strong></td>
</tr>
</tbody>
</table>

Henry H. Budd  
Accountant
Welcome Address*

On behalf of the Virginia Department of Agriculture and Commerce—and our Division of Animal Health and Dairies—I want to welcome you back to Virginia. Please note that I said “back to Virginia,” and not “to Virginia!”

Although it took a little digging, Dr. Bendix—our State Veterinarian—determined that your association held its eleventh annual meeting September 16-17, 1907, in Richmond in the “parlors” of the Murphy Hotel. And he has a copy of the official proceedings to prove it!

At that time your association was known as “The Interstate Association of Live Stock Sanitary Boards.” This title was changed in 1910 to the “U.S. Livestock Sanitary Association”—a name which lasted many years until the adoption of your present title, “U.S. Animal Health Association,” in 1968.

Dr. Bendix shares my pleasure at having this opportunity to be one of your hosts at this meeting which—in terms of your association—is your 78th. At the time of your last meeting in Virginia, Doc was about six weeks old. And, by the end of this month, he will round out 24 years as Virginia’s State Veterinarian—which I think must be some kind of record! We in the Department are proud that he has served as your President; for several years was your Secretary-Treasurer; and is currently your Secretary.

Being somewhat of a history buff, I was interested to learn that the records of the 1907 Richmond meeting show that your President—who was then Dr. D.F. Luckey of Columbia, Missouri—highlighted the association’s concern with animal diseases in his annual address. He also discussed briefly those diseases, both past and present, which were the association’s principal interests, and whose eradication was its primary reason for existence. I can do no better than to quote you direct from Dr. Luckey:

“We have come together again today to further plan the siege of battle against the many contagious diseases of livestock. We have already witnessed the banishing from this continent of two very important ones, namely: contagious pleuro-pneumonia and foot and mouth disease. We have witnessed the almost complete annihilation of glanders, and have watched the control of Texas fever to the point of such perfection that few of the stock raisers of this country today realize there is any danger of loss from it. Yet, we still have before us three great foes which are worthy of our best mettle, and there is little doubt that these are, named in the order of their importance—tuberculosis, hog cholera and the fever tick. Worthy of mention we also find contagious abortion, rabies, black-leg, actinomycosis, and dozens of parasitic diseases.”

Moving the clock forward to today, Texas fever is of course a thing of the past. Bovine tuberculosis, while not completely eradicated, is hopefully on the way out. All of the 50 states are now hog-cholera free as a result of your most recent effort. Thirty-five of the states are now brucellosis-free or, to use Dr. Luckey’s term, “contagious abortion free.” And I understand that you are currently organizing yourselves—along with your companion associations—for a final assault to eradicate this disease from our nation.

The efforts of your association over the years have done a great deal to improve our nation’s animal agriculture. A quick review of the record reveals that in 1973 Virginia farmers’ cash receipts from farm marketings totalled over $878 million. Of this amount $493 million, or about 56 percent, was derived from
sales of livestock and livestock products—a gain of 28 percent from the previous year and a new record. Without a doubt, your association’s contributions to the nation’s animal agriculture—and therefore to the nation’s general welfare—have been considerable. Furthermore, we confidently expect your future accomplishments to be equally impressive.

In closing, I want to say again that we are more than happy to have you back in Virginia once more. Virginia is the cradle of our nation, and has given the country more presidents than any other state. While you are our guests, please take advantage of every opportunity to visit some of the state’s many places of interest and historic shrines. Natural Bridge—one of the seven natural wonders of the world—isn’t far from Roanoke. Much of our nation’s history can be attributed to events that took place in Jamestown, Williamsburg, Appomattox, Fredericksburg, and Richmond.

Enjoy yourselves while you are here—have a good meeting and a safe return—and don’t wait so long to come back again!

Response to Address of Welcome by Dr. Glenn B. Rea

Commissioner Carbaugh, President Timm—

I don’t know just what thoughts crossed your mind, Commissioner, as you first glanced at the audience, but it occurs to me that from the way they are seated that someone passed the word that Dr. Tom Zwiegart was going to give the alter call. Everyone is seated from the middle to the rear of the auditorium. We have the “amen corner” made up of the indentured servants, and if we can just get President Timm to pass the plate, we can have an old-fashioned revival.

You mentioned in your opening remarks that at the last Virginia meeting of this organization—then called the USLSA—held in 1907, that Dr. Luckey spoke of many of the same diseases and the goals that are still a challenge to us today. However, some of the diseases that plagued Dr. Luckey’s contemporaries have been conquered in the interim, i.e., Foot and Mouth Disease, Vesicular Exanthema, Fever Tick, Glanders, Dourine, Fowl Plague and, more recently, Sheep Scab and Hog Cholera. Great strides have been made against Tuberculosis and Brucellosis. Though our tasks seem limitless, and our goals unattainable, we still keep striving for achievement.

Those of us gathered here represent all fifty states and several foreign countries. We who knwo something of national economics, geography and history are very cognizant of the economic importance of Virginia, as well as its natural beauty and the contribution of its people to the history of the United States even to its very genesis.

Although this meeting is notorious as one of diligence and attention to business, our ladies look forward to sampling some of the attractions offered here, not only of a social nature but cultural as well. I wouldn’t be at all surprised if some of the registered participants strayed from their appointed tasks on occasion for the same purpose. If you happen to recognize any of us doing such things, I hope you will be discreet when you next attend a NASDA meeting. Many of us are here at the taxpayers’ expense. Although I am sure that the good citizens of our several states and countries are getting their money’s worth, it might be upsetting to some few of them if they thought we were deriving pleasure from our labors.

Once again, Commissioner, we thank you for your warm words and your proffered hospitality.
PRESIDENT ELECT'S ADDRESS


Members and guests, it is a privilege and a pleasure to address you as President-Elect of this great association. I am very humble in following a livestock industry member who has been a strong President. Mr. Timm has been a very vigorous leader of this association.

I would like to take this opportunity to thank the Ketchum Company and Mr. Powers, the National Band and Tag Company, the Haas family, and our Ella and Bill for making our association meetings successful and pleasurable.

This will probably be the shortest President-Elect's address in the history of our association because of my unorthodox way of public speaking and because I have no gems of wisdom for you as did my past friend Frank Wheeler, when he said that State-Federal Relations were like sex—when they were bad they were good. I wonder if Frank were still alive would he make the same statement, because APHIS realignment has been more chaotic than anything that has happened during my time as a regulatory person. There is less communication and cooperation between State and Federal people working on the same programs than I have ever known previous to this time. Ladies and Gentlemen, all of our cooperative programs are in real jeopardy due to the nature of the times and the apparent inability of USDA to secure proper funds and personnel to fulfill their commitments to these programs.

There is much haggling and dissent among regulatory people and industry as to what our future course in brucellosis eradication will be. Unless the program is properly funded I see nothing for it to do but to fail, and this will certainly jeopardize the future beginning of any new cooperative programs. It is time that we regulatory people put our personal ambitions aside and look at ourselves in the mirror and think daily that we are working at the taxpayers' expense and that all that we have to offer is a service. Unless this is done efficiently and pleasantly, we have failed the people we are working for.

I do not mean to be a predictor of gloom, because there are good things in USDA that we should be proud of. The Emergency Diseases Staff with their Epic Room is probably the most outstanding in the world to deal with emergency situations. We all can be proud of the fact that we have, for the most part, completed eradication of one disease from this nation's livestock industry, and that is hog cholera. We have also dealt with Newcastle outbreaks that have entered this country from time to time. If we could in some way complete the eradication of brucellosis from our livestock industry, then certainly we could continue to hold our head high and feel that we have been successful in our approaches to disease situations that we have been faced with. The moving of the barrier zone for screwworm further south into Mexico appears to be a real step in the right direction, and hopefully it will prevent the reentry of this parasite into the States in future years.

There is a great need for Federal legislation to regulate the importing of all exotic animals through quarantine facilities so that they may be inspected for ecto-parasites and other conditions which might endanger our livestock industry. I am planning to request that the Import-Export Committee take this into consideration in making a recommendation to USDA officials.
I would also like to follow a recommendation that was made by my good friend, Dr. Mitch Mitchell while he was President of our association, in rotating the chairman of committees about every third year. I hope that none of you will become offended by my decision to do this with some of the committees.

I would like to thank the individual members who have joined us since the last meeting, and also to thank the allied organizations that have joined us since that time.

I promised to make this brief and I will. In closing, I would like to tell you a story which should apply to all of us in its meaning:

(Smith Joke)

* * * * *

At this time I would like to ask Mr. Timm to come forward and accept these tokens of appreciation from our association. Mr. Timm, you have been a credit to our association and, as I said previously, you have been a strong, forceful and successful president of the U. S. Animal Health Association. I hope you will accept these tokens in appreciation of you from our great association.
I gratefully receive this acknowledgement of my service. At the least these last four years can be considered as continuing education for Olin Timm, but I do hope that I have also contributed to the organization.

Jim, I enthusiastically relinquish to you the responsibility of committee assignments. The other responsibilities I pass to you with less enthusiasm, because this year has not been dull and I have enjoyed it. I know you will do well in all you undertake.

May I also thank the Committee Chairmen for the work they have done for the Association.
PRESIDENT'S REMARKS

President
American Association of Vet. Laboratory Diagnosticians

President Timm, President-Elect Andrews, Commissioner Carbaugh, Dr. Carbrey, Dr. Bendix, distinguished guests and friends—it is indeed a pleasure to again speak to you.

During the past year, our organization has taken some very significant action. We have “come of age” so to speak.

We have made ourselves a dues-paying Association, thereby taking the Association far along the road to being as self-supporting as a scientific group can be. We shall always need the moral support of USAHA.

We have expanded our laboratory-accreditation plans and action, to the point that we now have 17 laboratories with recognized qualifications for accreditation.

We have honored one of our most dedicated founders by establishing an honorary award in his name.

We have decided to relieve our supporting organization of the burden of publishing our papers and are expanding our service to include our own proceedings book.

We have authorized the separate publication of revised information on anaerobic organism identification.

We have in progress work on publication of detailed information on bovine abortion causes.

When we consider that in the short span of seventeen years we have grown from a small group of about 20 who attended the first conference to a dues-paying group of well over 300 with over 150 in attendance at this morning’s session, I think we can consider that we have “come of age”. In fact, we are one year early since the “legal age” of today’s individual is eighteen.

It has been a great pleasure to make these few remarks in the presence of such a distinguished company.

Thank you and good night.
REPORT OF THE COMMITTEE ON NOMINATIONS, RESOLUTIONS AND INTERNAL AFFAIRS

Chairman: W. C. Tobin, Denver, Colorado

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

PRESIDENT: Dr. J. F. Andrews—Georgia
PRESIDENT-ELECT: Dr. H. E. Goldstein—Ohio
FIRST VICE-PRESIDENT: Dr. A. E. Janawicz—Vermont
SECOND VICE-PRESIDENT: Dr. L. E. Bartelt—California
SECRETARY: Dr. W. L. Bendix—Virginia
TREASURER: Dr. J. C. Shook—Pennsylvania

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

Nominations presented to general membership on Tuesday morning, October 15, 1974. Posted on Association bulletin board until Wednesday morning, October 16, 1974 and acted upon at Wednesday’s general session as provided in the Association’s Constitution.
Slate unanimously elected as presented.
UNITED STATES ANIMAL HEALTH ASSOCIATION

Resolution No. 1

Seventy-Eighth Annual Meeting

Held at Roanoke, Virginia Dates October 13 - 18, 1974

Source

Subject Matter

Foot Rot in Sheep

BACKGROUND INFORMATION

The committee on Diseases of Sheep of the U. S. Animal Health Association has long stressed the need for research in Foot Rot in sheep. The National Wool Growers Association in their priority list has placed Foot Rot as "most needing research in first position. Foot Rot research is low on the ARS of USDA and has not been reflected in the needs of the industry and the funds have not been allocated.

RESOLUTION

The United States Animal Health Association recommends to USDA that funds for Foot Rot research be increased and given top priority.

Secretary
BACKGROUND INFORMATION

The Committee on Import-Export calls attention to the fact that there are insufficient appropriated funds available to Veterinary Services for the Department of Agriculture to provide for adequate diagnostic tests, laboratory screening, frequent veterinary inspections and other desirable surveillance during required port of entry quarantine for animals of overseas origin that are subject to the import health regulations of the Department. This lack of funds available to Veterinary Services places excessively restrictive limitations on the acquisition of necessary port of entry personnel to handle import animals during quarantine at the port of entry, also the expansion and improvement of existing import animal quarantine facilities are hampered by the lack of funds available to Veterinary Services.

Such limitations on funds available to Veterinary Services thereby acts to severely restrict the number of import animals allowed to enter the United States from overseas countries and this in turn has resulted in a backlog of some 3,000 breeding cattle awaiting importation and the commitment for port of entry space for a year in advance.

The United States Animal Health Association believes that the livestock growers in the United States benefit from increased importations of genetically desirable strains of meat producing animals through increased meat production and better feed utilization.

RESOLUTION

Therefore, be it resolved that the United States Animal Health Association encourage and support Veterinary Services in implementing so-called "user charge" fees to be levied on animal importations so that such funds revert to Veterinary Services to supplement appropriated funds so as to bring about the benefits and improvements herein cited.

Resolved further that individual members of this Association actively solicit the support of congressional members for implementation of the so-called "user charges" and that the officers of this Association cause copies of this Resolution to be sent to appropriate committees of both the House and the Senate that are involved with matters relating to Agriculture and Appropriations.

[Signature]
Secretary
UNITED STATES ANIMAL HEALTH ASSOCIATION

Resolution No. 3 - Seventy-Eighth Annual Meeting

Held At Roanoke, Virginia Dates October 13 - 18, 1974

Source Brucellosis Committee of USAHA

Subject Matter Brucellosis

BACKGROUND INFORMATION

The Western States are applying measures for effective brucellosis control in cattle and swine and a goal of total brucellosis. Brucellosis has been known to exist in the Yellowstone National Park bison since 1917, during which time no measures of control have been exercised by park officials or the U. S. Department of Interior.

The U. S. Department of Agriculture as the Federal agency responsible for the eradication of brucellosis in the United States and the U. S. Department of Interior as the Federal agency responsible for management of animals and people in Yellowstone Park have reached an impasse in judgement as to the risk Yellowstone Park bison pose to the cattle of surrounding states and to brucellosis eradication in the United States. The United States Animal Health Association is concerned for the present or future stigma that these diseases bison might have on the total brucellosis eradication program.

RESOLUTION

Be it resolved that the U. S. Departments of Agriculture and Interior enter into a cooperative range research program to conduct transmissibility studies of bison brucellosis to cattle by intermingling the two (2) species under appropriate pre-determined conditions to adequately determine the answer to this controversial uncertainty, and

Be it further resolved, that funding to support the costs of acquiring the bison and cattle, of their confinement and care be borne by the two (2) Federal Departments as a research study of importance to the health of animals of the United States.

Secretary
I need to increase foreign animal disease surveillance and for greater laboratory disease identification responsiveness to protect our livestock industry. In planning for emergency animal disease epizootics it is clear that extensive technical laboratory support is needed within or near the problem area. Current centralized information is needed as to location of existing diagnostic laboratories with specific information on their capabilities including size, biological security status, and personnel staffing.

RESOLUTION #3

Be it resolved that USAHA recommends to USDA, APHIS, the forming of an emergency program laboratory unit at Ames, Iowa to be staffed with sufficient personnel to conduct necessary examinations and to maintain close liaison with state, university, and other laboratories including sufficient personnel contacts for the unit to have current knowledge of laboratory capabilities in the various states.

Secretary
BACKGROUND INFORMATION

An urgent need for procurement of essential information relating to the inter-relationship of numerous exotic diseases transmissible between wildlife, domestic livestock, and poultry has been recognized and documented via resolutions adopted by the International Association of Game, Fish and Conservation Commissioners (IAGFCC), The National Association of State Departments of Agriculture (NASDA), The Southeastern Association of Game and Fish Commissioners (SAGFC), and the Southern Animal Health Association (SAHA), and;

Whereas, the United States Animal Health Association (USAHA) concurs with the need and concern expressed by the above named associations.

RESOLUTION # 1.

Be it resolved that the United States Animal Health Association urge Congress to support a minimum annual appropriation of $500,000 in the U. S. Department of Agriculture's budget to continue and accelerate through cooperative effort the vital function of the Southeastern Cooperative Wildlife Disease Study.
BACKGROUND INFORMATION

An urgent need for procurement of essential information relating to the inter-relationship of numerous exotic diseases transmissible between wildlife, domestic livestock, and poultry has been recognized and documented via resolutions adopted by the International Association of Game, Fish and Conservation Commissioners (IAGFCC), The National Association of State Departments of Agriculture (NASDA), The Southeastern Association of Game and Fish Commissioners (SAGFC), and the Southern Animal Health Association (SAHA) and the United States Animal Health Association (USAHA) concurs with the need and concern expressed by the above named associations;

RESOLUTION

NOW BE IT RESOLVED, that USAHA urges Congress to support a minimum annual appropriation of $500,000 in the U. S. Department of Agriculture's budget to continue and accelerate through cooperative effort the vital function of the Southeastern Cooperative Wildlife Disease study.
UNITED STATES ANIMAL HEALTH ASSOCIATION

Resolution No. 7

Seventy-eighth Annual Meeting

Held at Roanoke, Virginia

Dates October 13-18, 1974

Source

Subject Matter Mastitis

BACKGROUND INFORMATION

Post-milking dipping of teats in an effective, non-irritating, residual germicide coupled with dry cow therapy has been proven to be an effective means of mastitis control and there is presently no control over the efficacy or safety of the many products offered for sale as teat dip, and some teat dips products have been implicated in serious herd mastitis problems.

RESOLUTION

NOW, THEREFORE BE IT RESOLVED THAT the U. S. Animal Health Association recommends that the Bureau of Veterinary Medicine, F.D.A., in consultation with the National Mastitis Council and other appropriate authorities establish without delay criteria for effective teat dips and institute measures whereby teat dips shall be proven safe and effective prior to marketing.
UNITED STATES ANIMAL HEALTH ASSOCIATION

Resolution No. 8 - Seventy-Eighth Annual Meeting
Held at Roanoke, Virginia Dates Oct 13-18, 1974
Source
Subject Matter Foreign Animal Diseases

BACKGROUND INFORMATION

1. Hog Cholera was diagnosed in a backyard herd of swine in Hidalgo County in 1973.
2. VEE penetrated into the United States from Mexico in July of 1971. Cost of eradicating this disease in Texas was about $19,000,000.
3. Velogenic viscerotropic Newcastle disease was introduced into El Paso, Texas in 1974.
4. Texas fever and fever ticks have been introduced into Texas along a buffer zone abutting the Texas-Mexican border on several occasions.

Regulations to Safeguard Against Importation of Animal Sub-human Ectoparasites

It is now established that the dog louse fly or kid fly, Hippobosca longipennis, an ectoparasite of African felids and canids was unintentionally introduced into the United States in May, 1970 and at later dates. The fly is known to be present at wild animal parks or zoos in California, Oregon, Texas and Georgia.

RESOLUTION

Be it resolved that the United States Animal Health Association request the United States Public Health Service's Animal and Plant Health Inspection Service formulate regulations that would require all non-primates imported into the United States be subjected to inspections for ectoparasites. Said regulations should further require that all animal shipping crates and bedding materials be subjected to treatment (spraying, dusting or dipping) with suitable pesticides as determined by the expertise existing in the department to prevent future importations of ectoparasites from foreign countries.
UNITED STATES ANIMAL HEALTH ASSOCIATION

Resolution No. 9
Seventy-eighth annual Meeting
Held at Roanoke, Va.
Dates Oct. 13-18, 1974
Source
Subject Matter: Foreign Animal Diseases (Regulations to Safeguard Against Importation of Animal Ectoparasites)

BACKGROUND INFORMATION

It is now established that the dog louse fly or kid fly, *Hippobasca longipennis*, an ectoparasite of African felids and canids was unintentionally introduced into the United States in May, 1970 and at later dates. The fly is known to be present at wild animal parks or zoos in California, Texas, Oregon and Georgia.

RESOLUTION

BE IT RESOLVED that United States Animal Health Association request the Department of Agriculture's Animal and Plant Inspection Service formulate regulations that would require all mammals imported into the United States be subjected to treatment(spraying, dusting or dipping) with suitable pesticides as determined by the expertise existing in the department, to prevent future importations of ectoparasites from foreign countries.
There is a need for continuing a Venezuelan Equine Encephalitis surveillance program in the United States and recognizing the cyclic nature of VEE and its history of extensions into new territories, there is a recognized need to protect our foreign horse markets and international racing interests; we recognize the zoonotic aspect of VEE which places horses and other equidae as the major amplifying host of the virus with subsequent very real exposure of man to this virus;

BE IT RESOLVED that the United States Animal Health Association urges that the Secretary of Agriculture continue surveillance of VEE through:
1) Liaison with Mexican Animal Health Officials; and 2) Investigations and laboratory confirmation of horse illness and deaths suggestive of encephalitis; and 3) monitoring serological responses in certain small mammals and trapping of mosquitoes in States bordering old Mexico.
Certain devastating diseases and parasites of animals and poultry are currently endemic in Mexico but not the United States and in the past Venezuelan Equine Encephalomyelitis, hog cholera, velogenic vicerotropic Newcastle disease, screw worms, and tick fever have escaped Mexico and entered this country and highly organized illicit movements of animals, poultry personnel, materials or food products of animal origin are allegedly widespread across the international borders in both directions.

NOW THEREFORE BE IT RESOLVED that this committee recommends the establishment of a high intensity animal and poultry disease and parasite surveillance zone on the United States side of the Mexico-United States borders with sufficient inspectors, veterinarians, and laboratory support to quickly detect and stamp out animal and poultry diseases and parasites that may gain further access into this country and further, that in these efforts the active participation of Mexican government agencies be respectfully solicited.
BACKGROUND INFORMATION

The socio-economic problem was a major factor in the exotic Newcastle disease eradication program in California, and it appears clear that socio-economic factors will have a major impact on any wide scale future animal disease emergency eradication program.

RESOLUTION

THEREFORE BE IT RESOLVED, that the USAHA urge the USDA, APHIS to initiate studies immediately to determine socio-economic impact of the major foreign animal diseases which are most likely to gain entry into the United States.
UNITED STATES ANIMAL HEALTH ASSOCIATION

Resolution No. 13 Seventy-eighth Annual Meeting
Held at Roanoke, Virginia Dates October 13-18, 1974
Source Subject Matter Horses

BACKGROUND INFORMATION

In an effort to control EIA, 17 states now require and, in the near future, several more are expected to require that all horses have a negative EIA test prior to entering the state. USDA does not now require that imported horses be tested or authorize means whereby all importers may obtain recognized tests in the United States prior to importation.

Infected horses have been and continue to be imported thus compounding domestic control problems and this problem has been for many months and remains in an "open ended" position, a prompt solution is imperative;

RESOLUTION

THEREFORE, BE IT RESOLVED that the U. S. Animal Health Association recommends that the Secretary of Agriculture immediately exercise interim authority to require that all horses and other equidae offered for importation into the United States be officially tested for Equine Infectious Anemia and if found negative and otherwise eligible be allowed to enter the United States.
Presently a seven day quarantine with temperature recording is necessary for all horses imported into the United States from the Western Hemisphere (Canada 60-day residence or longer expected) and vaccination for VEE using TC 83 Vaccine has been shown to withstand the challenge of epidemic VEE virus;

RESOLUTION

BE IT RESOLVED that the United States Animal Health Association urges the Secretary of Agriculture to take action to change the present requirements relating to VEE on horses and other equidae offered for importation from the Western Hemisphere (except U.S. horses returning from Canada or horses kept in Canada during the preceding 60 days) to require that such animals have temperatures recorded on initial inspection at the quarantine station and on the day of release (when dourine, glanders or equine piroplasmosis test results are known). If temperatures do not exceed 102°F and the temperature spread does not exceed 2°F and the animal is clinically well and otherwise eligible for release that it be released for importation into the United States with the additional stipulation that the animal be accompanied by a satisfactory certificate showing evidence of vaccination with TC 83 vaccine not less than 14 days nor more than 12 months prior signed by a veterinarian.
BACKGROUND INFORMATION

Zebras frequently are offered for importation into this United States. Zebras do harbor the blood parasites, Babesia caballi and Babesia equi as inapparent infections;

RESOLUTION

BE IT RESOLVED that the United States Animal Health Association urge that the Secretary of Agriculture promptly implement a requirement that would cause all zebras offered for importation to be tested by the Complement Fixation test and only those found negative to that test be entered.
BACKGROUND INFORMATION

Tuberculosis in zoo and exhibition animals has become a problem of increased frequency and this serves as a potential danger of spread of this disease to our livestock population and this also serves as a public health hazard.

RESOLUTION

THEREFORE BE IT RESOLVED that consideration be given to the development of interstate and intrastate regulations for the control of movement of these animals.
BACKGROUND INFORMATION

Meat and poultry inspections regulations were amended regarding the disposition of bovine tuberculin reactors and this has resulted in nearly every packing plant refusing to slaughter known reactors and this has resulted in severe economic losses to the livestock industry.

RESOLUTION

THEREFORE BE IT RESOLVED that action be taken by the State and Federal Departments of Agriculture to increase the rate of indemnity for tuberculin reactors to a more adequate level.
Resolution No. 18

Seventy-eighth Annual Meeting

Held at Roanoke, Virginia

Dates October 13-18, 1974

Source

Subject Matter Tuberculosis and Johne's Disease

BACKGROUND INFORMATION

Meat and poultry inspection regulations were amended regarding the disposition of swine found tuberculosis at slaughter and this has resulted in severe economic losses to the swine industry;

RESOLUTION

THEREFORE BE IT RESOLVED that adequate funds be made available to implement a swine tuberculosis control and eradication program and that research into this disease be accelerated.
UNited States Animal Health Association

Resolution No. 19 Seventy-Eighth Annual Meeting
Held at Roanoke, Virginia Dates October 13 - 18, 1974
Source
Subject Matter Parasites and Parasiticides

Background Information

The cooperative cattle scabies eradication program has successfully eradicated psoroptes and sarcoptic cattle scabies from the greater part of the United States and thus has contributed greatly to directly reducing losses, caused by disease, to producers and indirectly greatly benefited consumers which include all our citizens.

Due to the fiscal difficulties now being suffered by Veterinary Services, APHIS, USDA, there is great danger that funds and Federal participation will be withdrawn from the cattle scabies eradication program.

Resolution

Therefore, be it resolved that the USDA be urged to continue its funding and participation in the cattle scabies program so as to protect the considerable success enjoyed up to this date and so that cattle scabies, like sheep scabies, can be completely eradicated from the U. S.

Proposed Revision of Constitution and Bylaws - Board of Directors

Line 70—After the word “officers,” add the following, “the immediate Past President,”

Approved by the Executive Committee at the meeting in Roanoke, Virginia, October 13-18, 1974.
REPORT OF THE AD HOC EPIZOOTIC ATTACK COMMITTEE

Chairman: L. E. Bartelt, Sacramento, California
Co-Chairmen: R. Bankowski, Davis, California; C. L. Campbell, Tallahassee, Florida; Joe Finley, Encinal, Texas; H. E. Goldstein, Columbus, Ohio; R. P. Hanson, Madison, Wisconsin; R. E. Omohundro, Hyattsville, Maryland; T. G. Murnane, Washington, D.C.; F. A. Hayes, Athens, Georgia.

First the Committee commends USDA Veterinary Services Emergency Programs for its development of regional emergency teams that may be called to duty in an emergency, and, their efforts to have viable units by holding test exercises to develop the concept to provide the nucleus of a strike force capable of effective timely response.

After considering options of control with those of eradication and weighing them against historical results combined with economic impacts, this committee concluded that USDA should in conjunction with the states, and the livestock and wildlife industries involved, use every means available to keep eradication as the goal for those foreign exotic diseases such as Foot and Mouth, African Swine Fever, and others in a group of 20 or more which USDA Emergency Programs consider significant.

Three primary topics for deliberation taken up were:
1. Emergency Disease Manuals for field use.
2. Test exercise for purpose of developing policy option principles in the face of a difficult emergency disease outbreak.
3. Need to keep available current centralized knowledge on the location and type of existing local laboratory facilities, fields in which each laboratory has personnel trained, and the degree to which security could or is being accomplished at each facility.

Manuals available for review were Emergency Animal Disease Eradication Guide, African Swine Fever and Hog Cholera Field Manuals, and a preliminary draft of a Newcastle Disease Field Operations Guide.

It was recommended that pocket disposable editions be developed for field personnel use so that they could be used at the site of field outbreaks. Check lists to guide personnel are needed and to aid effectiveness certain diseases could be combined into one handbook such as Hog Cholera and African Swine Fever.

Many additional specific suggestions on the existing manuals were made as to contents designed to improve their function. These were discussed with USDA personnel at the meeting.

While the manuals discussed the importance of wildlife in the spread of epizootic diseases and stressed the importance of gaining cooperation of all local state and Federal agencies involved, the wildlife sections of these manuals have not been endorsed by any segment of those agencies responsible for the wildlife resources of this country. For the proposed participation to be realistic wildlife interest must be involved in policy making.

Neither can it be overemphasized that a majority of the wildlife resources of the United States come under State jurisdiction which must be taken into account in the preplanning for handling of a devastating foreign
animal disease.

For the second topic a test exercise was partially developed to illustrate many policy problems faced if Foot and Mouth should break in one of our more concentrated dairy populations. Need to develop socio-economic information prior to emergencies and to have the services of one or more full-time economists during a major outbreak was stressed.

Proven tested and tried eradication principles should be used, but any task force should have the policy development potential in its leadership to bring in other concepts where both the economic and biological feasibility of eradication efforts are not compromised.

To complicate thinking on policy development, the test exercise considered a hypothetical foot and mouth outbreak in a ten-mile area in diameter containing 125,000 dairy cows in about 300 dairies with the largest single dairy complex having 8,000 cows on 120 acres. Consideration was given to conventional slaughter and disposal methods along with salvage options and their economic impact on feasibility in accomplishing a goal of eradication.

Current planning is necessary to handle possible future outbreaks if we are to meet our new environmental legal restrictions, but it was believed that new existing technology could be brought to bear on disposal methods where salvage may not be feasible or consistent with eradication goals. It appears that these disposal systems could be developed to meet legal requirements under emergency conditions.

The final topic considered the need for existing laboratory location information necessary for quick decision in the face of an emergency disease outbreak. It was felt that biological security emphasis would dictate the use of an existing laboratory if one was available or to establish an emergency temporary laboratory within the operations area for any major outbreak.

Four proposed resolutions were considered by the committee and are being forwarded to the resolutions committee for their consideration.
THE MINNESOTA DISEASE REPORTING SYSTEM FOR FOOD PRODUCING ANIMALS

Diesch, S. L., Martin, F. B., Johnson, D. W., and Christensen, L. T.
St. Paul, Minnesota

History

In 1970, members of Infectious Disease Committee (IDC) of the Minnesota Veterinary Medical Association (MVMA) were requested to develop a meaningful disease reporting system for the collection of infectious disease information. This followed the discontinuation of a reporting system which consisted of a post-card and monthly questionnaire (mail back) system by practicing veterinarians to the Minnesota Livestock Sanitary Board (MLSB) indicating number of cases of selected diseases. This system had been in effect since 1955. It was considered largely unsuccessful by a response of 6-8% during 1968-1970. Since population data (denominator) was not available and incidence could not be determined, meaningful data was not being obtained. This system had been jointly sponsored by the MLSB and the MVMA. The committee felt that it is the responsibility of all members of the veterinary profession and MLSB, by license and by law, to maintain the health of animals by prevention, control and eradication of disease. For many years the livestock industry, the veterinary medical profession, education and research institutions and disease control agencies have operated without meaningful morbidity and mortality data on diseases of animals.

The purpose of collecting information on infectious diseases developed by the committee was as follows:

1. Surveillance of disease is essential to the private practitioner to recommend proper preventive measures to the livestock industry and other animal owners.
2. To assist in control and prevention of disease the agencies must have knowledge of when, where and under what conditions disease occurs. It is the responsibility of an agency to compile, edit, publish and distribute this information.
3. Improved definition and control of the disease problems will reduce economic loss to the livestock industry.
4. It will encourage a better understanding of the value of veterinary service and early attack on disease.
5. Defines the problems for veterinary research and justifies research.
6. Reporting gives us:
   a. Indicators of incidence and changing trends.
   b. Geographic distribution of diseases.

Drs. Diesch and Johnson are members of the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul and Co-Chairman of the Infectious Disease Committee of the Minnesota Veterinary Medical Association. Dr. Martin is Director of the Statistical Center and member of the Department of Applied Statistics, University of Minnesota and a consultant member of the Infectious Disease Committee, MVMA. Dr. Christensen is a member of the Brucellosis Division of the Minnesota Livestock Sanitary Board, St. Paul and a member of the Infectious Disease Committee, MVMA.
c. Knowledge of emergence of new diseases.

d. Basis for certification of lack of diseases.

During 1971 four systems of disease reporting were developed and implemented, (1) Food producing animals, (2) Poultry (turkeys), (3) Equine, and (4) Small animals. This paper will describe only the System for Food Producing Animals.

**Development of the System.**

The basis for the present operating system is statistical estimation of population wide rates based on small but adequate probability samples of farmsteads having food producing animals. The animals selected in this small sample are subjected to actual total disease surveillance, which is quite impossible on a state wide basis.

The flow of information is based on disease morbidity information reported monthly by practicing veterinarians and their clients to the District Veterinarian (State or Federal) into the MLSB (located in St. Paul). There the information is tabulated and quarterly and annual reports printed out to be returned by reverse flow to the veterinarians, farmers and livestock industry.

**Schematic of Disease Reporting:**

```
+-----------------+       +-----------------+       +-----------------+
|             |       |             |       |             |
| Farm        |       | Farm        |       | Farm        |
|             |       |             |       |             |
| Practicing Veterinarian |       | Practicing Veterinarian |       | Practicing Veterinarian |
|             |       |             |       |             |
| District Veterinarian |       | District Veterinarian |       | District Veterinarian |
|             |       |             |       |             |
| Minnesota Livestock Sanitary Board |       | Minnesota Livestock Sanitary Board |       | Minnesota Livestock Sanitary Board |
| St. Paul |       | St. Paul |       | St. Paul |
|             |       |             |       |             |
| Computer Center |       | Computer Center |       | Computer Center |
| U of M       |       | U of M       |       | U of M       |
| St. Paul     |       | St. Paul     |       | St. Paul     |
```

The forms and guideline used in reporting by veterinarians and farmers were developed by members of the IDC with input from practicing veterinarians and members of the MLSB and Veterinary Services, APHIS, USDA. Individual report forms for the veterinarians and the farmers were developed for beef herds, feeders, dairy herds, swine, and sheep. (Forms and guidelines used are attached). In development of the list of the diseases, efforts were made to make it complete, but not so complicated that the veterinarian and farmer involved would not participate. Of utmost importance is that each month, animal populations found on each farm is reported. (Guidelines used for reporting disease are attached.) However, the
reporting does not truly indicate population at risk, because exposure experience and vaccination information status may be unknown.

Statistical Design:

The plan or design of the sampling procedure had to realize a number of constraints. The population studied is intended to be all food producing animals in the state of Minnesota which reside on farmsteads maintained as clients of members of the veterinarians of the MVMA. The sampling unit is an animal month. (See attached guidelines.) An animal kept under surveillance for one month constitutes one sample point. The monthly reports are compiled to prepare a quarterly (seasonal reviews) and a special annual report.

The quarterly reports show incidence, defined here to be the number of new cases of a disease observed, divided by the number of animal quarters kept under surveillance. An animal quarter is simply defined to be the sum of 3 animal months (sampling units) occurring in that quarter and can be construed to possibly involve different animals. This flexibility allows for the expected loss or gain of a few reporting farmsteads at any month of the year and movement of animals onto and off the farmland. The example report (attached) for the dairy herd category in the first quarter of 1973 indicates that 4955 adult cow quarters of 14,865 cow months were observed. It does not indicate the exact number of distinct cows observed which might be slightly larger, due to these dynamic forces.

The sampling plan was largely determined by the structure available for the efficient flow of information and voluntary cooperation. This resulted in a multistage cluster sampling procedure with approximately uniform density in relation to the geographical distribution of animal production activity in the State. An equal sharing of the burden of this activity is almost a necessity. So an equal number of practices are sampled in each district and an equal number of farmsteads are sampled in each practice. If all these clusters are about equal in size (i.e. equal number of practices per district, equal number of farms per practice, and equal number of animals per herd) simple estimators of rates, in number of cases per thousand defined by

\[
\frac{\text{number of disease cases observed}}{\text{number of animal quarters observed}} \times 1000
\]

are reasonably unbiased. Sophisticated estimation techniques have been developed but the intricate programming required to implement them has not at this time been developed. In light of considerable opportunity for preferential biases inherent in this voluntary system very sophisticated calculation would serve to be a bit optimistic.

Implementation

Implementation began on July 1, 1971, with forty practicing veterinarians selected by the IDC. These 40 veterinarians had been recommended by officers of local Veterinary Medical Associations and District Veterinarians. The veterinarians indicated their willingness to cooperate if the reporting would result in meaningful information. Each was invited
to participate on a voluntary, unpaid basis. They were each asked to submit a list of clients that they felt would report. From this list the IDC selected up to 15 farms which were invited to participate by the practicing veterinarian. The forms were identified by code numbers. Only the practitioner knew the farmer identity.

Contact was made with the identified practitioner by a letter from the MLSB. Subsequently, the District Veterinarians delivered reporting forms and explained the system to each practicing veterinarian located in his district. The veterinarian developed his own method of implementing the system with his farmer clients.

During the second year — 1972-73, the veterinarians reporting the previous year were asked to participate for another year, the majority consented. Additional veterinarians for reporting were selected in order to reduce the number of farms reported by a veterinarian from 15 to 8, thereby reducing the work-load of reporting for each reporting veterinarian to obtain the desired 500 farms. However, this increased the work load of the District Veterinarian.

For the 1973-74 reporting year a method of selection similar to that of the previous year was used.

For the 1974-75 reporting year the selection method was changed. A list of all veterinarians engaged in large animal practice was compiled. The selection was made from a table of random numbers. Some of the veterinarians selected by chance had previously reported for at least two years. Each veterinarian was notified of his selection by the MVMA and subsequently, contacted by the District Veterinarian. Each veterinarian who volunteered to participate was asked to submit a list of twenty or so farm units on which he could reasonably expect good cooperation with the owner. From each veterinarian’s list, several farmsteads were selected at random by each of the District Veterinarians.

**Reporting Results.**

<table>
<thead>
<tr>
<th>Year</th>
<th>Veterinarians Desired</th>
<th>Veterinarians actually Participating</th>
<th>Farms Desired</th>
<th>Farms Reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td>1971-72</td>
<td>40</td>
<td>28</td>
<td>500</td>
<td>200</td>
</tr>
<tr>
<td>1972-73</td>
<td>57</td>
<td>39</td>
<td>500</td>
<td>338</td>
</tr>
<tr>
<td>1973-74</td>
<td>60</td>
<td>23</td>
<td>500</td>
<td>182</td>
</tr>
<tr>
<td>1974-75</td>
<td>50</td>
<td>27</td>
<td>500</td>
<td>*</td>
</tr>
</tbody>
</table>

*Data unavailable

**Cost of Program**

The St. Paul Campus Computer Center of the Minnesota Agricultural Experiment Station routinely process the quarterly or annual data at a nominal annual expense and returns computer (IBM 360-30) output to the MLSB for their use in publication and distribution. An Agriculture Experiment Station research grant funded the initial costs of programming the computation package which is kept on a very low maintenance basis for
periodic use on the quarterly and annual reports. An efficient coding scheme used by the MLSB puts the raw data into the program input format ready for routine keypunching and subsequent print-out. The assigned cost of this program by the MLSB (state) for 1973-74 fiscal year is personnel $21,379 and supplies $5,284, Total cost $26,663. There is, in addition, the contributed time of the practicing veterinarians and the farmers.

Utilization of Data

Since the valid incidence rates for most diseases are not available, it was necessary to accumulate baseline information before utilization of data of quarterly and annual incidence data. This straight numbers form of reporting has not proven to be very useful to practicing veterinarians. They have requested a more descriptive type of interpretation be made of the data. The IDC and the MLSB have begun efforts to compare quarterly incidence rates to detect a change in disease incidence. The high incidence of diseases such as enteric disease of calves has pointed out critical problem areas.

Some interpretation of reporting information has been published in widely circulated farm magazines and extension newsletters. This information should be of direct value and interest to the livestock producers and all industries and agencies which work with and for the producers.

The information has been utilized as supportive documentation of the disease problems in the development of research proposals. We are of the opinion that this is the most reliable information available.

This data has been utilized by the MLSB. The incidence rates of swine dysentery were used for the Governors Committee in Swine Health Problems. Division heads of the MLSB watch disease incidence. The MLSB publishes and distributes quarterly and annual reports. In the future the MLSB will point out patterns of disease in seasonal incidence and increasing and decreasing occurrence. This will determine disease problems requiring regulatory attention.

Veterinary Services personnel of APHIS (USDA) review the reports. The information gained does enter into their cooperative programs with the State.

An example of the value of some of the data reported is as follows:

One can estimate the size of the calf scours problem by checking the Minnesota agricultural statistics. If 378 calves per 1,000 from the 971,000 milk cows that calved in 1972 had scours, this would mean 367,038 dairy calves were involved. Nearly half the calves with scours die.

In 1972 mastitis was reported in 454 cases per 1,000 animal years. Of these, 10% were diagnosed as to causative organisms. *Streptococcus agalactiae* mastitis, which can be cured and eliminated from the herd, represented 40% of the mastitis diagnosed. The more difficult to control, *Staphylococcus aureus* mastitis, represented only 14% of this group.

Respiratory disease (pneumonia, IBR, shipping fever, etc.) were reported in 435 out of 1,000 dairy animals. Of 70% of the cases identified further, over half were reported as calf pneumonia.

*Refers to 1000 animals observed for one year.
External parasites (lice) was also a common condition reported in 1972. The incidence was 396 per 1,000 dairy animal years. Abortions at the rate of 75 per 1,000 dairy female years indicates tremendous losses.

Of swine diseases, mange was the most frequently reported disease in 1972 and 1973, 271 cases per 1,000 swine in 1972, compared with 217 in 1973. Diarrheal diseases remained about the same, 73 cases per 1,000 swine in 1972 and 65 cases per 1,000 in 1973.

Abscesses, decreased from 45 per 1,000 to 17. Fewer cases of swine influenza were reported from July through September, '73 than in the same period in '72.

In sheep, the reported cases of internal parasites remained about the same for the third quarter of both years — 793 cases per 1,000 animals in 1972, compared with 746 in 1973.

As in 1972, respiratory diseases remained a most serious condition in beef herds in 1973. There were 510 cases per 1,000 in 1972. Most of these occurred in calves and heifers.

In cattle, more cases of pink-eye were reported in 1973 (161 per 1,000) than in 1972 (129 per 1,000). The opposite was true for footrot — a decrease from 69 cases per 1,000 in 1972 to only 8 per 1,000 in 1973. Reported cases of diarrheal diseases remained about the same — 18 per 1,000 and 21 per 1,000 for the third quarters of these years, respectively.

Problems with System

Although the veterinarians' cooperation and willingness to report has been excellent, tabulating data and mailing in reports for agencies does not receive a high priority with many practitioners and their clients. However, the Minnesota system has been very successful in obtaining the contributed input of a number of practicing veterinarians and farmers. Motivating and continuing the reporting system has required an increasing amount of effort from the District Veterinarians, since disease reporting is only one of many responsibilities of the District Veterinarian, only limited time effort (5%) is given to the system.

During the past four years excellent support has been given by the leadership and members of the MVMA. Their cooperation and efforts have been an important aspect of this system.

We realize that disease data available from the Veterinary Diagnostic Laboratory, Meat Inspection, and Control and Eradication programs of the MLSB and VS of APHIS has not been adequately utilized in the development of a more complete system.

Most of the morbidity reporting in this system is based on clinical diagnosis. As previously mentioned we do not, in many instances, receive a precise picture of the population risk, or the number of susceptible animals in a farm population.

Diseases with low incidence have a reasonably high probability of not being reported. For example, leptospirosis which is known to occur each month in dairy cattle was not reported in the first quarter of 1973.

We have developed and encouraged the use of forms by the farmer to report disease observed and treated. How much of this disease has been verified by the practicing veterinarian is unknown.
It is increasingly difficult to obtain voluntary cooperation of practicing veterinarians. Some who have reported for two or three years may decide that they have made their contribution to the system and now it's someone else's obligation.

A consideration of financial payment to the veterinarian, or farmer or both has been discussed, but not implemented. It appears that the veterinarian and farmer should likely be remunerated for their efforts.

**Future Needs**

Since the system is now in its fourth year of implementation the greatest need is a critical evaluation of the system to determine if the system has useful reliability in spite of its potential for preferential biases. A proposal has been submitted to the USDA to support a critical evaluation of the Minnesota system. The system itself needs greater encouragement, recognition and financial support from Central Governmental Agencies of Agriculture and Health.

There is a need to study and determine the monetary losses associated with the disease morbidity being reported. The need remains for greater interpretation and utilization of data collected — to give practical feed-back to the local level and all aspects of government.

It is the authors opinion that the Minnesota system should be considered a potential model for use in other states or nationally.

**Acknowledgements**

The authors wish to acknowledge the cooperation and support given by the following individuals.

Dr. J. G. Flint, Secretary and Executive Officer, and Supervisory and District Veterinarians of the Minnesota Livestock Sanitary Board.

Dr. A. G. Spreitzer, District Veterinarian and Section Veterinarians of Minnesota for Veterinary Services, APHIS, USDA.

Participating veterinarians of the Minnesota Veterinary Medical Association.

Cooperating farmers of Minnesota.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIGLETS</td>
<td>SWINE</td>
</tr>
<tr>
<td>Abscesses</td>
<td>4.61</td>
</tr>
<tr>
<td>Abortion or Reproductive Diseases</td>
<td></td>
</tr>
<tr>
<td>Arucllosis</td>
<td></td>
</tr>
<tr>
<td>Leptospirosis</td>
<td></td>
</tr>
<tr>
<td>MHA (Mastitis, Mastitis, Agalactiae)</td>
<td>0.72</td>
</tr>
<tr>
<td>Pseudorabies</td>
<td></td>
</tr>
<tr>
<td>Sudden Stillbirths, Nunnifications, Embryonic Deaths, and Infertility</td>
<td>5.97</td>
</tr>
<tr>
<td>Etiology Undetermined</td>
<td></td>
</tr>
<tr>
<td>Arthritis Diseases</td>
<td></td>
</tr>
<tr>
<td>Corynebacteria</td>
<td>1.89</td>
</tr>
<tr>
<td>Erysipelas</td>
<td>2.27</td>
</tr>
<tr>
<td>Mycoplasma Arthritis</td>
<td>0.09</td>
</tr>
<tr>
<td>Streptococcal Arthritis</td>
<td>0.09</td>
</tr>
<tr>
<td>Etiology Undetermined</td>
<td>17.18 23.08</td>
</tr>
<tr>
<td>Dermatitis and External Parasites</td>
<td></td>
</tr>
<tr>
<td>Exudative Epidermatitis</td>
<td>3.78</td>
</tr>
<tr>
<td>Mange: Specify Etiology if Determined</td>
<td>101.55 163.08</td>
</tr>
<tr>
<td>Pediculosis</td>
<td>74.09 103.08</td>
</tr>
<tr>
<td>Ringworm</td>
<td>21.42 2.51</td>
</tr>
<tr>
<td>Swinepox</td>
<td>85.13 2.56</td>
</tr>
<tr>
<td>Etiology Undetermined</td>
<td>16.67 10.67</td>
</tr>
<tr>
<td>Diarrheal Diseases</td>
<td></td>
</tr>
<tr>
<td>Colibacillosis</td>
<td>65.22</td>
</tr>
<tr>
<td>Eczema</td>
<td>0.28</td>
</tr>
<tr>
<td>Intermedulic Encephalomyelitis</td>
<td></td>
</tr>
<tr>
<td>Transmissible Gastroenteritis</td>
<td></td>
</tr>
<tr>
<td>Etiology Undetermined</td>
<td></td>
</tr>
<tr>
<td>Eperythrozoonosis</td>
<td></td>
</tr>
<tr>
<td>Neurologic Diseases</td>
<td></td>
</tr>
<tr>
<td>Listeriosis</td>
<td></td>
</tr>
<tr>
<td>Rabies</td>
<td></td>
</tr>
<tr>
<td>Tetanus</td>
<td></td>
</tr>
<tr>
<td>Viral Encephalomyelitis</td>
<td></td>
</tr>
<tr>
<td>(Vomiting and Wasting Disease)</td>
<td></td>
</tr>
<tr>
<td>Etiology Undetermined</td>
<td></td>
</tr>
<tr>
<td>Parasitism—Internal</td>
<td></td>
</tr>
<tr>
<td>Lungworms</td>
<td></td>
</tr>
<tr>
<td>Roundworms</td>
<td></td>
</tr>
<tr>
<td>Other: Specify Etiology if Determined</td>
<td></td>
</tr>
<tr>
<td>Etiology Undetermined</td>
<td></td>
</tr>
<tr>
<td>Respiratory Diseases</td>
<td></td>
</tr>
<tr>
<td>Atrophic Rhinitis</td>
<td></td>
</tr>
<tr>
<td>Bull Nose</td>
<td></td>
</tr>
<tr>
<td>Mycoplasma Pneumonia</td>
<td></td>
</tr>
<tr>
<td>Pyloriitis</td>
<td></td>
</tr>
<tr>
<td>Etiology Undetermined</td>
<td></td>
</tr>
<tr>
<td>Diseases Seen Infrequently</td>
<td></td>
</tr>
<tr>
<td>Anthrax</td>
<td></td>
</tr>
<tr>
<td>Hug Chorea</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td></td>
</tr>
<tr>
<td>Vesicular Exanthema</td>
<td></td>
</tr>
<tr>
<td>Vesicular Stomatitis</td>
<td></td>
</tr>
</tbody>
</table>
### MINNESOTA INFECTIOUS DISEASE REPORTS FOR SHEEP HERDS

1ST CALENDAR QUARTER, 1973

IN NO. OF CASES PER THOUSAND ANIMALS

<table>
<thead>
<tr>
<th>Number of Lambs Observed</th>
<th>Number of Sheep Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>588</td>
<td>720</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lambs</th>
<th>Sheep</th>
<th>Etiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.44</td>
<td></td>
<td>Undetermined</td>
</tr>
<tr>
<td>6.80</td>
<td>1.39</td>
<td>Enterotuxemia</td>
</tr>
<tr>
<td>5.55</td>
<td></td>
<td>Arthritis</td>
</tr>
<tr>
<td>35.67</td>
<td>249.88</td>
<td>Contagious Ecthyma</td>
</tr>
<tr>
<td>3.40</td>
<td></td>
<td>Diarrhal Diseases</td>
</tr>
<tr>
<td>6.80</td>
<td>4.16</td>
<td>Etiology Undetermined</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lambs</th>
<th>Sheep</th>
<th>Etiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.94</td>
<td></td>
<td>Mastitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neurologic Diseases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Listeriosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Menigo-encephalitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rabies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tetanus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Etiology Undetermined</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parasites, internal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coccidiosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fowlis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemochysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lungworms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oestrous Ovis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tapeworms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichostongyles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Etiology Undetermined</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pododermatitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Respiratory Diseases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enzootic Pneumonia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Progressive Pneumonia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pasteurellosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sporadic Pneumonia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Etiology Undetermined</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diseases seen uncommonly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anthrax</td>
</tr>
</tbody>
</table>

THE MINNESOTA DISEASE REPORTING SYSTEM
<table>
<thead>
<tr>
<th>FEEDERS</th>
<th>FEEDERS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.54</strong></td>
<td><strong>0.26</strong></td>
</tr>
<tr>
<td>ABSCESES</td>
<td>PNEUMOENCEPHALITIS</td>
</tr>
<tr>
<td>ACTINOBACILLOSIS</td>
<td>LISTERIOSIS</td>
</tr>
<tr>
<td>0.26</td>
<td>PSEUDO RABIES</td>
</tr>
<tr>
<td>ACINORRHINIS</td>
<td>PARTIES</td>
</tr>
<tr>
<td>ARTHRITIS</td>
<td>TITANUS</td>
</tr>
<tr>
<td>0.26</td>
<td>THROMBOEMBOLIC-PNEUMOENCEPHALITIS</td>
</tr>
<tr>
<td>ETIOLOGY IF DETERMINED</td>
<td>ETIOLOGY UNDETERMINED</td>
</tr>
<tr>
<td>0.77</td>
<td>PARASITISM-INTEPHAL</td>
</tr>
<tr>
<td>ETIOLOGY NOT DETERMINED</td>
<td>0.77</td>
</tr>
<tr>
<td>CLAUDRIAL DISEASES</td>
<td>COCCIDIOSIS</td>
</tr>
<tr>
<td>BLACK LEG</td>
<td>FLUKES</td>
</tr>
<tr>
<td>ENTEPTOXEMIA</td>
<td>HAEMONCHOSIS</td>
</tr>
<tr>
<td>MALIGNANT EDema</td>
<td>LUNGWORMS</td>
</tr>
<tr>
<td>ETIOLOGY UNDETERMINED</td>
<td>TAPEWORMS</td>
</tr>
<tr>
<td>DIARRHEAL DISEASES</td>
<td></td>
</tr>
<tr>
<td>1.80</td>
<td>TRICHOHYALYSIS</td>
</tr>
<tr>
<td>BVD</td>
<td>ETIOLOGY UNDETERMINED</td>
</tr>
<tr>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>SALMONELLOSIS</td>
<td>74.64</td>
</tr>
<tr>
<td>7.44</td>
<td>TRICHODERMA</td>
</tr>
<tr>
<td>ETIOLOGY UNDETERMINED</td>
<td></td>
</tr>
<tr>
<td>DERMATITIS AND EXTERNAL PARASITIS</td>
<td>10.00</td>
</tr>
<tr>
<td>CUTANEOUS MYIASIS</td>
<td>PODD DERMATITIS</td>
</tr>
<tr>
<td>30.47</td>
<td></td>
</tr>
<tr>
<td>HYPERDERMA</td>
<td>RESPIRATORY DISEASES</td>
</tr>
<tr>
<td>BVD</td>
<td>4.10</td>
</tr>
<tr>
<td>4.10</td>
<td>CALF DIPHTHERITE (NECROTIC LARYNGITIS)</td>
</tr>
<tr>
<td>WARTS</td>
<td>2.05</td>
</tr>
<tr>
<td>19.24</td>
<td>MALIGNANT CATARRHAL FEVER</td>
</tr>
<tr>
<td>KINGWORMS</td>
<td>19.75</td>
</tr>
<tr>
<td>4.36</td>
<td>PASTURELLISIS</td>
</tr>
<tr>
<td>INFECTIOUS KERATITIS</td>
<td>46.17</td>
</tr>
<tr>
<td>LEPTOSPIROSIS</td>
<td>SHIPPING FEVER</td>
</tr>
<tr>
<td>ETIOLOGY UNDETERMINED</td>
<td>4.36</td>
</tr>
<tr>
<td>MALIGNANT LYMPHOMA (LEUKEMIA)</td>
<td>ETIOLOGY UNDETERMINED</td>
</tr>
</tbody>
</table>

**Number of Feeders Observed:** 789
MINNESOTA INFECTIOUS DISEASE REPORTS FOR BEEF HERDS
1ST CALENDAR QUARTER, 1973
IN NO. OF CASES PER THOUSAND ANIMALS

CALVES HEIFERS ADULTS

5.79 9.66 50.62 ANIMAL DEATHS
14.42 ABORTIONS
4.96 UNICELLULOSIS
19.35 IDF
27.03 LEPTOSPIROSIS
3.27 TRICHOHYMAMIS
14.69 Vibriosis
10.3 OTHER CAUSES NOT LISTED ABOVE
6.66 7.53 ETIOLOGY UNDETERMINED
19.32 ACTINOMYCOSIS
10.3 ACTINOMYCOSIS
10.3 ARTHRITIS
4.96 ETIOLOGY IF DETERMINED
3.27 ETIOLOGY NOT DETERMINED
4.96 CLOSTRIDIAL DISEASES
19.35 H Clash LEG
27.03 Leptodermia
3.27 MALIGNANT EDema
3.27 ETIOLOGY UNDETERMINED
14.42 DIARRHEAL DISEASES
4.96 EVD
4.96 CILIACIALOSIS
24.69 PARATUBERCULOSIS
27.03 SALMONELLOSIS
14.42 WINTER DYSENTERY
3.27 ETIOLOGY UNDETERMINED
14.42 DERMAITIS AND EXTERNAL PARASITES
3.27 CUTANEOUS MYIASIS
3.27 HYMNIFORTA
35.27 MANIPELIDENY IF DETERMINED
17.31 PUBICULOSIS
4.96 PSORIACOSIS
42.91 20.99 RHINOMYIASIS
4.96 ETIOLOGY UNDETERMINED
8.02 INFECTIOUS KAPITIS
1.74 LETHALIS
1.74 INFECTIOUS KAPITIS
2.5 INFECTIOUS KAPITIS
2.5 INFECTIOUS KAPITIS
1.74 MELITIS

CALVES HEIFERS ADULTS

19.35 27.03 3.27 MASTITIS
19.35 3.27 CULITIDES
27.03 19.35 STAPHYLOCOCCAL
3.27 STR. GALACTICUM
14.42 ETIOLOGY UNDETERMINED
10.3 NEUROLOGIC DISEASES
3.27 MENINGOENCEPHALITIS
4.96 LISTEROSIS
24.69 PSUEDO RABIES
14.42 RABIES
3.27 TETANUS
3.27 PSEUDOMEMBRANOCERENNOENCEPHALITIS
19.35 ETIOLOGY UNDETERMINED
27.03 PARASITOSES INTERNAL
19.35 COCCIDIOSIS
3.27 FLUKES
3.27 HALMUNCHOSIS
3.27 LUNGWORKS
3.27 TAPEWORMS
3.27 TRICHOSTRONGYLES
19.35 ETIOLOGY UNDETERMINED
14.42 PODODERMATITIS INFECTIOUS FOOTROT
3.27 PYLUNPHARINX
14.42 PSPIRATORY DISASES
3.27 BVD
3.27 BARN PNEUMONIA (ADULTS)
3.27 CALF (GENEAL Y.
19.35 INZUOTIC PNEUMONIA (CALVES)
10.3 INR
19.35 MALIGNANT CATARRHAL FEVER
27.03 PSEUDOMEMBRANOCERENNOENCEPHALITIS
19.35 SHIPPING FEVER
19.35 SPOKLOCIDIC PNEUMONIA (ADULTS)
14.42 ETIOLOGY UNDETERMINED
14.42 DISEASES SEEN INFREQUENTLY
19.35 ANAPLASIAS
19.35 ANTHRAX
14.42 BLUF TRINGUL
19.35 TUBERCULOSIS
19.35 VISCULAR STOMATITIS
19.35
### MINNESOTA INFECTIOUS DISEASE OUTS FOR DAIRY HERDS
#### 1ST CALFMAIL QUARTER, 1973
#### IN NO. OF CASES PER THOUSAND ANIMALS

<table>
<thead>
<tr>
<th>Animal Type</th>
<th>Adult</th>
<th>Heifer</th>
<th>Calves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17.10</td>
<td>2.02</td>
<td>4.04</td>
</tr>
<tr>
<td></td>
<td>73.52</td>
<td>13.32</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>0.81</td>
<td>0.34</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.04</td>
<td>4.41</td>
</tr>
<tr>
<td></td>
<td>106.36</td>
<td>9.69</td>
<td>60.91</td>
</tr>
<tr>
<td></td>
<td>10.37</td>
<td>1.71</td>
<td>6.15</td>
</tr>
<tr>
<td></td>
<td>4.44</td>
<td>0.38</td>
<td>7.47</td>
</tr>
<tr>
<td></td>
<td>4.04</td>
<td>0.38</td>
<td>2.01</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.38</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>2.01</td>
<td>0.04</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>1.54</td>
<td>0.04</td>
<td>4.93</td>
</tr>
<tr>
<td></td>
<td>1.32</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>14.13</td>
<td>1.15</td>
<td>6.55</td>
</tr>
<tr>
<td></td>
<td>1.71</td>
<td>1.03</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>1.03</td>
<td>0.77</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>1.01</td>
<td>0.77</td>
<td>1.15</td>
</tr>
</tbody>
</table>

**Calves**
- MASTITIS
- COLIBACILLOSIS
- STAPHYLOCOCCAL
- TETANUS
- INFECTIOUS FOXTROT
- BARN PNEUMONIA (ADULTS)
- CALF DIPHTHERIA (NECK, LARYNX)
- ENZOOTIC PNEUMONIA (CALVES)
- MALIGNANT CATARRHAL FEVER
- SHIPPIING FEVER
- INFECTIOUS KERATITIS
- LEPTOSPIROSIS
- MALIGANT LYNPHOMA (LEUKEMIA)
- ANAPLASMOSIS
- ANTHRAX
- BLUE TONGUE
- TURFUCULOSIS
- VESICULAR STOMATITIS

**Adults**
- COLIBACILLOSIS
- STAPHYLOCOCCAL
- TETANUS
- INFECTIOUS FOXTROT
- BARN PNEUMONIA (ADULTS)
- CALF DIPHTHERIA (NECK, LARYNX)
- ENZOOTIC PNEUMONIA (CALVES)
- MALIGNANT CATARRHAL FEVER
- SHIPPIING FEVER
- INFECTIOUS KERATITIS
- LEPTOSPIROSIS
- MALIGANT LYNPHOMA (LEUKEMIA)
- ANAPLASMOSIS
- ANTHRAX
- BLUE TONGUE
- TURFUCULOSIS
- VESICULAR STOMATITIS

<table>
<thead>
<tr>
<th>Animal Type</th>
<th>Adult</th>
<th>Heifer</th>
<th>Calves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>73.52</td>
<td>13.32</td>
<td>4.44</td>
</tr>
<tr>
<td></td>
<td>4.44</td>
<td>13.32</td>
<td>4.44</td>
</tr>
<tr>
<td></td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>4.44</td>
<td>4.44</td>
<td>4.44</td>
</tr>
<tr>
<td></td>
<td>4.44</td>
<td>4.44</td>
<td>4.44</td>
</tr>
<tr>
<td></td>
<td>4.44</td>
<td>4.44</td>
<td>4.44</td>
</tr>
<tr>
<td></td>
<td>4.44</td>
<td>4.44</td>
<td>4.44</td>
</tr>
<tr>
<td></td>
<td>4.44</td>
<td>4.44</td>
<td>4.44</td>
</tr>
</tbody>
</table>

**Number of Calves Observed:** 2974
**Number of Heifers Observed:** 2603
**Number of Adults Observed:** 4955
1. A reporting farm consists of a reporting client's total livestock operation and it may include more than one species, more than one farm.

2. Report on each of the types of livestock (beef feeder cattle, beef cow-calf, dairy, swine and sheep) present on a farm only when ten or more head are present during the month.

3. Be sure population figures for each age group are present on the reporting form. Use the maximum number of animals present during the month.

4. If no disease is observed on a given farm during a reporting month, submit the monthly report with the livestock population on that farm.

5. Report actual number of animals that were clinically ill.

6. Count each case only once.
   a. Animals becoming ill during a month and continuing ill into subsequent month are not reported on the following month's report.
   b. Animals recovering within one month and becoming clinically ill ill again with same disease within the month — report only once. If they become ill with same disease in following month — report as a new case.

7. If a monthly report is not received from an owner for any reason, reports received in subsequent months from the same owner should be submitted.

8. Reports from owners received after your reports are submitted should be tabulated as occurring in the month diseases occurred and submitted with your next monthly report.

9. Laboratory confirmation is not required for reporting a disease condition.

10. When a sample has been submitted to the diagnosis laboratory, date of diagnosis shall be day you receive report from laboratory. Sample sent on 29th of month report returned on 5th of following month, the date of diagnosis shall be 5th.

11. If under any category of disease on the basis of clinical evidence or on basis of laboratory confirmation the etiology appears to be other than those listed, enter number of affected animals opposite Etiology Undetermined and write in the suspected or identified etiologic agent.

12. The number code used on the farmer's report form and veterinarian’s form are the same to facilitate transfer of the information. As an example — the owners report lists cases of "408 Early Calving" which would be entered on the veterinarians report under "Abortions 408 Etiology undetermined". However if the veterinarian had been consulted on the abortions and had made a diagnosis of Leptospirosis, the number of Lepto cases diagnosed should be entered at "Abortions, 404 Leptospirosis". The other abortions which were not diagnosed by the veterinarian would be entered at "408 Etiology Undetermined".

13. Monthly reporting dates:
   a. Livestock owner reports to his veterinarian by the 5th of the month.
## Infectious Disease Reporting Form for Swine Month of ________ 197-

**Important:** Record total number of animals in each age category

- **Young pigs (up to 8 months of age).**
- **Breeding Stock (over 8 months of age).**

Laboratory confirmation or diagnostic tests (check proper column if done)

<table>
<thead>
<tr>
<th>Record number of affected animals in appropriate age column</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DISEASE CONDITIONS</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>100 ABSCESS:</td>
</tr>
<tr>
<td>102 Abortion or Reproductive Disease:</td>
</tr>
<tr>
<td>104 Brucellosis</td>
</tr>
<tr>
<td>171 Mycoplasma arthritis</td>
</tr>
<tr>
<td>172 Streptococcal arthritis</td>
</tr>
<tr>
<td>173 Swine coccidiosis (stillbirths, Mummification, embryonic deaths and infertility)</td>
</tr>
<tr>
<td>174 Etiology undetermined</td>
</tr>
<tr>
<td>175 Corynebacteria</td>
</tr>
<tr>
<td>176 Erysipelas</td>
</tr>
<tr>
<td>177 Mycoplasma pneumonia (VPP)</td>
</tr>
<tr>
<td>178 Streptococcal arthritis</td>
</tr>
<tr>
<td>179 Etiology undetermined</td>
</tr>
<tr>
<td>181 Exudative dermatitis</td>
</tr>
<tr>
<td>124 Range: specify etiology if determined</td>
</tr>
<tr>
<td>125 Pseudomonas</td>
</tr>
<tr>
<td>127 Trichomonas</td>
</tr>
<tr>
<td>129 Etiology undetermined</td>
</tr>
<tr>
<td>130 Enterotoxemia (Clostridium perfringens Type C)</td>
</tr>
<tr>
<td>131 Salmonellosis</td>
</tr>
<tr>
<td>132 Transmissible Gastroenteritis</td>
</tr>
<tr>
<td>133 Vibrio cholera</td>
</tr>
<tr>
<td>134 Etiology undetermined</td>
</tr>
</tbody>
</table>

### DISEASE CONDITIONS
- 170 Eperythrozoonosis:
- 138 Neurologic Diseases:
- 140 Rabies
- 141 Tetanus
- 191 Viral encephalomyelitis

### Parasites, Internal
- 143 Etiology undetermined
- 147 Lungworms
- 192 Roundworms
- 193 Other: specify etiology if det.
- 150 Etiology undetermined

### Respiratory Diseases
- 194 Atrophic rhinitis
- 195 Bull nose
- 196 Mycoplasma pneumonia (VPP)
- 188 Polyarthritis
- 161 Spordic pneumonia
- 197 Swine influenza

### Diarrheal Diseases
- 162 Etiology undetermined

### Diseases Seen Infrequently
- 164 Anthrax
- 169 Hog cholera
- 166 Tuberculosis
- 168 Vesicular exanthema
- 167 Vesicular stomatitis

---

**The Minnesota Disease Reporting System**

b. Practicing veterinarians submit reports to district veterinarians by 10th of the month.

c. District veterinarians submit reports to Livestock Sanitary Board Of-
## INFECTIOUS DISEASE REPORTING FORM FOR SWINE

**MONTH OF __________, 197__**

**OWNER'S NAME**

**Important:** Record total number of animals in each age category

- Young pigs (up to 8 months of age)*
- Breeding stock (over 8 months of age)**

<table>
<thead>
<tr>
<th>Record number of affected animals in appropriate age column</th>
<th>Breeding Stock**</th>
<th>DISEASE CONDITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piglets*</td>
<td></td>
<td><strong>100</strong> ABSCESSES (soft swelling anywhere on body).</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>174</strong> ABORTIONS (sows having their pigs early).</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>179</strong> ARTHRITIS (swollen joint or joints and lameness).</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>194</strong> ATROPHIC RHINITIS (bloody noses, sneezing piglets, deformed snouts etc.).</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>186</strong> &quot;BLOODY SCOURS&quot; (Market pigs with watery bloody diarrhea).</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>125</strong> LICE</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>124</strong> MANGE (roughened, thick skin and itching).</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>171</strong> MMA (sows not letting down milk, swollen udders).</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>197</strong> PNEUMONIA (flu - pigs of all ages coughing).</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>196</strong> PNEUMONIA (VPP - pigs one (1) month of age).</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>121</strong> SCOURS (baby pigs or older swine).</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>173</strong> STILLBORN &amp; MUMMIFIED PIGS (pigs come at term but are dead or brown and dried up).</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>185</strong> TGE (baby pigs scouring, vomiting and dying).</td>
</tr>
</tbody>
</table>

**OTHER DISEASE CONDITIONS**
**INFECTIOUS DISEASE REPORTING FORM FOR BEEF FEED-LOTS, MONTH OF __________, 197__**

**Important:** Record total number of animals

--- Feeder cattle.*

Laboratory confirmation or diagnostic tests (check proper column if done).**

<table>
<thead>
<tr>
<th>Feeders*</th>
<th><strong>DISEASE CONDITION</strong></th>
<th>Feeders*</th>
<th><strong>DISEASE CONDITION</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 300      | ABSCESSES             | 308      | ARTHRITIS             |
| 309      | ACTINOBACTERIOSIS (wooden tongue) | 307      | Etiology if determined |
| 310      | ACTINOMYCOSES (lumpy jaw) | 306      | Etiology not determined |
| 311      | ARTHRITIS             | 305      | Etiology undetermined |
| 312      | Blackleg              | 304      | Enterotoxemia (type B, C or D) |
| 313      | Enterotoxemia (type B, C or D) | 303      | Malignant edema |
| 314      | Malignant edema       | 302      | Etiology undetermined |
| 315      | Malignant edema       | 301      | Enterotoxemia (type B, C or D) |
| 316      | BVD                   | 300      | Etiology undetermined |
| 317      | Salmonellosis         | 299      | Etiology undetermined |
| 318      | Etiology undetermined | 298      | Dermatitis and External Parasites |
| 319      | Etiology undetermined | 297      | Malignant edema |
| 320      | Etiology undetermined | 296      | Enterotoxemia (type B, C or D) |
| 321      | Etiology undetermined | 295      | Enterotoxemia (type B, C or D) |
| 322      | Cutaneous myiasis     | 294      | Etiology if determined |
| 323      | Hypoderma (cattle grubs) | 293      | Etiology if determined |
| 324      | Mange:                | 292      | Etiology if determined |
|          | Etiology if determined | 291      | Etiology if determined |
| 325      | Pediculosis           | 290      | Etiology if determined |
| 326      | Ringworm              | 289      | Etiology if determined |
| 327      | Warts (papillomatosis) | 288      | Etiology if determined |
|          | Etiology undetermined | 287      | Etiology if determined |
| 328      | Warts (papillomatosis) | 286      | Etiology if determined |
|          | Etiology undetermined | 285      | Etiology if determined |
| 329      | Etiology undetermined | 284      | Etiology if determined |
| 330      | INFECTIOUS MENINGITIS (Pink eye) | 283      | Etiology if determined |
| 331      | LEPIOTRIOSIS          | 282      | Etiology if determined |
| 332      | MALIGNANT Lymphoma (Leukemia) | 281      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 280      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 279      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 278      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 277      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 276      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 275      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 274      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 273      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 272      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 271      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 270      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 269      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 268      | Etiology if determined |
|          | MALIGNANT Lymphoma (Leukemia) | 267      | Etiology if determined |

---

* Dist. V #  Pract. #  Farm #
INFECTION DISEASE REPORTING FORM FOR BEEF FEED-LOT

MONTH OF ______, 197__

OWNER'S NAME

Important: Record total number of animals.

_________ Feeder cattle for reporting month.*

<table>
<thead>
<tr>
<th>Record number of affected animals*</th>
<th>DISEASE CONDITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>ABSCESSES (soft swelling anywhere on body).</td>
</tr>
<tr>
<td>368</td>
<td>ARTHRITIS (swollen joints and lame).</td>
</tr>
<tr>
<td>321</td>
<td>DIARRHEA (scours)</td>
</tr>
<tr>
<td>351</td>
<td>FOOT ROT (swollen foot or feet and lame).</td>
</tr>
<tr>
<td>350</td>
<td>INTESTINAL WORMS (&quot;wormy&quot;)</td>
</tr>
<tr>
<td>325</td>
<td>LICE</td>
</tr>
<tr>
<td>310</td>
<td>LUMPY JAW (firm swelling jaw)</td>
</tr>
<tr>
<td>330</td>
<td>PINKEYE</td>
</tr>
<tr>
<td>360</td>
<td>PNEUMONIA OR SHIPPING FEVER (breathing hard, coughing, runny nose).</td>
</tr>
<tr>
<td>327</td>
<td>RINGWORM</td>
</tr>
<tr>
<td>328</td>
<td>WARTS</td>
</tr>
<tr>
<td>309</td>
<td>WOODEN TONGUE (soft swelling of tongue and mouth).</td>
</tr>
<tr>
<td></td>
<td>OTHER DISEASES</td>
</tr>
</tbody>
</table>
### Infectious Disease Reporting Form for Sheep, Month of ________

**Important:** Record total number of animals in each age category.

- Lambs (up to 8 months of age).*
- Adults breeding stock (over 8 months of age).*
- Laboratory confirmation or diagnostic tests (check proper column if done).*

<table>
<thead>
<tr>
<th>Lambs</th>
<th>Adults</th>
<th>Lab.</th>
<th>DISEASE CONDITIONS</th>
<th>Lambs</th>
<th>Adults</th>
<th>Lab.</th>
<th>DISEASE CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td></td>
<td></td>
<td>Abscesses:</td>
<td>260</td>
<td></td>
<td></td>
<td>MASTITIS:</td>
</tr>
<tr>
<td>201</td>
<td></td>
<td></td>
<td>Abortions:</td>
<td>261</td>
<td></td>
<td></td>
<td>Neurologic Diseases:</td>
</tr>
<tr>
<td>202</td>
<td></td>
<td></td>
<td>Brucellosis</td>
<td>262</td>
<td></td>
<td></td>
<td>Listeriosis</td>
</tr>
<tr>
<td>204</td>
<td></td>
<td></td>
<td>Leptospirosis</td>
<td>263</td>
<td></td>
<td></td>
<td>Meningo-encephalitis</td>
</tr>
<tr>
<td>206</td>
<td></td>
<td></td>
<td>Vibriosis</td>
<td>264</td>
<td></td>
<td></td>
<td>Rabies</td>
</tr>
<tr>
<td>208</td>
<td></td>
<td></td>
<td>Etiology undetermined</td>
<td>265</td>
<td></td>
<td></td>
<td>Tetanus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anthrax:</td>
<td>266</td>
<td></td>
<td></td>
<td>Etiology undetermined</td>
</tr>
<tr>
<td>207</td>
<td></td>
<td></td>
<td>Psittacoid (chlamydial)</td>
<td>267</td>
<td></td>
<td></td>
<td>Parasites, Internal:</td>
</tr>
<tr>
<td>276</td>
<td></td>
<td></td>
<td>Streptococcal</td>
<td>268</td>
<td></td>
<td></td>
<td>Coccidiosis</td>
</tr>
<tr>
<td>269</td>
<td></td>
<td></td>
<td>Other: Specify etiology if determined</td>
<td>269</td>
<td></td>
<td></td>
<td>Flukes</td>
</tr>
<tr>
<td>270</td>
<td></td>
<td></td>
<td>Etiology undetermined</td>
<td>270</td>
<td></td>
<td></td>
<td>Haemorrhagic fever</td>
</tr>
<tr>
<td>271</td>
<td></td>
<td></td>
<td>Blue Tongue</td>
<td>271</td>
<td></td>
<td></td>
<td>Lungworms</td>
</tr>
<tr>
<td>272</td>
<td></td>
<td></td>
<td>Clostridial Diseases:</td>
<td>272</td>
<td></td>
<td></td>
<td>Oestrous ovis</td>
</tr>
<tr>
<td>212</td>
<td></td>
<td></td>
<td>Black leg</td>
<td>273</td>
<td></td>
<td></td>
<td>Tapeworms</td>
</tr>
<tr>
<td>213</td>
<td></td>
<td></td>
<td>Enterotoxemia (Pulpy Kidney disease)</td>
<td>274</td>
<td></td>
<td></td>
<td>Trichonocardia</td>
</tr>
<tr>
<td>214</td>
<td></td>
<td></td>
<td>Malignant edema</td>
<td>275</td>
<td></td>
<td></td>
<td>Etiology undetermined</td>
</tr>
<tr>
<td>215</td>
<td></td>
<td></td>
<td>Etiology undetermined</td>
<td>276</td>
<td></td>
<td></td>
<td>Pneumonia</td>
</tr>
<tr>
<td>270</td>
<td></td>
<td></td>
<td>Contagious Ecthyma (Sore mouth):</td>
<td>277</td>
<td></td>
<td></td>
<td>Enzootic pneumonia (Lambs)</td>
</tr>
<tr>
<td>222</td>
<td></td>
<td></td>
<td>Cutaneous myiasis</td>
<td>278</td>
<td></td>
<td></td>
<td>Progressive pneumonia</td>
</tr>
<tr>
<td>224</td>
<td></td>
<td></td>
<td>Mange: Specify etiology if determined</td>
<td>279</td>
<td></td>
<td></td>
<td>Pasteurelosis</td>
</tr>
<tr>
<td>289</td>
<td></td>
<td></td>
<td>Sheep keds</td>
<td>280</td>
<td></td>
<td></td>
<td>Sporadic pneumonia</td>
</tr>
<tr>
<td>221</td>
<td></td>
<td></td>
<td>Etiology undetermined</td>
<td>281</td>
<td></td>
<td></td>
<td>Etiology undetermined</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diarrheal Diseases:</td>
<td>222</td>
<td></td>
<td></td>
<td>Diseases Seen Infrequently:</td>
</tr>
<tr>
<td>217</td>
<td></td>
<td></td>
<td>Colibacillosis</td>
<td>223</td>
<td></td>
<td></td>
<td>Anthrax</td>
</tr>
<tr>
<td>218</td>
<td></td>
<td></td>
<td>Paratuberculosis</td>
<td>224</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>219</td>
<td></td>
<td></td>
<td>Salmonellosis</td>
<td>225</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>221</td>
<td></td>
<td></td>
<td>Etiology undetermined</td>
<td>226</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
THE MINNESOTA DISEASE REPORTING SYSTEM

INFECTIONIOUS DISEASE REPORTING FORM FOR SHEEP

MONTH OF ____________, 197_

OWNER'S NAME

Important: Record total number of animals in each age category.

______ Lambs (up to 8 months of age)*.

______ Breeding stock (over 8 months of age)**.

<table>
<thead>
<tr>
<th>Lambs*</th>
<th>Breeding Stock**</th>
<th>DISEASE CONDITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>279</td>
<td></td>
<td>ARTHRITIS (swollen joint or joints and lameness).</td>
</tr>
<tr>
<td>221</td>
<td></td>
<td>DIARRHEA (scours).</td>
</tr>
<tr>
<td>213</td>
<td></td>
<td>ENTEROTOXEMIA (acute death loss in feeder lambs).</td>
</tr>
<tr>
<td>251</td>
<td></td>
<td>FOOT ROT (sore and swollen feet, lameness, undermining of hoof).</td>
</tr>
<tr>
<td>250</td>
<td></td>
<td>INTESTINAL WORMS (persistent diarrhea, poor condition).</td>
</tr>
<tr>
<td>208</td>
<td></td>
<td>LAMBLING EARLY (abortions or stillborn lambs).</td>
</tr>
<tr>
<td>224</td>
<td></td>
<td>MANGE (loss of wool and itching).</td>
</tr>
<tr>
<td>280</td>
<td></td>
<td>MASTITIS (udder hot, swollen, milk abnormal).</td>
</tr>
<tr>
<td>262</td>
<td></td>
<td>PNEUMONIA (breathing hard, cough, runny nose).</td>
</tr>
<tr>
<td>289</td>
<td></td>
<td>SHEEP KEDS.</td>
</tr>
<tr>
<td>270</td>
<td></td>
<td>SORE MOUTH (pustules and scabs around mouth).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OTHER DISEASES</td>
</tr>
<tr>
<td>DISEASE CONDITION</td>
<td>Calves</td>
<td>Heifers</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>400 ABORTIONS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>401 BVD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>402 Brucellosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>403 IBR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>404 Leptospirosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>405 Atypical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>406 Vibriosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>407 Other causes not listed above</td>
<td></td>
<td></td>
</tr>
<tr>
<td>408 Etiology undetermined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>410 ACTINOMYCOSES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>411 ARTHRITIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>412 Aortic disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>413 Enterotoxemia (Type B, C or D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>414 Malnutrition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>415 Etiology undetermined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>421 Cutaneous myiasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>422 Hypoderma (cattle grubs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>423 Mange: Etiology if determined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>424 Pediculosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>425 Pneumonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>426 Pulmonary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>427 Ringworm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>428 Warts (malignant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>429 Etiology undetermined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>430 Diarrhea: Diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>431 Etiology undetermined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>432 INFECTIOUS RHEUMATITIS (pink eye)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>433 Leptospirosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>434 MALIGNANT Lymphoma (Leukemia)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>435 MALIGNANT Lymphoma (Leukemia)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>436 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>437 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>438 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>439 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>440 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>441 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>442 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>443 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>444 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>445 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>446 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>447 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>448 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>449 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>450 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>451 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>452 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>453 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>454 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>455 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>456 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>457 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>458 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>459 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>460 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>461 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>462 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>463 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>464 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>465 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>466 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>467 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>468 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>469 TUBERCULOSIS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
INFECTIONOUS DISEASE REPORTING FORM FOR DAIRY AND BEEF COW-CALF HERDS

MONTH OF _________, 197__

OWNER'S NAME

Check one:
Beef
Dairy

Important: Record total number of animals on farm for each age category.

___ Calves (up to 1 year of age).*
___ Heifers and other animals (1 year - 2 years of age).**
___ Adults (2 years and over).***

Record number of affected animals in appropriate age column.

<table>
<thead>
<tr>
<th>Calves*</th>
<th>Heifers**</th>
<th>Adults***</th>
<th>DISEASE CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>400 ABScesses (soft swelling anywhere on body).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>408 ARTHRITIS (swollen joint or joints and lameness).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>408 CALVING EARLY (calves born dead or abortions).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>421 DIARRHEA (scours).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>451 FOOT ROT (swollen foot or feet and lameness).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>450 INTESTINAL WORMS (&quot;wormy&quot;).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>425 LICE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>410 LUMPY JAW (firm swelling of jaw).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>436 MASTITIS (swollen udder, abnormal milk).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>454 PNEUMONIA - Adults (breathing hard, coughing, runny nose).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>456 PNEUMONIA - Calves (breathing hard, coughing, runny nose).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>462 PNEUMONIA - Heifers (breathing hard, coughing, runny nose).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>427 RINGWORM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>428 WARTS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>409 WOODEN TONGUE (soft swelling of tongue and/or around mouth).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>430 PINNATE</td>
</tr>
</tbody>
</table>

OTHER DISEASE CONDITIONS

FORM 65 (3-72)
THE MINNESOTA DISEASE REPORTING SYSTEM

<table>
<thead>
<tr>
<th>Year</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</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>2015</td>
<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>2016</td>
<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>2017</td>
<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>2018</td>
<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>2019</td>
<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>

**DAIRY COW CALF**

- **Type of Herd (Beef/Dairy)**
- **Area Code**
- **Vet Code**
- **Farm No.**
- **Month**
- **Year**
- **Calves Total Count**
- **Heifers Total Count**
- **Adults Total Count**
- **Disease Code**
- **Lab Confirm**
- **Calves Disease Count**
- **Heifers Disease Count**
- **Adults Disease Count**
<table>
<thead>
<tr>
<th>Type of Herd</th>
<th>Area Code</th>
<th>Vet Code</th>
<th>Farm No.</th>
<th>Month</th>
<th>Year</th>
<th>Feeder Total Count</th>
<th>Second Total Count</th>
<th>Third Total Count</th>
<th>Disease Code</th>
<th>Lab Confirm</th>
<th>Feeder Disease Count</th>
<th>Second Disease Count</th>
<th>Third Disease Count</th>
</tr>
</thead>
</table>
# The Minnesota Disease Reporting System

<table>
<thead>
<tr>
<th>Type of Herd</th>
<th>Beef/Dairy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area Code</td>
<td></td>
</tr>
<tr>
<td>Vet Code</td>
<td></td>
</tr>
<tr>
<td>Farm No.</td>
<td></td>
</tr>
<tr>
<td>Month</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td></td>
</tr>
<tr>
<td>Young Pigs</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Breeding Stock</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Third Total Count</td>
<td></td>
</tr>
<tr>
<td>Disease Code</td>
<td></td>
</tr>
<tr>
<td>Lab Confirm</td>
<td></td>
</tr>
<tr>
<td>Piglets</td>
<td></td>
</tr>
<tr>
<td>Disease Count</td>
<td></td>
</tr>
<tr>
<td>Breeding Stock</td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td></td>
</tr>
<tr>
<td>Third Disease Count</td>
<td></td>
</tr>
<tr>
<td>Type of Herd</td>
<td>Area Code</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>SHEEP</td>
<td></td>
</tr>
</tbody>
</table>
REPORT OF THE COMMITTEE ON MEAT AND POULTRY HYGIENE

Chairman: E. D. Baker, Madison, Wisconsin
Co-Chairman: J. K. Payne, Washington, D.C.


Mr. Chairman:

Four regional Meat and Poultry Hygiene Committee meetings were held during the year. Topics originating from these regional meetings were submitted to this committee for further action.

After review and discussion, the committee considered the following items on its agenda.

1. Proposed National Meat and Poultry Inspection Program

The National Meat and Poultry Inspection Advisory Committee has recommended that Federal and State meat inspection programs be incorporated into a consolidated national inspection system. These recommendations and a plan for implementation were submitted to the USDA for approval. Due to statutory limitation, the USDA is not in a position to accept the proposal as submitted. Your committee recommends that the National Advisory Committee continue to explore all means of achieving the goals of the proposal which included the means for greater efficiency of manpower utilization, improving uniformity of program application and improvement of communication.

2. Re-Establishment of the Technical Subcommittee for Meat and Poultry Inspection

The National Meat and Poultry Inspection Advisory Committee formerly had an active subcommittee which was concerned with the technical aspects of meat and poultry inspection. The subcommittee was discontinued when the National Advisory Committee was reorganized. The present Advisory Committee has members with technical backgrounds in meat and poultry inspection. Thus it was determined that the technical subcommittee was no longer necessary. State Meat and Poultry Inspection officials are of the opinion that there is inadequate technical input into the present Advisory Committee.

Your committee recommends that the National Association of State Meat and Food Inspection Directors (NASMID) serve in a consultative capacity to the National Advisory Committee. Proposals generated by the NASMID would be presented to the National Advisory Committee for consideration. Furthermore, the Association would welcome the opportunity to assist the
committee on technical matters involving meat and poultry inspection. It is further recommended that a member of NASMID be appointed by the Secretary of Agriculture to membership on the Advisory Committee.

3. Tissue Residue Report

Based on data furnished by FDA, 1.7% of all slaughter animals sampled contained violative tissue drug residues in FY 74. This compares with 2.4% for FY 73. Although much progress has been made, there is still need for increased surveillance and improved education regarding directions for drug use and withdrawal.

In FY 74, the specific reason for violative residue was not determined in 39% of the investigations. Where the cause was determined, 78% of the violators did not observe withdrawal directions.

The Committee recommends the current and ongoing development of State-FDA-USDA cooperative tissue residue investigation.

The development of this program would serve to:

1. Avoid duplication of State and Federal effort; conservation of time, money, and manpower; and improvement of compliance with the regulations.
2. Promote a uniform educational investigational protocol for producers and others in order to better identify the violator, the specific residue, and cause of residue.
3. Promote better communication between interested regulatory agencies.

The Cooperative State-Federal residue monitoring program which is now established will enhance the regulatory efforts of State and Federal Inspection Programs.

4. Supervising the Performance System (SPS)

During the past year, Meat and Poultry Inspection Program (MPIP) has placed considerable emphasis on the role of the supervisor in inspection programs.

In order to overcome traditional misconceptions regarding the relationship between the technical and the human resource management aspects of inspection programs, MPIP moved to integrate the two. The results of integration amounts to a method of supervision that incorporates basic human behavioral principles into a system which is equally applicable at all levels of supervision and management. It provides supervision with a framework for analyzing performance problems, determining their causes and a method of developing effective correctives.

All levels of management in the Federal program from the Washington level down to the first level supervisor have been trained.

Five state programs are scheduled to receive training by June 30, 1975. It is estimated that 20 sessions will be needed to cover all state programs. Your committee strongly urges that USDA, MPIP provide the states with training under “Supervising the Performance System.”

5. Revision of the Committee's Name

Your committee recommends that the name of this committee be changed to give recognition to the added responsibilities to include fish hygiene.
Therefore your committee has moved that the name be changed to “Food Animal Hygiene and Inspection Committee.”

6. *Swine Tuberculosis*

Your committee believes that there is a need to review all aspects of swine tuberculosis. Swine tuberculosis poses many problems for the producers, packers and meat inspectors.

Your committee recommends that the American Association of Extension Veterinarians be asked to organize and conduct a national workshop on tuberculosis in swine.

7. *Amendment to the Federal Meat Inspection Act*

Your committee recognizes the inequity which exists against USDA certified state inspected meat and poultry establishments.

The Federal Meat Inspection Act amended in 1967 prevents state inspected meat and poultry from entering interstate commerce or entry into federally inspected plants for further processing. This denies the economic advantage to both State and Federally inspected firms who desire to transact business with each other. This inequity exists for State inspected products while foreign meat products are permitted entry into the United States and are distributed as US inspected products.

It is therefore recommended that the US Animal Health Association actively support effective legislative changes in the Wholesome Meat Act to eliminate this inequity existing in 40 states involving thousands of State inspected plants which have been declared and updated annually as being fully equal to Federal inspection. It is reiterated that such legislative change to the Wholesome Meat Act would permit such business transactions only within the same State until State inspected product has been reinspected and accepted and passed by the USDA as meeting the same criteria required for foreign products when reinspected at US ports of entry.

I respectfully submit this report and recommend that it be approved.

E. D. Baker
Chairman
REPORT OF THE COMMITTEE ON STATE-FEDERAL RELATIONS

Chairman: J. F. Andrews, Atlanta, Ga.


The state-federal Relations Committee met in Washington, D.C., March 11-14, 1974. The committee wishes to express appreciation to all Federal personnel who gave freely of their time and effort at this meeting. We hope that mutual benefits will result for all agencies and associations concerned. The State-Federal Relations Committee presents the following statement for mutual consideration and guidance and for approval by the Executive Committee of this association:

Veterinary Services

Many diseases and situations were discussed during this meeting. For many years our association has urged the movement of the barrier zone for screwworms from the U.S.-Mexico border to the Isthmus of Tehuantepec. We are pleased that real progress in this direction is being made. Our association has been concerned for many years regarding emergencies that could occur when one or more exotic diseases are introduced into the United States. We certainly were pleased with the handling of the recent outbreaks by the Emergency Diseases Staff. The reorganization and realignment of APHIS has been received with considerable apprehension. In the past year it has been functioning with varying degrees of success in the individual states. We do urge that consideration be given to the reduction of the monthly and quarterly reports that we are presently having to make concerning program activities.

This committee and our association have had grave concern about the diversions in U.S.D.A. of monies from one program to another and the priorities placed on these programs. We notice improvement in this situation and so far as we are able to ascertain, brucellosis monies for this fiscal year are being spent on brucellosis. We have always agreed with the concept of brucellosis eradication and not brucellosis control. It has been pointed out that the major brucellosis infections are largely confined to 10 or 12 southeastern or Gulf Coast states. A problem is associated with the incidence in these states and is not limited to these states or geographical areas. It poses a constant threat of introduction of infection into states that have obtained Free or Modified Certified Free status. We still believe that the brucellosis program should be given top priority and be properly funded. Unless this matter is given immediate attention, there is a real possibility of losing the program nationally. This would not only be a serious impediment to our livestock industry, but would also have great influence on the beginning of any future cooperative programs.

Veterinary Biologics

The U.S. Animal health Association Committee is extremely pleased to
know of the progress that is being made in the expansion of present biologic testing facilities and the construction program for additional facilities at Ames, Iowa.

The committee noted that the funds for the expansion of facilities for evaluating quality, safety, potency and efficacy of veterinary biologics has been included in the 1975 budget. The updating of the Federal Serum-Toxin Act was received with interest and the committee is very much aware that the livestock industry generally does not wish to continue to use biologics of questionable efficacy and endorses the principle that all biologics marketed should be conclusively established to be safe and efficacious and that the quality of the products should be governed by requirements that are technically feasible. It was also noted that there is a decrease in the number of new commercial licenses issued, also a decrease in the number of veterinary biological product licenses issued. It also was proposed that the Biological Licensing and Standards Staff of Veterinary Services consider the establishment of an advisory committee made up of members from the livestock industry, veterinary practitioners, USAHA members and research workers from which input could be obtained as to the need for new tools for combatting livestock diseases and field reports on the use of presently licensed products would be forthcoming to the APHIS staff.

Meat Inspection

The State-Federal Relations Committee commends the Meat Inspection personnel for their efforts in enhancing the interworking relations with the State Meat Inspection program personnel.

The Committee commends the USDA and FDA for reducing the time of notifying state officials on residue violations. It also was noted that there is need for legal counsel to study the authority for quarantines, embargoes, and hold orders on livestock suspected of residues. We commend USDA MPIP for the Los Alamos Study on sophisticated animal identification procedures. We strongly recommend that leadership and funding be extended for further study of electronic animal identification.

Veterinary A.R.S.

There has become a trend in A.R.S. to replace veterinary personnel with other professionals and the State-Federal Relations Committee is somewhat discouraged by this, and do strongly recommend, due to better training in this field, that every consideration be given placing veterinarians in key positions in A.R.S.

We commend A.R.S. for the five foreign animal diseases which they have given top priority. We trust that this research will continue at least at the present rate, or possibly be increased. The Committee is extremely interested in knowing more about African Swine Fever and S.V.D. We certainly feel that more information should be gleaned in regard to Imidocarb as a cleansing agent for anaplasmosis and piroplasmosis. The Committee feels that appropriations should be made to A.R.S. for the specific purpose of doing further research on brucellosis in the hope of developing more knowledge regarding the diagnosis and management of brucellosis infected herds. This is an immediate, urgent need if the brucellosis program is to survive.
The Committee commends USDA with the work that A.R.S. has done and by their being able to obtain more space and capabilities for their research services.
Mr. Chairman:

The Committee on Livestock Commerce met with 26 members and guests in attendance and again reviewed recommendations made and approved by the USAHA over the past several years.

Various formats and uses of documents accompanying livestock in commerce were discussed. The committee could detect no increase in the use of the uniform health certificate. This certificate is being used by some 30 states and some of these have not adopted the veterinary certification statement as recommended by this organization and the AVMA.

We strongly recommend that this uniform certificate and the veterinary certification statement thereon be adopted and used by all states.

The Committee recommends that all states implement a "certificate of examination" or "certificate of inspection" for use at livestock markets. Such certificate could follow the same format as that used for health certificates but be more definitive of its actual purpose.

The livestock Transportation Certificate as recommended by the Association in 1972 was reviewed. It was reported that a survey taken after the annual meeting that year resulted in 26 states stating they would accept this document. The Committee found no use of the prescribed certificate but found that some states have devised a certificate serving similar functions but not conforming to the desired uniformity. Since considerable interest was shown in the use of this certificate the committee has recommended that a new survey be conducted by Veterinary Services to update information as to use and acceptance of this livestock Transportation Certificate in its adopted format. Results of the survey will be distributed to each state. The Committee recommends that states adopt the use of this certificate and make it available within their state.

The Committee noted that this Livestock Transportation Certificate is not intended to be a certificate of health but only a statement as to the general condition of the livestock on day of issuance and as a notification to the state of destination of the shipment.

A very brief discussion was held regarding small animal health certificates. It is hoped that this committee will work jointly with the rabies com-
mittee during the next year to develop a recommended uniform small animal certificate.

Discussions revealed that in a number of cases the federal form 1-27 is being used improperly. We would like to emphasize that the proper use of Form 1-27 is for the movement of animals which are diseased, exposed to disease, or otherwise restricted because of a disease. We recommend that those using Form 1-27 adhere to the instructions printed on the cover of the pad. We particularly emphasize that part of the instructions which indicate a preaddressed envelope be enclosed with the copy being mailed to the destination of the shipment so that a completed copy of the 1-27 will be returned to the state of origin. This will enable the state of origin to know that the shipment arrived at the proper destination.

Identification of cattle under Part 71 CFR was discussed. It was noted that 3 letter codes of identification being used by Texas are being confused with the standard ear tags when the letters and numbers are recorded on documents. The committee recommends some corrective measures, to eliminate this confusion, be taken.

Backtag identification of only a part of a log shipped from a market to slaughter does not meet the requirements of 71.18 CRF. This regulation requires that a backtag be applied to each animal so shipped.

The committee reviewed legislation which was introduced in the present session of Congress relating to the Humane Handling of animals in transit. It was felt the provisions of bill HR 15843 introduced by Congressman Foley, while commendable in its intent, contained many provisions which would result in undue burden on the livestock industry. The committee feels certain that this or similar legislative proposals will be reintroduced at subsequent sessions, until passed. The committee recommends USAHA be prepared to make positive recommendations on this subject. In this regard, we recommend that the President of USAHA appoint an Ad Hoc Committee consisting of representatives from the Animal Welfare Committee and the Committee on Livestock Commerce, to study humane handling of livestock and other animals while in commerce and possible legislation relating thereto. This constitutes the report of the Committee on Livestock Commerce. I respectfully submit the report for approval by the Executive Committee.

PERMIT FOR MOVEMENT OF ANIMALS

INSTRUCTIONS

PRESS HARD — YOU ARE MAKING 5 COPIES

*USE A SEPARATE FORM FOR EACH SPECIES
*PRESS HARD — YOU ARE MAKING 5 COPIES
*INSERT COVER UNDER EACH SET TO USE AS WRITING REST
*COMPLETE EACH APPLICABLE ITEM — OMISSIONS WILL VOID THE PERMIT
IF SHIPMENT IS RESTRICTED FOR MORE THAN ONE DISEASE
*LIST EACH DISEASE IN ITEM 8, AND RELATED DATA IN ITEMS 9, 10, & 11. CONSULT VS MEMORANDUMS FOR THE APPROPRIATE DISEASE PROGRAM INSTRUCTIONS.

DISTRIBUTION OF FORM:
*PART 1 — ORIGINAL — TO ACCOMPANY SHIPMENT
*PART 2 — MAIL TO DESTINATION OF SHIPMENT
   ENCLOSE A PREADDRESS ENVELOPE TO WHERE THIS COPY SHOULD BE MAILED — AFTER ITEMS 26 THRU 30 HAVE BEEN COMPLETED]
*PART 3 — TO STATE OF DESTINATION (VS OFFICE)
*PART 4 — TO STATE OF ORIGIN (VS OFFICE)
*PART 5 — RETAIN BY ISSUING OFFICIAL
<table>
<thead>
<tr>
<th>No.</th>
<th>075413</th>
</tr>
</thead>
</table>

**State Where Issued:**

1. **Name & Address of Shipper on Condition Include Species:**

2. **Name & Address of Owner at Time Condition Diagnosed:**

3. **Location of Premises of Origin (County & State):**

4. **Consignee (Name & Address, Include Zip Code):**

<table>
<thead>
<tr>
<th>Ear Tag No.</th>
<th>Breed</th>
<th>Sex</th>
<th>Other Ident.</th>
<th>Ear Tag No.</th>
<th>Breed</th>
<th>Sex</th>
<th>Other Ident.</th>
</tr>
</thead>
<tbody>
<tr>
<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>
</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></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>
</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></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Valid Only for Above Destination:**

<table>
<thead>
<tr>
<th>17. Animals to Be Moved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear Tag No.</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Signature of Inspector:**

19. **Date Issued:**

20. **Time Issued:**

21. **Date Signed:**

22. **Time Signed:**

I hereby certify that I have inspected the animals described on this certificate and find them eligible to move in accordance with the requirements of State and Federal regulations.

I understand that it is a violation of Federal law to move the animals described herein out of the State in which they are located except in compliance with the provisions of applicable Federal Regulations. I also understand that such animals must comply with existing State, local, and Federal regulations governing movement of livestock and poultry.

I have arranged or will arrange for a copy of this certificate to accompany the interstate shipment of the above described animals.

**Signature of Owner or Shipper:**

24. **Title:**

25. **Date Signed:**

**Signature of Inspector:**

26. **Date Signed:**

27. **No. Animals Received:**

28. **Date Slaughtered/Quarantined:**

**Date Cleaned & Disinfected:**

**Signature of Inspector:**

**DISTRIBUTION AS MARKED**
**HEALTH CERTIFICATE**

**OKLAHOMA DEPARTMENT OF AGRICULTURE**

**ANIMAL INDUSTRY DIVISION**

**LIVESTOCK MARKET INSPECTION**

<table>
<thead>
<tr>
<th>B. SPECIES</th>
<th>C. NUMBER ANIMALS IN SHIPMENT</th>
<th>D. AREA STATUS</th>
<th>E. HERD OR FLOCK STATUS</th>
<th>F. CARREER</th>
<th>G. VACCINATION OR DIPPING</th>
</tr>
</thead>
<tbody>
<tr>
<td>CATTLE</td>
<td></td>
<td>MODIFIED ACCREDITED (T)</td>
<td>ACCREDITED HERD NO.</td>
<td>AIR, RAIL, TRAIN</td>
<td>VACCINATED OR DIPPED FOR</td>
</tr>
<tr>
<td>SHEEP</td>
<td>PURPOSE OF MOVEMENT</td>
<td>MODIFIED CERTIFIED (S)</td>
<td>CERTIFIED HERD NO.</td>
<td>1441L, WATER</td>
<td></td>
</tr>
<tr>
<td>SWINE</td>
<td>BREED</td>
<td>FREE OF</td>
<td>VALUATED HERD NO.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>POULTRY</td>
<td>FEEDING</td>
<td>ENRICHED OF</td>
<td>QUALIFIED NEGATIVE HERD TEST DATES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTHER</td>
<td>SLAUGHTER</td>
<td>INFECTED WITH</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10. OTHER MARKET

11. C AND D VEHICLE

12. INDIVIDUAL ANIMAL IDENTIFICATION AND TEST DATA

<table>
<thead>
<tr>
<th>BAR TAG NO.</th>
<th>TATTOO OR OTHER PERMANENT IDENTIFICATION</th>
<th>REGISTRY NUMBER OR DESCRIPTION</th>
<th>TUBERCULIN TEST (1/10000)</th>
<th>Mycobacterium (1/10000)</th>
<th>VACCINATOR</th>
<th>OTHER TESTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LINE 1</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 2</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 3</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 4</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 5</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 6</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 7</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 8</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 9</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 10</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 11</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 12</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 13</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 14</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
<tr>
<td>LINE 15</td>
<td></td>
<td></td>
<td>DATE</td>
<td>HOUR</td>
<td>LABORATORY</td>
<td>DATE</td>
</tr>
</tbody>
</table>

13. CERTIFICATION

I CERTIFY THAT THESE ANIMALS HAVE BEEN INSPECTED UNDER MY SUPERVISION IN ACCORDANCE WITH ANIMAL HEALTH DIVISION STANDARDS AND FOUND FREE FROM SIGNS OF INFECTIOUS, CONTAGIOUS, OR COMMUNICABLE DISEASES, BALE FEVER, Tinea, AND BODILY INFESTATIONS.

14. SIGNATURE OF SUPERVISING VETERINARIAN

NOTE: THE REGULATIONS OF THE STATE OF DESTINATION SHOULD BE CONSULTED BEFORE THE SHIPMENT IS MADE.
LIVESTOCK TRANSPORTATION CERTIFICATE

DATE: ___________________________ TIME: ___________________________ TYPE OF CARRIER: ___________________________

<table>
<thead>
<tr>
<th>KIND OF LIVESTOCK &amp; IDENTIFICATION</th>
<th>SWINE</th>
<th>STEERS</th>
<th>HEIFER CALVES</th>
<th>FEEDING</th>
<th>Slaughter</th>
<th>RESALE</th>
</tr>
</thead>
</table>

PERMIT NUMBER

ORIGIN:  □ Ranch □ Public Market □ Collection Points PURPOSE: FEEDING Slaughter RESALE

NAME OF OWNER AND ADDRESS: ____________________________________________________________

ORIGIN OF LIVESTOCK IF DIFFERENT FROM ADDRESS: _________________________________________

SIGNATURE OF OWNER: ________________________________________________________________

CONSIGNED TO:

NAME AND ADDRESS: _________________________________________________________________

DESTINATION OF LIVESTOCK IF OTHER THAN ADDRESS

The animals described did not originate in a quarantined herd and are to be shipped in accordance with applicable Federal and State regulations.

This certificate is valid for (1) shipment only, not to exceed 96 hours from time of issuance and is void at destination.

INSPECTOR: ___________________________

This certificate issued under the authority of the State Department of Agriculture

Chief Livestock Official
Rabies Committee Report

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


The Rabies Committee met on October 15, 1974 with a total of 18 members and guests present. Participation in this year's deliberations included three new members: Dr. Bruce Kaplan, veterinary practitioner from Louisville, Kentucky; Dr. J. T. Frank, veterinary pathologist, Quebec, Canada; and Dr. John Brown, Major, US Army of Walter Reed Institute of Research, Washington, D.C.

Two major accomplishments were reported by the Committee, each designed to improve and standardize the animal rabies vaccination program in the United States. First, the “Compendium of Animal Rabies Vaccines” (Table 1) was revised for use to standardize vaccination procedures of all licensed vaccines. Secondly, new and simplified certificates for rabies vaccination (Figure 1) and interstate movement (health certificate) (Figure 2) were developed during the year. The committee recommends their adoption in all the states.

As a result of the development of these certificates and the compendium, it is now possible to standardize the intra and interstate movement of dogs and cats. The cooperation of all state veterinarians, public health officials and veterinary practitioners is needed to attain this goal however. The committee will submit a cover letter as well as copies of the compendium and certificates to state agriculture and public health officials in every state explaining these forms.

The Committee acknowledged the earlier recommendations by the USAHA Committee on Rabies and Commerce to have a standardized rabies vaccination certificate to replace the health certificate for the interstate movement of dogs and cats. Until the necessary details to allow this to be done are completed, the new certificate for interstate movement developed by your Committee on Rabies is recommended for use in all states.

Other recommendations made by the committee were:
1. To reject a proposal that this committee amalgamate with one or two other committees to form a new one on Companion Animals.
2. That the distribution and sale of all animal rabies vaccines be restricted to veterinarians and certain designated local and state officials.

Three sub-committees were appointed to develop specific recommendations concerning: (a) methods to assist local governments in controlling the overpopulation of dogs and cats; (b) educational resource material for use by local and national news media concerning various aspects of rabies and stray animal control; (c) research priorities.

The Committee recognized the recent licensing and sale of the new
human origin rabies immune globulin to replace the equine origin anti-rabies serum in post-exposure human prophylaxis. This new product will prevent serum sickness in several thousand Americans annually who are severely exposed to rabid animals and must receive passive immunization.

Respectfully submitted by your rabies committee.

Table 1.

### Compendium of Animal Rabies Vaccine in the U.S.

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Companies Marketing</th>
<th>For Use in</th>
<th>Regimen Recommended for Primary Immunization</th>
<th>Revaccination Recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Modified Live Virus Vaccines (MLV)</td>
<td>FROMM (Rabovac)</td>
<td>Dogs</td>
<td>1 dose of 1 mL at 1 yr.</td>
<td>3 yrs</td>
</tr>
<tr>
<td></td>
<td>AMERILAB (Rabies Vaccine)</td>
<td>Dogs</td>
<td>1 dose of 2 mL &amp; 1 yr</td>
<td>3 yrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cats</td>
<td>1 dose of 1 mL</td>
<td>1 yr</td>
</tr>
<tr>
<td></td>
<td>NORDEN (EnduraVax-R)</td>
<td>Cats</td>
<td>2 doses of each 6 wk apart</td>
<td>1 yr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dogs</td>
<td>1 dose of 2 mL</td>
<td>3 yrs</td>
</tr>
<tr>
<td></td>
<td>JEN-SAL (ERA)</td>
<td>Cats</td>
<td>1 dose of 2 mL</td>
<td>2 yrs.</td>
</tr>
<tr>
<td></td>
<td>CONNAUGHT (ERA)</td>
<td>Cattle</td>
<td>1 dose of 2 mL</td>
<td>4 yrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sheep and Goats</td>
<td>1 dose of 2 mL</td>
<td>1 yr.</td>
</tr>
<tr>
<td>B) Inactivated Vaccines</td>
<td>JEN SAL (Rabies Vaccine)</td>
<td>Dogs</td>
<td>2 doses of 2 mL as</td>
<td>1 yr</td>
</tr>
<tr>
<td></td>
<td>BURNS-BIOTEC (BiaRab or Amgen-R)</td>
<td>Cats</td>
<td>2 doses of 1 mL as</td>
<td>1 yr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dogs</td>
<td>1 dose of 2 mL</td>
<td>3 yrs</td>
</tr>
<tr>
<td></td>
<td>BANDY (Rabies Vaccine)</td>
<td>Cats</td>
<td>1 dose of 2 mL</td>
<td>1 yr</td>
</tr>
<tr>
<td></td>
<td>DOUGLAS (SMBV)</td>
<td>Dogs</td>
<td>1 dose of 1 mL</td>
<td>1 yr.</td>
</tr>
<tr>
<td></td>
<td>FT. DODGE (Trimeus)</td>
<td>Cats</td>
<td>1 dose of 1 mL</td>
<td>1 yr.</td>
</tr>
</tbody>
</table>

*In mass vaccination programs the schedule of primary immunization may consist of only 1 inoculation given each year to all dogs between 3 months and 1 year of age.
# Fig. 1

**Georgia Department of Human Resources**

**CERTIFICATE FOR INTERSTATE MOVEMENT**

47 Trinity Avenue, Room 13-H, Atlanta, Georgia 30334

## Owner

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First</th>
<th>Middle Initial</th>
<th>Phone No.</th>
<th>Current License No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Address (Street or RFD)</th>
<th>City</th>
<th>Zip Code</th>
<th>County</th>
<th>Previous License No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Animal Description

<table>
<thead>
<tr>
<th>Tag No.</th>
<th>Breed</th>
<th>Color</th>
<th>Sex</th>
<th>Age</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Vaccine Used

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Name of Vaccine</th>
<th>Serial No.</th>
<th>Live</th>
<th>Killed</th>
<th>Vaccine Exp. Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- □ CEO
- □ TC
- □ TC
- □ Murine
- □ Caprine

<table>
<thead>
<tr>
<th>Vaccination Date</th>
<th>By</th>
<th>D.V.M.</th>
<th>License Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This Rabies Vaccination Expires ____________________________

## Consignee

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First</th>
<th>Middle Initial</th>
<th>Telephone No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Address (Street or RFD)</th>
<th>City</th>
<th>State</th>
<th>Zip Code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This is to certify that this animal was examined by me and is sufficiently healthy for shipment on this date. To my knowledge this animal has not been exposed to rabies and did not originate from a rabies quarantined area.

Licensed Veterinarian: ____________________________

D.V.M. ____________________________ Date ____________________________

Ga. License No. ____________________________

DPH/DCS(1)-4 (Rev. 7-74)
**CERTIFICATE FOR ANIMAL RABIES VACCINATION**

47 Trinity Avenue, Room 13-H, Atlanta, Georgia 30334

<table>
<thead>
<tr>
<th>Owner</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Last Name</td>
<td>First</td>
</tr>
<tr>
<td>Address (Street or RFD)</td>
<td>City</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal Description</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tag No.</td>
<td>Breed</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vaccine Used</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Name of Vaccine</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D.V.M. License Number

This Rabies Vaccination Expires

DPH/DCS(1)-4 (Rev 7-74)
1974 REPORT OF THE COMMITTEE ON IMPORT-EXPORT

Chairman: C. L. Campbell, Tallahassee, Florida.
Co-Chairman: C. K. Jewell, Trenton, N.J.


The Committee on Import-Export voiced its concern in last year’s report on the inadequacies of import facilities, particularly for breeds of cattle which are so sorely needed in the improvement of such cattle within the continental United States. Delays which had been experienced in replacing the Clifton, New Jersey, quarantine station, as well as provisions for the construction of the Fleming Key Maximum Security Import Station in Florida to further this aim, resulted in the Committee’s appointment of a sub-committee for the purpose of coordinating the activities of the various livestock entities in stimulating the interest of Congress in this field. It was quite disappointing to receive the report from the action sub-committee during the year ensuing since its establishment that the group had been further frustrated by additional logjams acting as a further deterrent in getting cattle into the United States to the tune of less than 600 having been imported during the past year.

The lack of existing import quarantine facilities to meet this demand for cattle has prompted importers in one case to construct facilities for all in-all out movements of cattle under situations where foot and mouth disease tests are not required. The proposed facility is to be constructed in an industrial complex at the edge of Payne Field near Everett, Washington. The importer has submitted plans for the facility which has received tentative approval by Veterinary Services. This facility would be under the direct supervision of VS personnel if and when it is completed. This, however, will not greatly relieve the cattle import problem.

The action sub-committee had even explored plans for the construction of temporary emergency housing facilities in the Clifton station, which would augment its capacity, from private funds. However, it was brought out that such construction might well be in conflict with the intent of Congress for the utilization of this station. In addition to this conflict it was stated that any expanded construction, be it temporary or not, would certainly represent to the citizenry of Clifton, who are desirous of phasing out the station, that this would be a breach of good faith by the Department.
REPORT OF THE COMMITTEE

None the less, although several sites proposed for re-location were evaluated this past year, for various reasons the Department was unable to obtain any of them. At the present time Stuart Airport at Newburgh, New York, is being evaluated as a possible site of re-location of the station at Clifton. If approved, the ensuring authorization, appropriation, designing and construction would encompass a time span of some three years at a minimum before utilization of these facilities could be expected.

Lengthy discussions ensued within the Committee as to the possibility of recognizing export quarantine requisites supervised by USDA personnel at debarkation points in the country of origin. The cost of diverting personnel for such purpose from this country would be beyond the present limits of the APHIS budget under existing ceilings. As a consequence it was determined that such costs might properly be absorbed by either the foreign country exporters or the importers from the United States under a contractual agreement, and to this end the Committee recommends that USDA pursue the feasibility of collection of reimburseable services for inspections in overseas quarantine facilities, in lieu of U.S. port of entry quarantine, in countries authorized by federal regulations to ship cattle to this country.

The Committee was advised that USDA is attempting to obtain authority to initiate a system of user charge fees to offset the costs of services provided by the Department for the inspection of livestock being imported and exported, and the Committee strongly supports the Department in its request to the Office of Management and Budget of user charge fees to be paid by the importer in connection with animal importation into the United States from foreign countries. A resolution confirming this intent is herewith submitted to the Committee on Nominations, Resolutions and Internal Affairs for consideration and adoption by this body.

The Committee reviewed the disease threat imposed to the United States through the importation of dry milk from foreign countries. The current regulations provide that this product may enter this country provided it is not diverted to livestock feed. This, however, does not preclude the practical problem of this imported product getting into animal food channels when it might be rejected for human consumption purposes. The safety of this product is now being considered by the Foot and Mouth Disease Research Laboratory at Plum Island, and it is recommended that dry milk be prohibited entry into this country unless the Plum Island laboratory determines it is a safe product so far as foot and mouth disease and rinderpest are concerned, or that it be permitted to go only to specifically approved plants for further processing to make it safe.

The Committee again considered the present eradication efforts of the several states directed toward the diseases of equine infectious anemia and equine piroplasmosis. With the going programs of elimination of these diseases within the continental boundaries of this nation, it is evident that efforts directed toward eradication thereof are futile if we continue to allow the introduction of these diseases from outside sources. It is therefore recommended that all animals susceptible to equine infectious anemia and equine piroplasmosis imported into this country be tested and found negative to these diseases before being released from the quarantine station.

Respectfully submitted, C. L. Campbell, Chairman
Dr. Don Van Houweling at a Food and Drug Administration Seminar held last June in Chicago said:

"To communicate with someone effectively requires recognition by the recipient that the information is important."

There is nothing wrong with that statement, but it reveals the fact that regulatory agencies both Federal and State assume too much about those with whom they want to communicate. Important or not important, the message must get the recipients' attention, and it must be presented in a manner which will encourage the recipient to receive it.

First it is necessary to get the recipients' attention. In this modern age of communication many others are vying through all the media to get the public's attention. Today, this organization, APHIS or FDA in order to get the attention of state regulatory officials, veterinarians and animal agriculturists may find it necessary to do what the old farmer did to get his mules' attention — hit him on the head with a pick handle first.

Both Federal and State regulatory people have been making commendable progress in some communication efforts in the past few years, but there is a great need for more improvement. For example: Take the Federal Register. This communications effort may meet necessary legal requirements, but failure of the appropriate agencies to interpret its gobble-de-gook for their appropriate audiences reveals what can only be considered a lot of professional arrogance. The Federal Register may make fascinating reading for a few old-preregulatory officials and Philadelphia lawyers. For the rest of us who want to know what is being said it is a continuous exercise in frustration.

At the Monday afternoon session of this organization's Brucellosis Committee, copies of "Proposed Rules" were distributed and members were urged to consider them carefully. Following is a single sentence selected at random from that document. This sentence contains 100 words, 4 semicolons, and one comma. With your permission, I shall attempt to read it. It is paragraph 78.3 — Handling of certificates or permits for movement of animals.

(a) Whenever the regulations in this Part require a certificate or a permit for movement of animals and the animals are moved by a transportation agency issuing waybills or other forms of billing covering the movement, the certificate or permit for movement shall be delivered to such transportation agency by the shipper or his agent at the time the animals are delivered for shipment; shall become the property of the transportation agency; shall be attached to the billing by the transportation agency; shall accompany such billing to the destination of the animals; and shall be filed with such billing for future reference.

If those Proposed Rule changes are as important as they appear to be, why hasn't somebody made an effort to make them readable. How in the world could extension agents, farmers, livestock dealers and veterinarians be expected to know their content. I have tried repeatedly to interpret various statements in the Federal Register for that audience only to be rebuked because I could not accurately convey the meaning expressed in that jungle of
words.

It would be unfair on my part not to congratulate USDA-APHIS Information Division on the recent USDA News features that have been sent more frequently in recent months to communicators. The story on EIA Testing published on October 3, 1974 is a good example. It appeared in three sheets. The first was a well written concise progress report, the second page told the reactor rate and the number of tests by individual states on a national map. The third page discussed the “A” branded horse in an interesting manner. It was entirely irrelevant in Pennsylvania, but in spite of that it was a highly satisfactory attention-getting communications effort. Such bits of information transmitted to farmers’ and veterinarians’ news media can be extremely useful. That communications effort arrived on my desk the same day I learned that several 4H horses which won at regional shows were found positive to Coggins Test and County Agents called me for assistance. The USDA information has been widely disseminated in Pennsylvania and its audience has been receptive to it.

Our own state Bureau of Animal Industry has made a great forward stride informing its district offices, local veterinary associations, and animal agriculture organizations by publishing a monthly summary of its activities. This has been a well received worthwhile communications effort for all concerned.

On the other hand, Federal agencies have done very little to alert veterinarians and animal agriculture regarding the introduction of Exotic Animal Diseases. I have seen miles of good colored movies showing all veterinary aspects of diseases like Foot and Mouth Disease, Rinderpest and Bovine Pleural Pneumonia. These movies are too technical and far too sophisticated and too long for other than specialist audiences. Why hasn’t USDA-AHPIS put together a good colored film showing animals with typical symptoms of a few of the most feared exotic animal diseases. At the same time the films could show graphically how the diseases might spread and outline the measures necessary to contain them. Livestock men, poultrymen, feed men, Vo-Ag teachers and County Agents could care less about the gross pathology and histopathology of these diseases. What a great comfort it would be to regulatory people if they felt with some degree of confidence that this group of people and even veterinarians might recognize an exotic disease when it appears. The statement has been made publicly many times that it’s no longer a question whether or not we will have an outbreak of Foot and Mouth Disease; it’s only “when” we will have the outbreak. A good simple colored film with wide distribution would greatly aid detection and eradication of exotic disease. It is difficult to get the money to do the tasks already underway, but it is terribly shortsighted to fail to take every opportunity to communicate about such matters as this.

Communications within this organization regarding its procedural methods are far from satisfactory. Committee deliberations and actions could be greatly expedited if those who have proposals to make would make better presentations. For example, a state group made its second annual journey to present a proposal to the Brucellosis Committee that might have been understood in 1950, but committee members lacking all of the facts have had to postpone action for at least thirty days and schedule a very expensive special
meeting to consider the matter again. This is not an attempt to comment on the wisdom of the proposal. It is simply inexcusable that the case could not have been presented to committee members well in advance of their session stating all of the relevant facts, the objectives, the public health implications, the precise procedures sought and the termination dates for the program.

Over the past twenty years I have participated in many committee meetings as chairman, committee member, or interested listener. I've heard the chairman admit to members who present resolutions that were presented to the Executive Committee the previous year or years that he was not aware of any action regarding that proposal, but with the groups' concurrence it would be offered again. It seems to me that it would be proper for the Executive Committee to make an effort to keep committee chairmen informed.

The National Mastitis Council and Livestock Conservation Incorporated have made important contributions to better communications to their audiences. The new USAHA Newsletter is a great step forward. It would be a better communications effort if it could be produced by a professional news writer. Perhaps, it would be a good idea to write information sheets on two levels: One for professional regulatory men and scientists; the other for the animal agriculture public. Both would be worth the time and the money spent. From my own point of view, the recent move by APHIS-Veterinary Services Division which relocated Federal Veterinarians in Charge from State to Regional Offices has had serious detrimental effects on communicating. I would appreciate it if any in this audience will raise his hand and let me know that his communications have improved with the new regional setup. (No hands were raised)

For me, Albany, New York is thousands of miles farther away than is Harrisburg, Penna. I used to call the Federal Inspector in Charge at Harrisburg for answers to questions and for counsel frequently using our state WATS line and we communicated freely and effectively. Calling out-of-state is a major project for me. The Penn State Agricultural Extension Service is now restricted to the receiving end of communications which consist entirely of mimeographed missiles. Our Bureau of Animal Industry no longer has face to face communication down the hall only difficult long distance telephone contact. I know that the situation is worse in other states.

I have tried to review some good things and some bad things involving this organization's communication needs. There never has been a greater need for better communications. For the failures I can blame only the professionals involved. Professionals are expected to be aware of and keep abreast of the body of knowledge they hold, but this does not separate them from craftsmen. The real professional is one who is dedicated to use his knowledge and expertise for the PUBLIC INTEREST. Failure to do that can destroy this organization, the veterinary profession, and halt progress in disease control and eradication. Communication is everybody's business. Every successful communication can only be of great benefit to all.

Suggestions Which Should Improve Communications:

1. Federal agencies (APHIS, FDA, EPA) should prepare readable interpretations of relevant material from the Federal Register for this own people, state regulatory people, agriculture and veterinary press and the extension service.
2. Good color sound films depicting symptoms and major facts about losses, means of spread and eradication procedures would greatly help in recognition and attack on exotic animal disease.

3. APHIS-VS should sponsor a Seminar or other meeting to work out ways to improve communications between their new regional offices and their former sources and audiences.

4. This organization should establish procedural guidelines for those who plan to bring before it proposals. Committees of this organization must have all the facts to study before they meet for intelligent action.

5. This organization should continue the Newsletter only recently begun. A modest subscription fee might very well be used to keep it solvent.

6. A new "sunshine approach" to the activities and motives of this organization could only help to improve communications and foster the job which is of concern to all of us: The control and eradication of animal and poultry disease to insure that the impact of disease will remain lower in the U.S. animal agriculture than it is anywhere else in the world.
REPORT OF THE COMMITTEE ON PROFESSIONAL RELATIONS
US ANIMAL HEALTH ASSOCIATION 1974

Chairman: R. C. Hammond, College Park, Maryland
Co-Chairman: N. B. Haynes, Ithaca, New York


The Committee on Professional Relations met on Wednesday afternoon in the Colonial Room of the Hotel Roanoke. Actions resulting from the committee meeting are as follows:

1. The committee is pleased that the USAHA has begun publication of the Newsletter. To improve the effectiveness of the Newsletter the committee recommends that all members and especially Chairmen of all Committees report their views and activities to the Secretaries office continually throughout the year.

2. The committee recommends that the association commend the editor of the USAHA Proceedings for the quality of the book. We further recommend that ways be explored to expedite publication of the Proceedings including possible use of a style guide for papers presented.

3. The committee recommends that the Federal Extension Service reinstate and fill the position of Federal Extension Veterinarian. The lack of this position has resulted in a serious break in rapid and effective animal health communications to the Extension Services of the various states.

4. The committee recommends that the USAHA commend Livestock Conservation Inc. for the excellent job they are doing in educating the public about food animal diseases.

5. The committee recommends that the USAHA commend the National Mastitis Council for the production and distribution of the excellent series of slides to be used in the campaign against mastitis.

6. The committee recommends that the USAHA commend the Animal Health Institute for their campaign to prevent drug residues in foods of animal origin.

7. The committee recommends that the USAHA invite the officers of allied livestock organizations to send official representatives of their organization to attend deliberations of the USAHA meeting. It is anticipated that they will participate in relevant committee meetings and carry information back to their parent organizations.

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

Chairman: J. C. MacFarlane, Braintree, Massachusetts
Co-Chairman: A. E. Decoteau, Waltham, Massachusetts

The committee met on Wednesday as scheduled with eight committee members and 16 non-members present; we submit the following report.

1. There is a need to provide adequate care for animals being transported by various methods to include air transportation.

ACTION: The Committee offers its sincere support to USDA in seeking legislation to amend the Animal Welfare Act to include all carriers of animals under the act.

The Committee further offers support to USDA in developing a uniform health certificate for dogs and cats moving interstate and in international trade.

The Committee urges recognition of the necessity for an accredited veterinarian to be responsible for signing and issuing the proper health certificate to persons shipping dogs and cats interstate.

Too frequently common carriers accept any certificate. Lists of approved accredited veterinarians must be presented to common carriers at points of origin. The Committee suggests that the above comments be accepted. This Committee commends the excellent job performed by the ATC (Animal Transportation Committee) group covering air transportation.

2. The Committee strongly recommends a higher level of funding be approved for APHIS to properly conduct its current animal welfare programs thereby strengthening the progress already made in the betterment of animal welfare.

3. The Committee recommends the strengthening of regulations concerning Class B. licensed dealers under the Animal Welfare Act of 1970.

The Committee is concerned over the recent surge of stolen dog rings; specific proven cases involving numerous stolen dogs and cats have disclosed inadequate record keeping and the ease with which Class B dealers can falsify records. A severe and distinct record keeping system is needed; otherwise, one of the true purposes for enactment of the Act will be to no avail.

4. The Committee recommends support of the Department of Interior extension of the Lacey Act with the understanding that a resolution be offered to improve the permit system for zoological parks. It is believed by the committee that zoos and aquariums should not be prevented from importing necessary exotic animals, particularly for propagation of species.

The Committee further recommends necessary legislation effecting the sale of exotic animals through pet shops.

Many exotic species such as pythons, poisonous species of reptiles, various
species of monkeys are not only dangerous to the potential naive owner, their idiosyncrasies in need for care are often misunderstood, thereby a danger to the species itself is apparent. These species may also be disease carriers and dangerous to the environment.

The committee also recommends that all exotic species be properly identified as to common name and potential danger on the outer portion of any crate in which interstate shipments occur. This would include reptiles not under the current jurisdiction of the Animal Welfare Act of 1970.

5. The Committee recognizes the increase in personal horse ownership and the increase in inadequate care of horses within the suburbs. This, of course, must be differentiated from the working horse.

The committee suggests a study be developed to secure information concerning this problem with recommendations for improvement of such conditions.

6. The Committee recommends action on the Dog Fighting and Cock Fighting problems which are on the surge of increase in the United States. This committee recommends full support to current legislation with emphasis that a proper law enforcement agency be awarded the Act to Administer when passed.

7. The Committee is concerned on the increase of exotic bird quarantine stations. Due to a constant danger of diseases to the welfare of all birds, this committee recommends a return to a complete ban on the importation of birds.

The Committee further recognizes that until such time as a ban can again be declared, it is a necessity that an adequate and proven system of identification be disclosed. It is apparent that without adequate identification systems, the true prevention of smuggling cannot be controlled. Certain species, such as psittacines, cannot be banded since their traits would dispose of a band rather quickly.

The Committee also recommends to USDA that further regulations deem it necessary for the importer to disclose the actual country of origin of all birds imported.

8. The Committee urges the need to utilize more effective procedures in rounding up of wild horses to prevent the great loss due to injuries and death.

There are currently restrictions from using mechanical means of rounding up wild horses. Current methods have resulted in many injuries and loss of horses by death. This committee recommends action which will allow certain mechanized procedures to be initiated insuring usage by qualified operators only.

9. This Committee lauds the current progress made by USDA in handling the Animal Welfare Act. The Committee also suggests the Humane Society of the United States and the American Humane Association work concurrently in developing an information campaign on the care of animals.

The committee further recommends to the Executive Committee that where other committees' action recommendations made by the Animal Welfare Committee, these actions be conveyed appropriately to this committee.
1974 REPORT OF PARASITIC DISEASES AND PARASITICIDES
COMMITTEE

Chairman: J. L. Hourrigan, Hyattsville, Maryland
Co-Chairman: R. L. Pyles, Albuquerque, New Mexico


This committee met in open session on Wednesday, October 16.

Southwest Screwworm Program

A total of 14,976 laboratory-confirmed cases of screwworms were reported from the United States during calendar year 1973. This was a significant reduction from the more than 95,000 cases which occurred during 1972. In 1973, the United States cases were distributed as follows: Texas 8,913; New Mexico 1,103; Arizona 4,714; Oklahoma 1; California 235; Nevada 6; Utah 1; Colorado 1; Iowa 1; and Louisiana 1. During the first nine months of 1974, the program has continued to improve, as evidenced by the 3,758 laboratory-confirmed cases reported in the United States this year, more than a two-fold decrease under the 8,334 cases reported for the same period last year. There have been 3,482 cases in Texas; 147 in Arizona; 31 in California; 6 in Oklahoma; 90 in New Mexico; and 2 in Arkansas through September of this year. There were 19,789 laboratory confirmed cases of screwwords in the Mexican portion of the screwworm barrier zone in 1973, a marked decrease from the more than 30,000 cases during the previous year. For the first 9 months of 1974, there were 6,765 cases reported from Northern Mexico, which is a significant decrease from the 15,851, which occurred during the same period in 1973.

Puerto Rico and the Virgin Islands

As a result of the cooperative program with the United States Air Force, the British and US Virgin Islands and the Puerto Rican Islands of Vieques, Mona, and Culebra continue to be free of screwworms. Screwworms have been reduced to below detectable levels throughout most of Puerto Rico with the exception of a lingering infestation confined to several municipalities in Southeastern Puerto Rico.

Mexico

The United States-Mexico Commission for the Eradication of screwworms is making good progress in establishing a screwworm eradication program in Mexico. Both governments have assigned key personnel to their field stations in various parts of Mexico. Construction of the sterile fly production plant at Tuxtla Gutierrez, Chiapas, is well underway. Sterile fly distribution centers are being established at Tampico, Guadalajara, and at Tuxtle Gutierrez. The problem of inflation is one of the most serious problems facing program officials in Mexico.
Cattle Fever Ticks

During Fiscal Year 1974 *Boophilus* ticks were collected from 21 herds of livestock located within the area under State and Federal quarantines along the Rio Grande River in Southern Texas and from 26 herds of livestock located outside this quarantined area. In addition to these infestations, 102 stray or smuggled cattle and 67 horses of Mexican origin were seized disclosing 23 infested cattle and one infested horse. During the year 42 cases of illegal movements of livestock were reported. Inspections of livestock included 1,292,334 cattle and 61,834 horses; 189,168 cattle were dipped and 6,161 sprayed; 12,960 horses were dipped, and 34,546 sprayed.

By the end of the year nearly all of the dipping vats had been charged with coumaphos (Co-Ral) permitted dip rather than arsenic dip.

*Amblyomma variegatum* (tropical bout tick) were reported for the first time in Puerto Rico.

Psoroptic Scabies of Sheep

The entire country remained free from psoroptic scabies of sheep. The most recent outbreak occurred in January 1970.

Psoroptic Scabies of Cattle

During fiscal year, 1974, psoroptic scabies was reported in 39 herds in nine states as follows: Nebraska 11, Texas 10, New Mexico 6, Kansas 4, Colorado 4, and Oklahoma, Indiana, Iowa and Arizona 1 each. During fiscal year 1973, 53 outbreaks were reported compared to 91 outbreaks in 1972.

The committee reviewed research data available on the efficiency of coumaphos (Co-Ral) against psoroptic scabies mites and recommends Co-Ral be added to the list of permitted dips. All cattle and horses being imported into the United States from Mexico are being dipped in toxaphene permitted dip. Previously animals from certain areas were dipped in toxaphene, and those from other areas were dipped in arsenic.
REPORT OF COMMITTEE ON EVALUATION AND DEVELOPMENT OF STATE-FEDERAL PROGRAMS

Chairman: John L. O'Harrar, Reno, Nevada
Co-Chairman: John G. Milligan, Montgomery, Alabama


The Committee on Evaluation and Development of State-Federal Programs met at 1:30 p.m., Thursday, October 17, 1974, with twelve Committee members and seventy-nine visitors present.

The Committee received a Resolution from the National Assembly of Chief Livestock Health Officials as follows:

Resolution No. 1
National Assembly of Chief Livestock Health Officials

Source — National Assembly — 1974 Meeting — Roanoke, Virginia
Subject — Federal Realignment of Veterinary Services
Assigned to — U.S.A.H.A. Committee on Evaluation and Development of State-Federal Programs

Background: The realignment of the personnel in the U.S.D.A., A.P.H.I.S., Veterinary Services staff from about 50 State offices and staffs to 18 regional offices and staffs, the dislocation of so many U.S. employees, the reduction in field personnel in many States is causing great concern in the State veterinary regulatory organizations. There seems to be a growing discontent on all sides throughout the country with this arrangement. This problem, coupled with a serious reduction of commitment of Federal resources, both in money and manpower, to the critical problem of Brucellosis in a major section of the country, has resulted in almost chaotic conditions in the Brucellosis eradication program, and resulted in very grave concern in most of the States as to the very survival of the program.

NOW, THEREFORE, The National Assembly of Chief Livestock Health Officials, in annual session at Roanoke, Virginia, October 14, 1974, hereby resolve as follows:

Resolution:

After several prolonged meetings on the entire subject, and after hearing all of the debate, pro and con, and after a years experience with the effects to date of the new system, it is the considered opinion of the membership that realignment has not and does not work, that as it now exists it is not practical, and that it is not responsive to joint State-Federal Program needs. We therefore herewith refer this matter to the Committee on Evaluation and Development of State-Federal Programs of the U.S.A.H.A. with a re-
quest that that Committee use its best efforts to seek the necessary corrective action.

Adopted on Motion by Dr. Regan, seconded by Dr. Quinn — 38 ayes, 0 nays, 3 not voting.

The resolution was fully discussed in open session with twenty-one members and guests speaking to the problem brought to light by the resolution as it pertained to their particular situation or situations they were knowledgeable about.

Responsible Federal officials were present at the meeting. The following proposal, in response to the resolution and discussion was presented by Dr. John Hejl, Deputy Administrator, APHIS, USDA, and adopted by the Committee: That the President of U.S.A.H.A. appoint a representative group from this association to meet with Federal officials for development of corrective measures and elimination of deficiencies in realignment as they relate to State-Federal Cooperative Programs. It is the intent of this Committee that the membership be advised of progress through communication with various members of the Executive Committee.

The Committee was pleased to hear the Administrator of APHIS state that Brucellosis eradication has been placed in top priority. We urge this priority be maintained to the successful conclusion in the eradication of Brucellosis. Failure in this area will result in jeopardy of all State-Federal programs.

We concur in the need for diversion of funds from other program activities due to the present budget allocations. However, we would caution program Administrators to avoid cutting other programs to the level that inadequate work can be accomplished with the result of programs deteriorating as has occurred in the Brucellosis program. We strongly urge that efforts be made through Office of Management and Budget to obtain adequate funds for Animal Disease Programs.

This Committee concurs with the recommendation of the State-Federal Relations Committee that the Brucellosis Program Review currently in progress be terminated. Funds used for that purpose can better be used for support of other activities in the Brucellosis program.
REPORT OF THE COMMITTEE ON PUBLIC HEALTH AND ENVIRONMENTAL QUALITY

Chairman: R. L. Parker, Atlanta, Ga.


The Committee heard the report of the sub-committee on “Flea Collars” presented by Dr. Robert Singer. The sub-committee was not able to locate any evidence of severe or permanent neurologic or other damage to animals wearing flea collars, except in animals with underlying pathological states, recognized or not. Dr. James H. Steele presented additional evidence, based on surveys of veterinary practitioners in Harris County, Texas, that substantiated the report of Dr. Singer. The Committee concluded that a definite problem exists only when flea collars are used on sick animals, and may exist if simultaneous medications are used with the collar, or if the collar is applied too tightly. Since several new chemical ingredients may be forthcoming in flea collars continued informal surveillance is indicated, but no public health problem is known to be associated with currently available flea collars.

At the invitation of the Committee, Dr. J. R. McDowell, Veterinary Medical Officer, Division of Veterinary Medical Review, Bureau of Veterinary Medicine, Food and Drug Administration, reported on the present status of animal products included in pet foods. Canned pet foods first appeared about 1925 and for the most part reflected efforts of the meat packing industry to salvage by-products. The Food and Drug Administration is charged with all regulatory activities related to animal feeds; under the Food, Drug and Cosmetic Act no distinction exists between human and animal foods. Administrative priorities have resulted in emphasis on control of human foodstuffs. Regarding any foodstuffs the FDA is concerned with adulterations and misbranding, including false claims.

At the present, more emphasis than in the past is being placed on the types of animal protein being used in pet foods. Complications arise when disposal of animal carcasses create conflicts with local zoning laws, the Clean Air Act, and a variety of economic factors, rendering or similar salvage procedures may be the only acceptable alternatives. In addition to concern for ingredients, the FDA is cooperating with the Animal Association of Feed Control officials in reviewing label statements regarding claims for specific benefits, nutritional balance and economic misbranding tending to mislead the purchaser.
Although at this time the FDA has no compliance program for pet foods, recalls and seizures for specific causes have been made. The question of inclusion of animal ingredients derived from sources other than by slaughter have been under review for a number of years. Regulation of this area is presently under consideration.

Dr. E. J. Lauritsen of Louisiana State University had furnished a proposal for a public supported impoundment facility for dogs and cats to the Committee. Since two other committees (Rabies and Humane Treatment) were known to be considering similar material the chairman appointed a sub-committee on Animal Regulation for liaison with the other committees. The sub-committee will be composed of Drs. R. L. Parker, William Hubbert, and James Kornder.

The need for surveillance of diseases of agricultural workers was discussed. The role of the U.S. Department of Labor (OSHA) in this area and the need to acquaint that agency with the farm industrial health problem, and the U.S. Department of Health, Education and Welfare (NIOSH) report on “Respiratory Disease in Agricultural Workers” as a partial response to the need were discussed.

The death of Dr. K. F. Meyer, a leader in many areas of veterinary medicine and animal health was noted. A biographical sketch of Dr. Meyer by Dr. J. H. Steele was published in the Journal of Infectious Diseases, Vol. 129 (Supplement), pages S-1 – S-12, May 1974.

Your Committee respectfully submits this report for your approval.
HOST ANIMAL EFFICACY AND LABORATORY POTENCY TESTS FOR NON SPECIFIC BACTERINS, MIXED BACTERINS, AND BACTERIAL ANTISERUMS

M. H. Bairey,* D.V.M., M.S. and N. K. Jungk,** M.A.

Introduction

Some 40 to 50 years ago certain bacterins started becoming commercially available composed of species of organisms, singly and in various combinations, primarily of the genera Aerobacter, Corynebacterium, Escherichia, Pasteurella, Pseudomonas, Salmonella, Staphylococcus, and Streptococcus. In 1939, by an agreement between the manufacturers and the U.S. Department of Agriculture, certain of these combinations were "fixed" in regard to their components and identified as specific "Mixed Bacterins," such as "Mixed Bacterin (bovine) Formula 1."29 These same organisms were also used in fixed combinations in the preparation of hyperimmune antisera.28 In 1958 there were 24 of these mixed bacterins and antisera licensed by the USDA.30 In addition to these products there continued to be available bacterins, similar to mixed bacterins, called by their genus/species names: such as, "Coli-Enteritidis Bacterin," "Pasteurella Bacterin," etc. These generally have been referred to as non specific bacterins17 since they have not been given an official designation as a mixed bacterin, yet include some of the same species of organisms. These bacterins, the mixed and non specific, are intended for use in stimulating resistance to microorganisms that are not generally considered primary pathogens. And, thus, such bacterins are distinguished from specific bacterins which induce immunity to specific diseases such as erysipelas and leptospirosis.10

During the most active era of veterinary immunology, extending over the last 25 years, this group of organisms making up the nonspecific bacterins, mixed bacterins, and bacterial antisera were given a low priority of attention. With certain exceptions they became marginally profitable to manufacture and considered of questionable value.1,5,6,2,3,9,10,14,16,19,51 Many manufacturers dropped their federal licenses and ceased manufacturing these products. This does not mean that as a group they are worthless or that future improvements will not be forthcoming. Quite to the contrary, experimentally and under specified conditions of commercial manufacture and use certain of these bacterins, particular components of these bacterins and antisera have been reported to be efficacious.2,4,6,11,13,15,22,25 Also, the demand for these particular products has continued strong31,33 indicating confidence by a segment of the livestock industry.

The value of these products has been questioned from time to time because of several reasons.10 Certain of the organisms involved have never been shown to be pathogenic and in all cases efficacy has been difficult to

*Head, Aerobic Products Section, USDA-APHIS-VSL, Ames, Iowa
demonstrate by controlled tests. Some organisms of this group do not cause a clinically definable primary disease but rather they are secondary invaders, or opportunists, which show up in conjunction with other health problems. Immunization and challenge tests have been difficult to perform since, in many cases, it has been found impossible to duplicate experimentally the clinical disease as seen under field conditions.

When the general interest, from many sources, in product efficacy was brought into focus by the Veterinary Services section of USDA-APHIS-VSL in late 1970, the non-specific bacterins, mixed bacterins, and bacterial antisera group of products was correctly chosen to receive first attention.

Organizational Program

At the 1971 annual Spring meeting of AHI-VBLC, an Ad Hoc Subcommittee of interested VBLC members was appointed by the VBLC Chairman to initiate and coordinate a review and study of this group of products and further to cooperate with the active research program on these products underway since early 1970 by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services Laboratories (USDA-APHIS-VSL). TABLE 1 shows the current participants in this research effort.

It became apparent that the problem of evaluation had to be approached on the basis of individual organisms rather than products. TABLE 2 shows the number of products under consideration and the organisms involved in the evaluation and review as it faced us in 1971. Our initial effort included a survey of diagnostic veterinarians, State Veterinarians, Veterinary Medical Schools, and State regulatory officials to learn the current frequency of isolation of these organisms from field disease problems. The survey also gave us certain collateral information to improve our understanding of the entire problem. Based on this information we evaluated the organisms and placed them into three groups as shown in TABLE 3.

Those organisms to be eliminated from the products were so designated for one or both of the following reasons: 1) they were judged not to be natural pathogens for animals and were not associated with animal health problems; 2) if they were occasionally pathogenic, their low incidence of occurrence would not justify the effort to develop effective immunization agents. Organisms in the group listed “Not Under Study,” while important pathogens, were placed there because only one or possibly two companies were interested in each case. Thus, there is work progressing on certain of these organisms by individual companies but not by the Ad Hoc Subcommittee or APHIS-VSL. Organisms listed as “Under Active Study” are official components of 10 bacterins and are used in manufacturing 3 antiseraums.

TABLE 4 shows the licensed products under consideration in April 1974. Note that the number of bacterins and antiseraums under consideration has been reduced and consolidated from a total of 32 in 1971 to 13 in 1974 and the number of organisms under study reduced from 15 to 7.

Investigative Procedure

Our approach, in all cases, following selection of the organisms for
study, has been as follows:

1. Investigation and selection of the proper bacterial isolates for bacterin preparation. This included examination and immunogenic evaluation of many strains. Agreement was necessary, based on the history and experimental facts, among companies and with APHIS-VSL before a final determination was made.

2. Preparation of reference bacterins, deposited in adequate quantities with Veterinary Services, for subsequent use by industry and APHIS-VSL in setting proper standards of potency of future bacterins.

3. Development of laboratory potency tests, generally and preferably laboratory animal protection tests, correlated with host animal efficacy and suitable for serial to serial releases of product.

4. Demonstration of host animal efficacy.

Due to the varying complexity, the nature of each problem, and the amount of attention given by the Subcommittee and APHIS-VSL progress has not been equal for each organism.

Laboratory Results and Discussion

The experimental work has been oriented toward the desired goals of establishing the efficacy of bacterins and antisera prepared from each of these organisms in the host animal and to develop a meaningful potency test that would reflect this efficacy on a serial to serial basis.

The organisms selected for initial studies and from which the present methods being utilized were developed were Pasteurella multocida and Salmonella typhimurium.

After evaluating several of the currently accepted methods of potency test procedures, an adaptation of the Ose-Muenster mouse protection test was selected. TABLE 5. This bioassay procedure is conducted by vaccinating 7 groups of 10 mice each with 1/20th of the recommended field dose twice 14 days apart. The immunity produced in the mice is challenged by inoculating 10 mouse groups with consecutive tenfold dilutions of a virulent culture of the organism being assayed. The challenge inoculation is given 7-12 days after the second vaccination.

The potency of the bacterin is measured by calculating the number of logs protection which is also referred to as the "protective index".

The logs protection is determined by subtracting the 50% lethal dose of the vaccinated mouse groups as calculated by the method established by Reed and Muench from the 50% lethal dose of the unvaccinated mouse groups.

A procedure for infecting sheep with a bovine isolate of P. multocida was developed and the efficacy of experimental reference bacterins was measured using sheep as the host animal. TABLE 6. Two bacterins were evaluated at various dilutions. Bacterin A was an experimental bacterin produced on solid media while Bacterin B was a larger lot of reference bacterin produced by commercial manufacturing methods. It can be noted from the table that a 100% infection rate was produced in both evaluations.

Bacterin A was produced at least 75% protection against this infective dose even when diluted to 1 to 3. The corresponding mouse potency was 6.5 logs protection or greater.
Bacterin B produced only 33% protection undiluted and it is postulated that an immune paralysis phenomena was produced as there was over 7 logs protection in mice. When this bacterin was diluted 1 to 5, 75% protection was produced with a mouse potency of 6.6 logs.

However, then Bacterin B was diluted 1:25 or more, there was no protection produced in sheep and the potency in mice dropped to 2.5 logs.

A similar procedure was developed in swine using a porcine isolate of *P. multocida* as cross protection between bovine and swine isolates could not be demonstrated. TABLE 7

The reference bacterin produced 100% protection against a 100% infection when given undiluted with a corresponding mouse potency of 5.8 logs. A 1:2 dilution gave 50% protection and a 1:4 dilution gave no protection in swine but a mouse potency of 3.1 logs.

Additional results in several laboratories indicated a mouse potency of 3.5 logs for both bovine/ovine and swine *P. multocida* bacterins would be indicative of a bacterin that would produce satisfactory protection in the host animal.

It was of interest to assay presently produced *P. multocida* containing bacterins to determine the potency of these products. Three different bacterins produced by three different manufacturers were selected for the initial examination. The results are recorded in TABLE 8.

The results indicate that the potency produced with Mixed Bacterin Bovine Formula I is less than in other preparations. The results also indicate that there is a variance between companies producing the same bacterin combination.

A procedure was developed for infecting calves with a bovine isolate of *S. typhimurium*. This procedure was used to evaluate the efficacy of a reference bacterin prepared by a commercial manufacturing procedure. Two replications were conducted in calves that were 6 weeks old at the time of the first vaccination by two different laboratories. TABLE 9

The results were very similar with solid protection being produced by the undiluted bacterin against a 75% and 100% infective challenge. The corresponding mouse potency was 4.4 logs or more.

Reduced protection was demonstrated when the bacterin was diluted 1:100 and very little protection was produced when the bacterin was diluted 1:1000. The corresponding mouse potency for the less effective bacterins was 3.1 logs or less.

One serial of commercial *S. typhimurium* containing bacterin was evaluated in calves and mice demonstrating 100% efficacy and 4.8 logs protection or greater. From these data it was determined that at least 3.5 logs protection was necessary by the mouse potency test to indicate an efficacious bacterin.

When presently available Salmonella containing Mixed Bacterins were tested, some company to company and serial to serial variation was observed. TABLE 10. However, none of these bacterins contained *S. typhimurium* but did contain antigenically related Salmonellae.

A procedure has been developed to infect swine with *S. choleraesuis*. Both an experimental bacterin and a commercially prepared reference bacterin have been evaluated by this procedure by two replications at each di-
olution. TABLE 11. The experimental Bacterin A protected undiluted, but showed some loss of protection when diluted 1:10. Reference Bacterin B appeared to produce some immune paralysis undiluted but protected the animals at 1:10. There was a reduction in efficacy when the bacterin was diluted 1:100. The mouse potency results were erratic but 2.2 logs appeared to correspond with reduced protection. Additional experiments are necessary with this organism to establish test parameters for the mouse potency test so as to establish the logs protection necessary for a bacterin to indicate that it will have efficacy in swine.

This fact was further illustrated when *S. choleraesuis* containing mixed bacterins were assayed by the mouse potency test. TABLE 12. Some bacterin and company to company variation was observed but erratic death patterns in mice may have been the cause of these variations.

One trial was conducted using a multi-component bacterial antiserum in sheep and doing comparative studies using the mouse potency test system passively. TABLE 13. This experiment was conducted for *P. multocida* by calculating both the sheep and mouse dose equally on a ml per kilogram basis. These results indicate that passive protection can be demonstrated in the host animal and that a mouse potency test is applicable and will correlate with the host animal efficacy. However, further experiments are necessary to establish what level of potency in the mouse test would indicate protection in the host animal.

**Summary**

In the course of our joint work, the VBLC-Ad Hoc Subcommittee and APHIS-VSL have held 5 major joint technical meetings since August of 1971 and 5 small informal meetings. This has resulted in substantial progress in the reorganization of Non specific Bacterins, Mixed Bacterins, and Bacterial Antisera in order to eliminate unnecessary components and products; reducing the number of such products to those for which we already have or hopefully will demonstrate host animal efficacy and make available as positive aids in the control of animal diseases.

The current status of our studies with these components are that host animal efficacy and mouse potency test procedures have been established and will be issued as proposed rule making in the Federal Register for *P. multocida* and *S. typhimurium*.

Host animal efficacy has been established for *S. choleraesuis* but further work is necessary to standardize the mouse potency test before a standard test procedure can be proposed.

Promise has been indicated with the Bacterial Antiserums and *S. dublin* but more host animal and potency test development is necessary before standard test procedures can be considered.

Several avenues for evaluation of *E. coli* have been pursued but the chances for success with this organism are still unknown.

Initial studies and experimentation have been started with *P. hemolytica* and *C. pyogenes* but sufficient progress has not been made as yet to estimate the probability of success.
### TABLE 1  PARTICIPANTS IN THE JOINT PROGRAM TO ESTABLISH HOST ANIMAL EFFICACY AND LAB POTENCY TESTS

US REGULATORY AGENCY: USDA-APHIS-VSL  

BIOLOGICAL INDUSTRY: AHI-VSLC-AD HOC SUBCOMMITTEE (1974)  

| Abbott (National) | Burns Biotec Colorado Serum | Fort Dodge Franklin |  
| Affiliated Armour | Cutter Diamond | Philips Roxane (Anchor) Pitman Moore |  
| Bayvet (Cutter-Haver) | | |  

### TABLE 2  ORGANISMS USED IN PREPARATION OF LICENSED PRODUCTS UNDER CONSIDERATION, 1971 (25 Bacterins and 7 Antiserums) (15 Organisms)

| AEROBACTER AEROGENES | SALMONELLA ENTERITIDIS |  
| CORYNEBACTERIUM PYOGENES | SALMONELLA SCHOTTMUELLERI |  
| ESCHERICHIA COLI | SALMONELLA TYPHIMURIUM |  
| PASTEURELLA MULTOCIDA | STAPHYLOCOCCUS ALBUS |  
| PSEUDOMONAS AERUGINOSA | STAPHYLOCOCCUS AUREUS |  
| SALMONELLA ABORTIVOEQUINA | STREPTOCOCCUS EQUUS |  
| SALMONELLA CHOLERAESUIS | STREPTOCOCCUS SPECIES |  
| SALMONELLA DUBLIN | |  

### TABLE 3  STATUS OF ORGANISM EVALUATION, 1974

<table>
<thead>
<tr>
<th>Under Active Study</th>
<th>Not Under Study</th>
<th>Eliminated from U.S. Licensed Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORYNEBACTERIUM PYOGENES</td>
<td>STAPHYLOCOCCUS AUREUS</td>
<td>AEROBACTER AEROGENES</td>
</tr>
<tr>
<td>ESCHERICHIA COLI</td>
<td>STREPTOCOCCUS EQUUS</td>
<td>PSEUDOMONAS AERUGINOSA</td>
</tr>
<tr>
<td>PASTEURELLA HEMOLYTICA</td>
<td>STREPTOCOCCUS SPECIES</td>
<td>SALMONELLA ABORTIVOEQUINA</td>
</tr>
<tr>
<td>PASTEURELLA MULTOCIDA</td>
<td></td>
<td>SALMONELLA ENTERITIDIS</td>
</tr>
<tr>
<td>SALMONELLA CHOLERAESUIS</td>
<td></td>
<td>SALMONELLA SCHOTTMUELLERI</td>
</tr>
<tr>
<td>SALMONELLA DUBLIN</td>
<td></td>
<td>STAPHYLOCOCCUS ALBUS</td>
</tr>
<tr>
<td>SALMONELLA TYPHIMURIUM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 4  LICENSED PRODUCTS UNDER CONSIDERATION, APRIL 1974

<table>
<thead>
<tr>
<th>10 Bacterins and 3 Antiserum (7 Organisms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLOSTRIDIUM CHAUVOEI PASTEURELLA BACTERIN</td>
</tr>
<tr>
<td>CLOSTRIDIUM CHAUVOEI-SEPTICUM PASTEURELLA BACTERIN</td>
</tr>
<tr>
<td>COLE-STAPHYLOCOCCUS STREPTOCOCCUS BACTERIN</td>
</tr>
<tr>
<td>CORNYEBACTERIUM PASTEURELLA BACTERIN</td>
</tr>
<tr>
<td>PASTEURELLA BACTERIN</td>
</tr>
<tr>
<td>SALMONELLA DUBLIN-TYPHIMURIUM BACTERIN</td>
</tr>
<tr>
<td>MIXED BACTERIN BOVINE FORMULA 1</td>
</tr>
<tr>
<td>MIXED BACTERIN BOVINE FORMULA 2</td>
</tr>
<tr>
<td>MIXED BACTERIN PORCINE FORMULA 1</td>
</tr>
<tr>
<td>MIXED BACTERIN PORCINE FORMULA 2</td>
</tr>
</tbody>
</table>

TABLE 5  MOUSE POTENCY TEST

1. Vaccinates = 7 groups of 10 mice each vaccinated with 1/20 of the recommended field dose.
2. Unvaccinated controls = 7 groups of 10 mice each.
3. Two vaccinations 14 days apart.
4. Immunity is challenged by inoculating groups of vaccinates and controls with tenfold dilutions of virulent bacterial culture 7 to 12 days after the second vaccination.
5. The 50% Lethal dose is calculated for both vaccinates and unvaccinated controls.
6. The 50% Lethal dose of the vaccinates is subtracted from the 50% Lethal dose of the unvaccinated controls.
7. The difference is recorded as the "Logs Protection" or "Protective Index".

TABLE 6  PASTEURELLA MULTOCIDA BACTERIN (Bovine and Ovine)

<table>
<thead>
<tr>
<th>Bacterin</th>
<th>Dilution</th>
<th>Sheep Protection</th>
<th>Infection Rate</th>
<th>Mouse Logs Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Undiluted</td>
<td>75%</td>
<td>100%</td>
<td>6.6</td>
</tr>
<tr>
<td>A</td>
<td>2:3</td>
<td>75%</td>
<td>100%</td>
<td>6.9</td>
</tr>
<tr>
<td>A</td>
<td>1:3</td>
<td>100%</td>
<td>100%</td>
<td>6.5</td>
</tr>
<tr>
<td>B</td>
<td>Undiluted</td>
<td>33%</td>
<td>100%</td>
<td>&gt; 7</td>
</tr>
<tr>
<td>B</td>
<td>1:5</td>
<td>75%</td>
<td>100%</td>
<td>6.6</td>
</tr>
<tr>
<td>B</td>
<td>1:25</td>
<td>0%</td>
<td>100%</td>
<td>2.5</td>
</tr>
<tr>
<td>B</td>
<td>1:50</td>
<td>0%</td>
<td>100%</td>
<td>2.3</td>
</tr>
</tbody>
</table>
### TABLE 7  PASTEURELLA MULTOCIDA BACTERIN (Porcine)

<table>
<thead>
<tr>
<th>Bacterin Dilution</th>
<th>Swine Protection</th>
<th>Infection Rate</th>
<th>Mouse Logs Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>100%</td>
<td>100%</td>
<td>5.8</td>
</tr>
<tr>
<td>1:2</td>
<td>50%</td>
<td>100%</td>
<td>*ND</td>
</tr>
<tr>
<td>1:4</td>
<td>0%</td>
<td>100%</td>
<td>3.1</td>
</tr>
</tbody>
</table>

*ND - Not done

### TABLE 8  MOUSE PROTECTION RESULTS OF P. MULTOCIDA CONTAINING BACTERINS

<table>
<thead>
<tr>
<th>Bacterin</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Pasteurella Bacterin</td>
<td>*&lt; 2</td>
</tr>
<tr>
<td>Clostridium Chauvoei Septicum Pasteurella Bacterin</td>
<td>3.5</td>
</tr>
<tr>
<td>Mixed Bacterin Bovine #1</td>
<td>&lt; 3.0</td>
</tr>
</tbody>
</table>

* Logs Protection
## Table 9

<table>
<thead>
<tr>
<th>Repetition</th>
<th>Dilution</th>
<th>Calf Protection</th>
<th>Infection Rate</th>
<th>Mouse Logs Protection Range</th>
<th>No. Reps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Undiluted</td>
<td>100%</td>
<td>100%</td>
<td>4.4 - 5.7</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Undiluted</td>
<td>100%</td>
<td>75%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:10</td>
<td>*ND</td>
<td></td>
<td>4.3 - 5.6</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>1:100</td>
<td>50%</td>
<td>100%</td>
<td>1.3 - 3.1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1:100</td>
<td>50%</td>
<td>75%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1:1000</td>
<td>0%</td>
<td>100%</td>
<td>0.2 - 0.6</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1:1000</td>
<td>25%</td>
<td>75%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Commercial</td>
<td>100%</td>
<td>100%</td>
<td>4.8 - 5.5</td>
<td>4</td>
</tr>
</tbody>
</table>

## Table 10

<table>
<thead>
<tr>
<th>Bacterin</th>
<th>Company</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Mixed Bacterin</td>
<td>4.8</td>
<td>4.1</td>
<td>3.3</td>
<td>3.8</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>Bovine #3</td>
<td>5.7</td>
<td>3.2</td>
<td>2.4</td>
<td>4.3</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Mixed Bacterin</td>
<td>5.1</td>
<td></td>
<td>3.2</td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine #2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed Bacterin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>Ovine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## TABLE 11
**SALMONELLA CHOLERAESUIS BACTERIN**

<table>
<thead>
<tr>
<th>Bacterin</th>
<th>Rep</th>
<th>Dilution</th>
<th>Swine Protection</th>
<th>Infection Rate</th>
<th>Mouse Loggs Protection Range</th>
<th>No. Reps</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Undiluted</td>
<td>100%</td>
<td>100%</td>
<td>3.7 - 4.1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Undiluted</td>
<td>100%</td>
<td>67%</td>
<td>4.4</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>Undiluted</td>
<td>25%</td>
<td>75%</td>
<td>2.2 - 4.3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Undiluted</td>
<td>50%</td>
<td>100%</td>
<td>5.1</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>1:10</td>
<td>50%</td>
<td>100%</td>
<td>3.1 - 4.3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1:10</td>
<td>67%</td>
<td>67%</td>
<td>4.7</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>1:10</td>
<td>75%</td>
<td>75%</td>
<td>1.7 - 3.9</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1:10</td>
<td>100%</td>
<td>100%</td>
<td>5.1</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>1:100</td>
<td>50%</td>
<td>75%</td>
<td>0.0 - 2.2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1:100</td>
<td>25%</td>
<td>100%</td>
<td>2.2</td>
<td>1</td>
</tr>
</tbody>
</table>

## TABLE 12
**MOUSE PROTECTION RESULTS OF S. CHOLERAESUIS CONTAINING BACTERINS**

<table>
<thead>
<tr>
<th>Bacterin</th>
<th>Company</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed Bacterin</td>
<td></td>
<td>2.5</td>
<td>1.3</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine #1</td>
<td></td>
<td>2.5</td>
<td>2.0</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed Bacterin</td>
<td></td>
<td>1.5</td>
<td>1.3</td>
<td>3.9</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine #2</td>
<td></td>
<td>2.4</td>
<td>3.3</td>
<td>3.5</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 13: Pasteurella Multocida Antiserum

<table>
<thead>
<tr>
<th>Antiserum Dosage</th>
<th>Sheep Protection</th>
<th>Infection Rate</th>
<th>Mouse Logs Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1.3 ml/kg</td>
<td>88%</td>
<td>75%</td>
<td>5.2</td>
</tr>
<tr>
<td>1.0 ml/kg</td>
<td>88%</td>
<td>75%</td>
<td>ND</td>
</tr>
<tr>
<td>0.3 ml/kg</td>
<td>63%</td>
<td>75%</td>
<td>2.8</td>
</tr>
<tr>
<td>0.15 ml/kg</td>
<td>38%</td>
<td>75%</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* Dosage was calculated on a ml/kg basis for both sheep and mice.

References

HOST ANIMAL EFFICACY


REPORT OF THE COMMITTEE ON BIOLOGICS

Chairman: R. B. Bushnell, Davis, Calif.
Co-Chairman: T. J. Grennan, Providence, R.I.

The meeting of the Biologics Committee was well attended by representatives of the livestock industry, APHIS, the State Animal Health Veterinarians, the biological industry, and the state universities.

In its 1973 report this committee expressed concern that the Animal Plant Health Inspection Service might remove certain fractions of licensed biological products from the market due to lack of proven efficacy. At this meeting a report was presented by Mr. Norman Jungk (Biologics Licensees Committee of AHI), and by Dr. Miles Bairey of the Veterinary Services Laboratory in which they outlined the progress made in developing efficacy and potency tests for fractions of those biologics concerned. Their report by addendum to the Biologics Committee report is made a part of these proceedings.

This cooperative effort between the biological industry and APHIS, and the spirit in which it was done, is commended by this committee.

Reports of anaerobic bacterin shortages during the past year were reviewed by this committee. A discussion of the situation suggested that the apparent shortages were primarily due to problems of distribution rather than restrictions imposed by changes in product testing procedures. It was suggested that seasonal demands for some products could result in shortages if sudden changes in testing procedures were imposed. It was, therefore, requested that the newly appointed subcommittee study the problem further.

This committee briefly discussed investigations of field problems associated with biologics and was pleased to learn of Veterinary Services' intent to document a procedure for handling such problems, which includes prompt notification of the manufacturer.

Realignment has had a beneficial effect on the ability of Veterinary Services to promptly and adequately make investigations of reported problems. This includes the capability for on-site investigations by Veterinary Services and the availability of the diagnostic capability at VSL:

APHIS regulations over the last several years have had a marked effect on biological research. Regulations to improve purity of products have stimulated development of new methods to produce live virus products and have stimulated the use of cell lines as well as frozen primary cells. This includes the development of practical methods to detect contaminating viruses and mycoplasma. Requirements to fully evaluate the safety and efficacy of
seed viruses have also stimulated much significant evaluation work. On the other hand, APHIS regulations have tended to retard research in the development and improvement of new products which have low potential sales volume as a result of the technical difficulties and economic problems of meeting all requirements.

The ram epididymitis bacterin is a current example of a product which may no longer be available. The producer has tentatively elected to surrender its license for this vaccine because the volume of sales does not warrant research to develop a serial to serial test for efficacy. However, the firm is still investigating the sales potential and the possibility of resuming production.

This vividly points to the specific problem that some of the smaller livestock industries do not have adequate economic impact to induce the biologics industry to produce specific products.

This committee recommends that land grant universities and the Agricultural Research Service give priority to the development of workable test procedures for efficacy of biological products which have a limited demand. Otherwise, small livestock industries, such as the sheep industry, will continue to encounter increasing difficulty in obtaining biological products.

This committee commends the efforts of APHIS in the circulation of the Veterinary Biologics Notices.

Because of the importance of these notices, it was felt that the circulation include those members of this Biologics Committee, as well as the national livestock producers organizations.

In an attempt to respond to some of the current problems the chairman appointed a Special Subcommittee on Biologics. Its responsibility is to establish liaison with the APHIS Veterinary Services and keep the industry and the veterinary profession appraised of new developments relating to logical products and alerting them to potential problems. Any problems related to this subject should be forwarded to one of the members of this subcommittee. These members are: J. W. Glosser, L. E. Hanson, D. A. Fuller, G. B. Peacock, Skip Thayer, and R. F. Hall. This subcommittee will disseminate this information to interested and affected groups and shall function as an action committee to help to speedily solve these problems.

1974 USAHA Committee on Biologics

Dr. Robert B. Bushnell, Chairman (P), Extension Veterinarian, Vet Med Annex, University of California, Davis, Calif. 95616
Dr. T. J. Grennan, Jr., Co-Chairman, Division of Animal & Dairy Industry, R. I. Dept. of Health, Davis Street, Providence, R.I. 02908
*Dr. G. V. Peacock (P), 912 Fortune Place, Edgewater, Md. 21037, 301-436-8721
Dr. M. T. Goff (P), P.O. Box 70, Ames, Iowa 50010, 515-232-0250
Dr. I. M. Paton (A), 3205 W. 84th Place, Leawood, Kans. 66206
*Dr. L. E. Hanson (P), R.R. 2, Urbana, Ill. 61801, 217-367-5392
Dr. N. B. Haynes (P), 214 Enfield Falls Rd., Ithaca, N.Y. 14850
Dr. J. Ralph Bishop (A), R.R. 4, Tipton, Ind. 46072
Information has been accumulating for some period of time that in California we are seeing an increasing incidence of clinical bluetongue in sheep which have been vaccinated. The time interval between vaccination and clinical disease, plus the severity of signs seen, indicates this is a result of natural exposure to the field strain and not a reversion of vaccine virulence.

I have observed clinical bluetongue and an isolate has been obtained from a flock in Sacramento County, California, which has experienced this problem for the last four years. For two years multiple vaccination (up to 3X) with Cutter's egg attenuated vaccine failed to prevent clinical disease. Limited cross immunity trials with the isolate at Davis by Dr. McKercher indicates that this is a variant strain from Bluetongue "8".

This flock has had a higher disease incidence this year following vaccination as recommended with the tissue attenuated vaccine of Colorado Serum Co.
Several reports in 1974 have indicated vaccination breaks with tissue attenuated vaccine. For example: Our Chico State University flock as well as flocks belonging to Mr. Patton, Al Vierra, Dick Gee, and Betty Rodgers, of the same area, as reported by their veterinarian, Dr. Dalzeil, have just experienced a high incidence of clinical disease (50% or greater) with concurrent death loss in some sheep which had been vaccinated two months earlier. Some of these flocks had a similar problem in 1973, and an isolate of bluetongue was obtained. Animals of several ages were affected, and the black-face sheep seem to be most susceptible.

Since we have seen a sudden increase in these reports in vaccinated sheep since the Cutter vaccine was withdrawn, and because a possible variant strain has been isolated, I feel the problem is twofold.

The tissue attenuated vaccine is not highly effective in controlling outbreaks due to bluetongue "8" for which it is designed. Secondly, we are encountering variant strains with antigenic differences against which we are not getting protection.

I request that responsible agencies make a cooperative effort to resolve this problem which has become of economic importance to the sheep industry. I suggest that the Denver Research Laboratory should supply the biological industry with attenuated strains of sufficient potency to produce immunity under field challenge.

A second issue of the Bluetongue Disease Problem has been brought to our attention by practicing veterinarians and producers who are required to test any animals negative to the test for bluetongue.

The validity and accuracy of the three tests available is under question, and justly so. The validity of the C.F., gel diffusion, and S.N. tests need to be compared.

Current official testing with the C.F. test indicates animals fluctuate almost weekly as to their positive and negative status. This has created an atmosphere of doubt and distrust among veterinarians and producers honestly attempting to comply with the export regulations.

As a profession, we need to define and resolve these two problems.
LEAD TOXICOSIS IN CATTLE*

C. M. Hibbs, D.V.M., Ph.D.

Summary
Three case reports of lead poisoning are presented to indicate the seriousness of exposure to this element. The need for constant vigilance against lead poisoning is obvious from these case reports. Lead poisoning can be prevented as well as any other disease providing we are constantly aware of the various sources of this toxin. Although the three cases presented in this manuscript do not involve lead batteries, perhaps they are one of the most common sources of lead to animals.

Lead continues to be the most common poison of cattle in our area. New sprays and medicaments of various kinds occasionally cause poisoning but are not as prevalent as lead poisoning. Others have reported a similar trend. During the fourth quarter of 1974 fiscal year, 4.5% of the city children screened had elevated blood lead levels.** False positives occur but lead is still a very common toxin of children, especially those from slum areas.

Automobile traffic in Palo Alto, California has been incriminated as the major source of environmental lead contamination. Lead concentrations at certain times were proportional to the traffic flow. Common sources of lead for livestock include lead paint, farm machinery grease, used crankcase oil, lead batteries, linoleum, lead arsenate pesticides, plumbing lead and lead lined drinking containers. Vegetation growing near busy intersections may have 500 ppm lead. The latter is not considered toxic to livestock. Hippies have developed lead toxicity following use of old bathtubs for preparing wine.

Toxic effects of lead generally occur as lead encephalopathy, gastroenteritis and/or degeneration of peripheral nerves. The central nervous manifestations are blindness and peculiar twitch of the ear or other muscles. This form is often observed in cattle. Gastroenteritis may or may not be associated with brain disturbance. The latter form (peripheral nerve degeneration) occurs primarily in the horse. Low doses of lead may be cumulative and produce chronic lead poisoning.

During 1970 through 1973 our laboratory received an annual average of 6+ cases of lead poisoning. The obvious cases probably do not get referred to the laboratory. Confirmation of lead poisoning is based on clinical history, often a lack of necropsy lesions, histologic findings and finally lead determination of liver, kidney, stomach contents and often the feed.

Representative cases observed in our laboratory are cited herein.

*Published as Paper No. 3950, Journal Series, Nebraska Agricultural Experiment Station.
Case 1. *(Combined lead and arsenic poisoning)*

Yearling heifers were kept in a pasture adjacent to a municipal sewage lagoon. Sewage overflowed into the pasture following heavy rain. At nearly the same time, the city had sprayed the peripheral weeds. The owner of the cattle suggested the possibility of spray drift to the pasture. Five heifers died while others were sick. (Owner reluctant to tell exact number of cattle in pasture).

Gross lesions consisted of ulcerative abomasitis, rumenitis, and mucoid enteritis. Rumen was filled with fibrous forage. One heifer had hemorrhagic gall bladder mucosa. The liver of one case was swollen with nearly a metallic sheen. One animal had renal surface hemorrhages.

Microscopic sections of liver were congested with mild swelling of hepatic cells. Kidney sections had severe nephrosis and some dilatation of renal tubules. Acid fast stain of kidney was negative.

Toxicology analysis of specimens from two heifers indicated liver arsenic 2.7 and 6.4 ppm while liver lead was 15.0 and 14.5 ppm. Kidney lead was 13.0 ppm, while kidney arsenic was 4.8 to 13.0 ppm.

Analysis of ditch water was negative for both lead and arsenic. Sewer water had 0.17 ppm arsenic and negative lead. Grass and weeds collected at edge of lagoon but in the pasture contained 183.3 ppm arsenic.

Findings of lead tissue residues above 10.0 ppm are considered significant. These arsenic levels are not diagnostic but reflect an exposure of animals to arsenic compounds. After visiting the area, we noted that not only was there drainage from the sewage lagoon but also from the city dump, which certainly could have been the source of lead. Diagnosis: lead and arsenic poisoning.

Case 2.

Owner of two dead calves brought to laboratory worked at a feed mill. Screenings and milling waste were often brought home to feed his calves and children's 4-H project calves. Recently the owner had swept wheat from a railroad car and mixed with oats and fed to calves.

Clinical signs given by referring veterinarian included bawling, pushing head into corner, blindness, salivation, temperature 102°+, depression and convulsions. Owner suspected urea toxicity. At least eight calves died.

Necropsy findings were essentially negative. Histologic section of brain had slight evidence of perivascular and perineuronal edema. Kidney sections were normal.

**Toxicology analysis was:**
- Liver — 150 ppm lead
- Kidney — 42 ppm lead
- Rumen contents — 40 ppm lead
- Mixed feed — 375 ppm lead

Comment: This case not only demonstrates a case of acute lead poisoning but also points out dangers of feed from railroad cars. Had this been grain for preparing human cereal or other human food, consumers could have had lead poisoning.
Case 3.

History of this case is somewhat vague. Owner apparently loses cattle during summer. Cattle are in a pasture near town. Municipal swimming pool water drains into the pasture where losses occurred. Deaths seem to follow recent draining of the swimming pool. Veterinarian and owner were concerned about chlorine poisoning. Children swimming and inadvertently drinking water seemed to eliminate that possibility.

Clinical history given during telephone conversation included blindness and central nervous system disturbance; therefore, we were concerned about lead toxicity. Tissues and water were submitted to the laboratory. Toxicology results were:

- Liver — 32.5 ppm lead
- Kidney — 105.0 ppm lead
- Water — lost in shipment to laboratory

Comment: This case is cited to show importance of keeping samples together so they do not get lost. This case represents a form of lead poisoning previously considered but not proven by other diagnostic laboratories.

REFERENCES

REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF CATTLE

The Committee on Infectious Diseases of Cattle met Thursday afternoon at 1:30 P.M. in the Cavalier room. More than 50 participants were in attendance. Dr. Anthony, Co-chairman of the committee, and I would certainly like to thank all those in attendance for their interest in the activities of the committee.

The parent committee accepts and endorses the report of the subcommittee on Artificial Insemination which is as follows:

The members of the subcommittee on AI conferred by mail and phone with APHIS personnel for several months while a new draft of the proposed regulation on the interstate movement of bovine semen was being prepared. All of the recommendations made by the subcommittee and approved by the parent committee last year were considered and incorporated essentially as presented. Four of the 5 subcommittee members who are present have read and approve the new draft of the proposed regulation.

The new proposal was sent to the Office of the General Council in early June 1974 but has not cleared. While no great problem is anticipated with General Council some minor changes may be necessary. The proposal must also have the approval of the Office of Management and Budget before it can be published in the Federal Register as a proposed rule making for public comment.

APHIS has indicated the annual cost for the administration of the regulation will not be great but when one considers the present financial situation of APHIS — possibility of cutting some programs now in operation — there is reason for concern for any new program.

The Canadian Health of Animals Division has indicated their intentions to impose restrictions on importation of bovine semen. The subcommittee believes this proposed regulation will meet their requirements. This further emphasizes the urgency of having health regulations for bulls producing semen for use in AI in this country.

The 1973 proceedings of USAHA include "The recommended uniform diagnostic procedures for qualifying bulls for the production of semen" prepared by a special committee of the American Association of Veterinary Laboratory Diagnosticians. Included therein the techniques are described for all tests procedures required in the proposed regulation on interstate movement of bovine semen.

The subcommittee recommends that the committee on Infectious Diseases of Cattle ask USAHA:
1. To strongly urge APHIS to make every effort possible to get the proposed regulation on Interstate Movement of Bovine Semen out of the Office of General Council and through the Office of Management and Budget so that it can be republished in the Federal Register.
2. To give high priority to the implementation of the regulation.

Respectfully submitted:
H. D. Anthony
David E. Bartlett
William Brown
F. W. Hansen
H. J. Bearden, Chairman

75
The committee heard a report of recent research efforts and developments on pinkeye at NADC. Trials using bacterins have not been successful in controlling this infection. Further trials on immunization are in progress and the findings will be reported at the next meeting. It was stated that treatment of infected eyes aids in the control of the carrier state of pinkeye.

Also presented was information on the IBR virus, noting that intranasally inoculated cattle had higher shedding rates of virus in nasal secretions than cattle inoculated by the intramuscular route.

New immunological test methods which correlate closely with the presence of leukemia virus are indicating the incidence of bovine leukemia to be greater than 10% in dairy cattle and approximately 1% in beef cattle in the Midwest. The committee emphatically urges the hearing of additional reports of research on bovine leukemia during the 1975 USAHA meeting.

Questions were raised as to the safety of MLV BVD vaccines when used in feeder cattle and in cow herds. There are no data supporting untoward reactions above the 1% reported frequency found experimentally.

Mastitis in dairy cows and the fact that it causes a 10% loss in milk production was discussed. Mastitis caused by *Streptococcus agalactia* can be diagnosed, treated, and controlled.

The problem of Weak Calf Syndrome was discussed. Research underway should aid in the evaluation of killed virus vaccine for controlling this condition. A possibility of a relationship to BVD has been noted.

A brief report was heard by the committee on the on-going research project studying the Sudden Death Syndrome at Kansas State University. It is felt at this time that more than an infectious agent is involved.

As usual, various other topics were mentioned in brief fashion and could be afforded only limited comment. As stated in previous committee reports, the scope of the title of this committee is very broad, necessitating that the agenda be limited. It was agreed by the entire group in attendance that no vaccine will ever be developed that will replace one key factor, this key factor is good management.

The committee respectfully submits this report for your consideration.

N. R. Swanson, DVM
Chairman
CULTURE OF ANAPLASMA MARGINALE IN LYMPH NODE CELLS*

Richard J. Hidalgo, DVM, Ph.D.

Summary

Monolayer cultures derived from bovine lymph nodes were used for propagation of *Anaplasma marginale*. Invasion of the host cells was attributed to pretreatment of the monolayers with diethylaminoethyl dextran and centrifugation of the inoculum onto the cells. Organisms in culture were demonstrated by the direct fluorescent antibody technique. This procedure was combined with the direct microscopic count for enumeration of organisms in culture.

Multiplication occurred in the cytoplasm of the lymph node cells and infection of individual cells was irregular. The greatest number of organisms occurred at 12 to 24 hours after inoculation and numbers decreased rapidly thereafter. Organisms persisted in small numbers for at least 7 days.

Inhibition of the multiplication and decreases in numbers of *A. marginale* occurred when either oxytetracycline HCl or specific rabbit antiserum was incorporated into the medium at the time of inoculation. The significance of the inhibition by these two substances is discussed.

Introduction

The *in vitro* propagation of *Anaplasma marginale* has become one of the major goals of researchers working on anaplasmosis of cattle. Continuous propagation of this organism in culture would permit the economical production of antigen for serologic tests and the development of attenuated and inactivated immunizing agents that would be free of troublesome erythrocytic antigens. In addition, an *in vitro* method of cultivation of *A. marginale* would be invaluable in basic studies on the nature of the organism and the mechanism of anemia produced during anaplasmosis.

Reports of laboratory propagation of *A. marginale* have appeared since 1915. Successes based on transmission of the disease to cattle inoculated with culture material and/or apparent increases in marginal bodies in culture were reported but never substantiated. In 1972, Marble and Hanks were able to produce anaplasmosis in an intact calf with inoculum that has been propagated through 20 continuous weekly passages in primary rabbit bone marrow cultures. In subsequent investigations, these workers were able to make 35 isolations of *A. marginale*, 20 of which were made in primary rabbit bone marrow cultures and 15 in a stable rabbit bone marrow cell line. Furthermore, they were able to produce anaplasmosis with culture material after 135 continuous weekly passages in the stable cell line. Marble and Hanks were not able to demonstrate the organism in culture by acridine orange (AO) or Romanowsky-type staining.

From the Department of Veterinary Microbiology and the Texas Agricultural Experiment Station, Texas A&M University, College Station, Texas 77843.

*A portion of the work described here has been accepted for publication in the American Journal of Veterinary Research. Therefore, much of the technical aspects will be omitted.*
In a recent report, Trueblood and Bear\(^7\) were able to propagate *A. marginale* in explants and organ cultures of bovine lymph node. They were able to produce anaplasmosis by inoculation of fluids from cultures maintained for up to 40 days. These researchers were able to prevent propagation of the organism in culture by incorporation of 200 \(\mu g/ml\) of either chlortetracycline or tylosin. Demonstration of *Anaplasma* was accomplished by Giemsa and fluorescent antibody (FA) stained smears but the authors stated that the reliability of the staining procedures was poor. Furthermore, they were of the opinion that demonstration of the organism by staining was not an acceptable criterion of multiplication.

The purpose of this report is to describe the propagation of *A. marginale* in bovine lymph node culture and the procedures developed for demonstration and enumeration of the organism in culture.

**Materials and Methods**

**Organisms.** The source of the inocula used in this work was the Texas Strain of *A. marginale* maintained under liquid nitrogen. Washed erythrocytes containing 86% parasitemia were stored in Hanks' balanced salt solution (HBSS) containing 10% dimethylsulfoxide. The inoculum consisted of infected red cell ghosts resulting from thawing, washing and rapid sedimentation of the infected erythrocytes.

**Cells.** Monolayer cultures were of cells in passage 5 through 30 that had been derived from bovine lymph node.

**Medium.** The medium used throughout the experiment was Minimal Essential Medium (Earle's salts) containing: 10% fetal calf serum, vitamins, non-essential amino acids, vitamin B12 (0.2 \(\mu g/ml\)), crystalline penicillin G and streptomycin sulfate.

**Inoculation of Cultures.** Lymph node monolayer cultures in plastic flasks and Leighton tubes were used for growth of the organism. Immediately prior to inoculation, cultures were treated twice with diethylaminoethyl dextran (DEAE-D) for 5 minutes each treatment. The DEAE-D solution was decanted and the inoculum suspended in growth medium was added. The inoculum was centrifuged onto the monolayers at 900xg for 1 hour at room temperature. With the exception of receiving inoculum, control cultures were treated identically.

**Demonstration of the Organism.** Coverslips for the qualitative study were removed from Leighton tubes at 24-hour intervals, fixed and stained by the direct FA technique, using rhodamine labeled normal bovine serum as a counterstain. Coverslips were occasionally stained with Giemsa and AO.

**Enumeration of Organisms.** The organisms per ml of culture were determined by the direct microscopic count modified as described below. Infected and control cultures for use in the quantitative studies were frozen at 6, 12 and 24 hours and at 24-hour intervals for 7 days. Each culture was then thawed, homogenized in a tissue grinder and 0.01 ml was delivered to a clean glass slide by means of a calibrated delivery syringe. The slide was placed over a template and the culture homogenate was spread over a one square cm area. The smear was air dried, fixed in absolute methanol and then stained by the direct FA technique using the counterstain. Growth curves were constructed using these data. Occasional counts were made on
culture fluids of infected cultures without disruption of the monolayers.

**Oxytetracycline Inhibition.** In order to determine the effect of oxytetracycline on *A. marginale* in culture, lymph node cultures were prepared, inoculated, incubated and counted as described with the exception that 200 ug/ml of oxytetracycline HCl was added to the growth medium of one half of the infected cultures.

**Effect of Antiserum.** The effect of specific antiserum on the growth of *A. marginale* was determined. Cultures were prepared, inoculated, incubated and counted as previously described with the exception that the growth medium of one half of the infected cultures contained a final 1:100 dilution of sterile antiserum. The antiserum was prepared in rabbits against a purified preparation of marginal bodies and had a complement-fixation titer of 1:64.

**Results**

When monolayers of lymph node cells on coverslips were stained by the FA procedure and AO immediately after inoculation and centrifugation, *A. marginale* was observed adhering to the cytoplasmic membranes. Thereafter the organisms appeared either clumped in inclusions or dispersed throughout the cytoplasm of cells (Fig. 1). The number of organisms per cell was quite variable and some cells did not contain organisms. The monolayers appeared to have the highest number of organisms, both from the standpoint of greatest number of infected cells and the highest number of *Anaplasma* per cell, at approximately 24 hours. The relative numbers of organisms in culture diminished rapidly after 24 hours but were still detectable in the cytoplasm for at least 7 days.

Cells of uninoculated control cultures stained a dull brick red color when stained by the counterstain of the direct FA procedure. This provided excellent contrast for the organisms stained by the fluorescein-labeled globulins. *Anaplasma marginale* when stained by the FA technique appeared to have an average diameter of 2.8 u. This was approximately twice the size of intracellular organisms stained by AO. Extracellular organisms appeared as faintly stained green to orange oval sacs containing dense green areas at one or both ends when stained by AO. Only the dense green portions of the organisms were visible within the cytoplasm of cells stained by AO (Fig. 2).

Use of specific staining of *A. marginale* with fluorescein-labeled globulins in combination with the direct microscopic counting procedure permitted enumeration of the organisms in culture homogenates. The use of rhodamine counterstain eliminated non specific staining of cellular debris and provided a good contrasting background for making counts. Counts made on cultures incubated for various periods of time were used to construct growth curves as shown in Fig. 3. The increase in numbers of organisms occurred rapidly with highest numbers observed at 12 to 24 hours. Numbers decreased rapidly thereafter to below that of the inoculum. Using data from several experiments the generation time of *A. marginale* was calculated to be approximately 17.1 hours.

The addition of oxytetracycline HCl to the growth medium resulted in a rapid and pronounced decrease in numbers of organisms in the cultures.
The effect of oxytetracycline on numbers of *A. marginale* in cultures as compared to infected control cultures is shown in Fig. 4.

The incorporation of specific rabbit antiserum into the growth medium of cell cultures prevented multiplication and resulted in a gradual decrease in numbers of organisms during the incubation period. A comparison of cultures containing antiserum and infected control cultures is shown in Fig. 5.

**Discussion**

In the first report of the propagation of *A. marginale* in the laboratory, Marble and Hanks were not able to demonstrate the organisms except by animal inoculation. The fact that these workers were able to produce the disease after several serial passages indicated that reproduction was occurring but numbers of organisms were apparently small. Trueblood and Bear were able to demonstrate the organism by Giemsa and the FA technique but stated that these methods were not sufficiently reliable for use in demonstration of multiplication of *A. marginale*. Reliable methods of demonstration and enumeration of the organism in cell culture are presented here.

Use of DEAD-D for treatment of host cells and centrifugation of the inoculum onto monolayers resulted in infection of the cells and multiplication of *A. marginale* in the cytoplasm. Without these techniques it was not possible to demonstrate either invasion or multiplication. This combination of techniques has been shown to increase numbers of *Chlamydia* in cell culture over either procedure alone. Qualitative studies made on coverslips stained by FA and AO indicated that the degree of multiplication within individual cells varied greatly. Cytopathic effects on the cells were not obvious. However, it is believed that cells containing large numbers of organisms in the cytoplasm were destroyed since the numbers of these cells decreased rapidly after 24 hours.

Quantitative studies of the growth of *A. marginale* in cell culture revealed that increases in numbers occurred rapidly with highest numbers occurring between 12 and 24 hours. The number of organisms then decreased rapidly. The rapid decrease in numbers of organisms per ml of culture homogenate and the rapid decrease in numbers of heavily infected cells observed during qualitative studies indicated that invasion of cells not originally infected during the inoculation procedure was minimal. Counts made on culture fluid of intact monolayers revealed that the number of extracellular organisms remained at a constant, low level throughout the experiment. This indicated that the survival of extracellular organisms released by lysis of heavily infected cells was of short duration.

The generation time of *Anaplasma* determined during the log phase of growth in several experiments varied considerable. However, the average generation time appeared to be in relatively close agreement with the 24 hour period required for a doubling of numbers of infected erythrocytes during acute infections observed in experimentally infected cattle. The *in vitro* generation time of this organism was similar to that of *Chlamydia grown in cell culture but the increase in numbers of Anaplasma was much lower*.

Procedures for demonstration and enumeration of *A. marginale* in cell
culture were reproducible. In the FA procedures, the specificity of the fluorescein-labeled counterstain resulted in an ideal method of demonstration of the organism. The direct counting procedure developed will be useful in determining the optimal system for cultivation of this organism. Cell types, media and growth conditions can be compared objectively using the quantitative method.

When compared to infected control cultures, cultures containing oxytetracycline had rapid decreases in numbers of *A. marginale*. Inhibition of growth by this antibiotic was included in this work because Trueblood and Bear\(^7\) used inhibition by a similar antibiotic, chlortetracycline, as evidence of multiplication in culture. These workers showed that cultures incubated with the antibiotic were non-infectious, whereas control infected cultures produced anaplasmosis in splenectomized calves. They also demonstrated that infected blood maintained in the presence of similar concentrations of either oxytetracycline or chlortetracycline at 4°C overnight was infective for calves. In addition to evidence of multiplication in culture, inhibition of multiplication of *Anaplasma* by oxytetracycline is of considerable practical importance. This indicates that use of this system for preliminary screening of drugs for use against this organism is a distinct possibility.

Demonstration of inhibition of multiplication of *A. marginale* in cell culture by specific rabbit antiserum is of importance from the research standpoint. Such a procedure could be used to determine serologic relatedness of *A. marginale* to such organisms as *Anaplasma centrale* and *Paranaplasma caudata*. In fact, the procedure could possibly be utilized to determine whether the latter organism is actually a separate organism or merely a stage in the life cycle of *A. marginale*. In addition, if inhibition of multiplication of *Anaplasma* can be demonstrated using serum from infected cattle, the procedure will be invaluable for basic studies on immunity to this organism in cattle.

Serial passage of the organisms in cell culture was not included in this work. In preliminary attempts at serial passage the organism was demonstrated in cells through three passages but numbers were reduced with each passage. Continuation of this work will include determination of the optimal host cells, medium and growth conditions for propagation of *A. marginale*. These optimal conditions should ultimately result in a higher degree of multiplication and serial propagation.
Fig. 1. Monolayers of bovine lymph cells stained by the direct fluorescent antibody technique and counterstained with rhodamine-labeled normal bovine serum. The upper photomicrograph is of uninoculated cells. The lower photomicrograph shows Anaplasma marginale in the cytoplasm. x540
Fig. 2. Bovine lymph node cells stained by acridine orange. The upper photomicrograph is of uninoculated cells. The lower photomicrograph shows Anaplasma marginale in the cytoplasm of cells and some extracellular organisms. x540
Fig. 3. Growth curve of Anaplasma marginale in bovine lymph node cells.

Fig. 4. Inhibition of growth of Anaplasma marginale in cell culture by oxytetracycline HCl.
Fig. 5. Effect of rabbit antiserum on the growth of Anaplasma marginale in lymph node cell culture.

REFERENCES
REPORT OF THE ANAPLASMOSIS COMMITTEE

Chairman: Bert Hawkins, Ontario, Oreg.
Co-Chairman: W. E. Brock, Stillwater, Okla.


The Anaplasmosis Committee met at 1:30 p.m., October 17, 1974, in the Hotel Roanoke, Roanoke, Virginia. Thirty persons were in attendance. The following research reports were presented:

Dr. T. O. Roby reported on the elimination of *A. Marginale* from cattle by treatment with imidocarb. This drug when administered in two doses was effective in eliminating infections. Following treatment resistance to reinfection remained for one year. The resistance also has been noted in cattle properly treated with chlortetracycline. It was noted that imidocarb has not been approved for use in cattle by the Food and Drug Administration.

Dr. M. Ristic reviewed Dr. Roby’s research on imidocarb and suggested anaplasmosis control program may be possible by the vaccination of cattle with attenuated *A. marginale* vaccine and then eliminating the infection with drug therapy. Vaccination would result in immunity and treatment would remove infection. Cellular immunity tests may demonstrate when protection is established. Research on this approach is continuing. It was suggested that information concerning Dr. Roby’s and Dr. Ristic’s research be mailed to Committee members.

Dr. B. R. McCallon reported on the anaplasmosis card test training meetings held last year. Personnel from every state attended and at least one laboratory in every state is now utilizing this new approved test. He also reported on a number of trials in which anaplasmosis was eliminated from infected herds by the removal of infected animals through treatment or slaughter. These herds remained free of infection as long as all replacements were anaplasmosis free. Treatment consisted of low level use of chlortetracycline in feed. Five mg./lb. of body weight daily for 30 to 60 days has generally been successful. Dr. McCallon indicated disappointment in the general acceptance of the Anaplasmosis Free Herd Program and hoped more producers would become interested in the future.

Dr. Hidalgo briefly discussed his research on propagating *A. marginale* in bovine lymphnode tissue culture. His paper presented in the general assembly will be published in the proceedings.
Dr. K. J. Peterson reported on research underway in Oregon. To better define the anaplasmosis vectors in the endemic areas of eastern Oregon two tick-proof platforms were constructed at the Squaw Butte Experiment Station. Twenty anaplasmosis free calves were placed on the platforms and ten control calves were maintained in the area surrounding the platform. Ten anaplasmosis sero positive cows were also maintained around one platform and nine around the other. No protection from flies were afforded the platform calves. Control cows and calves were watered in tanks adjacent to the platforms making it necessary for them to visit the area daily. Seven of eleven control calves around one platform and four of ten around other became sero positive during the trial which began on April 29 and terminated September 23, 1974. All platform calves remained negative to anaplasmosis card test. None demonstrated clinical signs of the disease. No anaplasma bodies were observed in blood smears, and packed cell volume and hemoglobin values remained within the normal range. This trial, with some modification, will be repeated at least one more year.

Dr. H. Renshaw reported on studies conducted to determine the incidence of anaplasmosis in Idaho. The incidence of infection varied in the areas sampled from .04 to 6.7%. All areas of the state have not yet been studied. He also reported on studies conducted to define wildlife reservoirs. One of two elk injected with blood from an A. marginale infected cow developed infection. Blood from this elk proved infective when injected into a susceptible calf.

The Committee agreed that research should be continued on (1) immunity; (2) drug therapy; (3) vector identification; (4) herd control programs; and (5) identification of wildlife reservoirs. It was also agreed that a report of the proceedings of the Anaplasmosis Researchers' Meeting be presented each year at the Anaplasmosis Committee Meeting.
45/20 VACCINATION IN A BRUCELLA INFECTED HERD

Winthrop C. Ray* and J. B. Hendricks**

Previous reports indicated that 45/20 bacterins provided protection against brucellosis comparable to that afforded by Strain 19 under controlled conditions3,4,12. These reports generated considerable interest in 45/20 bacterins among certain cattle industry representatives as a means to increase the resistance against brucellosis of beef cattle on range. Infrequent testing of some beef herds in range areas impeded the identification and removal of infected animals and commonly resulted in persisting herd infections. Vaccination with 45/20 bacterin was regarded by owners as a means whereby disease spread within the herd could be minimized without increasing the frequency of herd tests thereby facilitating the eradication of brucellosis without disruption of herd management practices.

Although 45/20 bacterins have been recommended and are used in brucellosis control and eradication programs in some countries,1,4,12 there had been no reports on the effectiveness of 45/20 bacterins in protecting cattle against brucellosis when maintained in an infected herd and subjected to continuing natural exposures. A field trial to answer this question was suggested but the wide usage of 45/20 bacterin was not considered desirable nor practical without first examining results attained under more closely supervised conditions.

This project was designed to provide information on the resistance to brucellosis provided by two doses of 45/20 bacterin in cattle repeatedly exposed to brucella by natural means over an extended period of time.

Materials and Methods

An infected herd of cattle was purchased for this investigation. The herd was classed as infected with blood tests for brucellosis revealed 15 reactors among 119 cattle tested. On six subsequent herd tests during the next 10 months, 33 additional reactors were identified and removed for slaughter. Approximately 1 year after the initial herd test, 58 animals were blood tested and 8 reactors were disclosed. Abortions were reported to have occurred shortly before this test. An abortion was also observed when the herd, including the 8 reactors, was moved to the Experiment Station for this investigation.

The 8 reactors remained with the herd at the Experiment Station for 7 days after arrival. On the first blood test conducted at the Experiment Station, 1 additional reactor was disclosed and separated from the herd. All reactors disclosed on later tests were left in the herd to duplicate potential field conditions.

The 49 serologically negative animals were examined for vaccination eartags and tattoos to identify Strain 19 calfhood vaccinated cattle. Nine

*Chief Staff Veterinarian, Brucellosis Epidemiology, Cattle Diseases, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, Hyattsville, Maryland.

**Regional Brucellosis Epidemiologist, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, Columbia, South Carolina.
cattle were identified as Strain 19 vaccinates but not until two had received one dose of 45/20 bacterin. The remaining 40 animals, judged to be non-vaccinated cattle, were randomly divided into a control group of 13 cows and a 45/20 bacterin group of 27 cows.

Blood samples were obtained on 6 occasions during the first 22 weeks and then at regular 4-week intervals throughout the remaining months of the investigation. The standard tube agglutination test (STT), brucellosis card test (CARD), mercaptoethanol tube test (ME), rivanol plate precipitation test (RIV), complement fixation test-Wisconsin method (CF-WIS), and complement fixation test-Hill's method (CF-HILL), were conducted on each sample. The test interpretations were as follows:

<table>
<thead>
<tr>
<th>Test</th>
<th>Brucella Vaccination History</th>
<th>Interpretations</th>
</tr>
</thead>
<tbody>
<tr>
<td>45/20 Bacterin and Controls</td>
<td>Strain 19</td>
<td></td>
</tr>
<tr>
<td>STT</td>
<td></td>
<td>Reactor</td>
</tr>
<tr>
<td>II:50 to II:100</td>
<td>II:100 to II:200</td>
<td>Suspect</td>
</tr>
<tr>
<td>ME &amp; RIV</td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>CARD</td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>CF-WIS</td>
<td></td>
<td>Reactor</td>
</tr>
<tr>
<td>CF-HILL</td>
<td></td>
<td>Reactor</td>
</tr>
</tbody>
</table>

Milk, udder secretions, uterine discharges, fetuses, and placental tissues were collected from some cattle for brucella culture using standard bacteriologic methods. Tissues were collected at slaughter for culture purposes from all animals from which brucella had not been recovered at an earlier date and from many animals which had previously been determined to be infected by isolation and cultivation of brucella. Seven animals died from various causes during the investigation. Five of these were not available for bacteriologic studies and involved one animal in the 45/20 bacterin group, one animal in the nonvaccinated control group, and three animals in the Strain 19 vaccinated group.

Inasmuch as brucella isolation attempts were not conducted with the same frequency as the blood tests, the brucellosis status of individual animals during the postvaccination period summarized in this paper are based primarily on the serologic results.

Intramuscular injections of 45/20 bacterin were given on February 23 and May 18, 1971, using a 12-week interval between doses.* The two injections were administered on opposite sides of the neck just anterior to the shoulder and approximately two-thirds of the distance from the dorsal line of the neck to the brisket.

The adult cattle were pastured as one group throughout the investigation. The pastures were adequate to prevent crowding except for January through April when supplemental feeding was necessary. Hay and some grain were fed during these months and the animals voluntarily crowded together in the feeding area.

Approximately 6 weeks postvaccination,** 4 heifers, 18 to 24 months of

*As recommended by N. A. Philips-Duphar, Amsterdam, Holland.
**Postvaccination refers to time interval starting with the administration of the first dose of 45/20 bacterin on February 23, 1971.
age, were added to the herd. These animals were the progeny of animals of earlier projects and had been vaccinated with two doses of 45/20 bacterin. The first dose was given when the heifers were between 10 and 12 months of age.

In May 1971, 3 months after the beginning of this investigation, 11 heifers were separated and held on pasture for 6 months before being returned to the herd. During this period, the heifers received two doses of 45/20 bacterin. None of the heifers were pregnant when added to the adult herd.

Results and Discussion

Of the 50 nonreactor adult cattle transported to the Experiment Station, one reacted to various serologic tests conducted a few days after arrival. Two additional animals, later determined to be Strain 19 vaccinated cattle, were temporarily included with the 45/20 group and received one dose of 45/20 bacterin. These 3 animals are not included in these data. The 15 heifers vaccinated with 45/20 bacterin are also excluded from this analysis because the first calvings in this group occurred after the primary 18-month evaluation period.

A summary of the brucellosis status of the remaining 47 animals is provided in Table 1. The status is based on interpretations of combined serologic and bacteriologic results with emphasis on the first 18 months following the first dose of 45/20 bacterin.

Seven animals in the 45/20 bacterin group were positive to one or more serologic tests during this 18-month period but were not confirmed to be infected by bacteriologic methods at any time during this investigation. Milk samples were collected for brucella isolation attempts from only 3 of these 7 cows and then on only one occasion for each animal. All 3 cows were serologic reactors at the time the milk samples were collected.

Three of the remaining 4 serologically positive cows were not subjected to brucella isolation attempts until sold for slaughter 28 to 31 months postvaccination. The serologic reactions had decreased significantly for all 3 animals but only 1 was completely negative at the time of slaughter.

The seventh animal died in November 1971, 9 months postvaccination, and was not subjected to bacteriologic examination.

In the 45/20 bacterin group only 2 animals (7.4 percent) remained serologically negative in this investigation. One of these cows had low titers to the CF-HILL test but reactions to this test were not observed after 18-weeks postvaccination. No specimens were collected for bacteriologic examination until the cows were slaughtered 28 and 32 months postvaccination. Both animals were culture negative for brucella at that time.

Five animals in the 45/20 bacterin group were serologically positive for varying lengths of time prior to August 8, 1972, but were not confirmed to be infected by bacteriologic methods until they were slaughtered approximately 1 year later. One of these animals became serologically positive within 6 weeks of the initial dose of 45/20 bacterin. After the initial peak, the titer decreased but continued to be positive to the various tests through August 8, 1972. Later, ascending titers to the various tests were recorded and the titer remained high until slaughter.

In the Strain 19 vaccinated group, only 2 of the 7 animals were positive
to any of the serologic tests during this investigation. One of these intermittently developed low titers to the CF-HILL test and was positive on two occasions to the card test (June 1971; January 1973). In June 1972 this animal was positive (+1:25) to the ME test. All other tests including bacteriologic isolation attempts were negative during the investigation.

The culture positive animal in the Strain 19 vaccinated group became serologically positive to all tests during the 14th month of the evaluation period. Irregular fluctuations in titer occurred to the various serologic tests until the animal was slaughtered a year later; however, the card, rivanol, and both CF tests remained positive.

In the nonvaccinated control group, 3 animals were serologically positive before August 8, 1972. Two of these cows were also confirmed to be infected by brucella isolations. The third animal was serologically positive from February 1972 to June 1973 at which time the animal was slaughtered and confirmed to be infected. A fourth animal in the control group became serologically positive after the 18-month evaluation period and was proved infected at slaughter. This was the only cow in the original group of 47 animals that became serologically positive after August 8, 1972. The relatively low infection rate observed for this group of nonvaccinated controls (30.7 percent) is similar to those of other studies referred to by Huddleson.6

In three separate investigations infection rates ranging from 25.5 to 34.0 percent were reported among nonvaccinated cattle subjected to natural exposure conditions while a fourth investigation indicated an infection rate of 6.2 percent.

A statistical analysis, using Fischer's Exact Probability method, of the serologic results for the 18-month postvaccination evaluation period indicated that there were highly significant differences in the reactor rates between the 45/20 bacterin group and the nonvaccinated control group (.00018) and between the 45/20 bacterin group and the Strain 19 vaccinated group (.001405). The difference in reactor rates between the 45/20 bacterin group and the combined Strain 19 vaccinated group and the nonvaccinated control group, which were not significantly different from each other (.594169), was also highly significant (.000002).

The statistical analysis, using Fischer's Exact Probability method, for the bacteriologic results indicated that significant differences occurred between the 45/20 bacterin group and the nonvaccinated control group (.013548), between the 45/20 bacterin and the Strain 19 vaccinated group (.028877), but no difference between the nonvaccinated control and Strain 19 vaccinated group (.593137). A highly significant different in the infection rates was found between the 45/20 bacterin group and the combined total for the Strain 19 vaccinated and the nonvaccinated control group (.002544).

An examination of the results on the 27 animals that received 45/20 bacterin indicates that the serologic titer patterns were unreliable for predicting which animals would prove to be bacteriologically positive (Table 2). The brucellosis status of animals with stable positive or negative serologic titers was not difficult to establish. Nine of 11 animals with persistently high titers to the various tests were confirmed as infected by isolating brucella. Also, 3 out of 4 animals developing serologically positive responses later on in the investigation were confirmed as infected.

Fluctuating, receding, and irregular serologic reactions were observed
with 10 of the 27 animals following the administration of two doses of 45/20 bacterin under the conditions prevailing in this investigation. Test results for selected dates on each of these 10 animals are presented in Table 3 as examples of problems with titer interpretation. The difficulty in making accurate serologic diagnoses of brucellosis on the basis of test results at one or more points in time is apparent. For example, animal No. 7 was culture positive in October 1971 but had receding titers to the serologic tests within 2 weeks. Low titers persisted for nearly 1 year before most tests were negative. However, individual serologic tests remained negative for only 1 or 2 months before reconverting to positive. Brucella isolations in April 1973 again confirmed the infected status of this cow.

Comparison of fluctuating and irregular titers with results reported by Meyer and Nelson on animals chronically infected with Strain 19 reveals few similarities. In their investigation, titer fluctuations were restricted almost entirely to the standard tube test. The card and the whey tests remained positive throughout their study. In general, the RIV test also remained positive but exceptions were noted for 2 animals in that investigation. The results in this investigation were different in that all of the tests reverted to negative at one or more points in time for some animals. In general, the ME test remained positive longer and at higher titers than other supplemental tests. The CF tests (both Hill's and Wisconsin) frequently were found to have incomplete fixation in succeeding dilutions. This was also observed in an investigation by Gue on animals vaccinated with 45/20 bacterins. In addition, prozones were frequently noted for the CF-HILL test. Some of the reported negative results with the CF-HILL test may have been the result of unrecognized prozones but this has not been established by rechecking those serums.

The 4 animals with delayed serologic reactions involve those animals which were still serologically negative on July 27, 1971. One of these animals converted to a reactor status on the next test date (September 7, 1971). The other 3 became serologically positive 6, 7, and 10 months respectively after the July 1971 date. The occurrence of these later reactors in February and May 1972 could be the result of exposure to infective discharges from infected cattle which aborted or calved beginning December 1971. The possibility of exposure occurring in March-June 1971 with accompanying long incubation periods cannot be ruled out, however.

The distribution of calvings and abortion for the 18-month evaluation period is summarized in Figure 1. Following the abortions which occurred in February 1971, only 2 infected cows terminated their pregnancies during the next 4 months. One of these was an abortion. The absence of calvings from July through November 1971 resulted from herd management practices limiting the breeding season. Following relocation of the herd to the Experiment Station, this practice was abandoned and the bull was allowed to remain with the herd.

From December 1971 through April 1972, 17 infected cows either aborted or calved normally. The first 6 infected cows that aborted during this period were serologic reactors on the previous June 1971 test and were in the 45/20 bacterin group. Obviously 45/20 bacterin in this instance did not protect these animals from infection or abortion. The seventh cow which aborted was a nonvaccinated control and was also a reactor on the
June 1971 test.

The administration of 45/20 bacterin as a diagnostic method has been suggested by several workers\[1,7,11,12\]. The rationale is that the first dose of 45/20 bacterin stimulates an anamnestic response which can be detected 6 to 12 weeks later by the Coombs Test, the CF test, or both. Although the Coombs Test was not included as one of the serologic methods used in this study, two CF test methods were included and can be examined for possible diagnostic test application.

In Table 4, the development of serologic responses is summarized for the 26 cattle contained in the 45/20 bacterin group on which bacteriologic examinations had been conducted.

The response 6 weeks after the initial dose of 45/20 bacterin indicates that the CF-HILL is more sensitive for detecting antibody response than the CF-WIS using these test interpretations. Approximately the same proportion of culture positive to culture negative animals developed CF titers at 6 weeks and at 12-weeks postvaccination. For example, on the test 6 weeks after the initial dose of 45/20 bacterin approximately 12 percent of both culture positive and culture negative animals showed positive CF-WIS test reactions whereas 44 and 50 percent respectively were positive to the CF-HILL test. At 12-weeks postvaccination 33 percent of the culture positive and 50 percent of the culture negative animals were positive to the CF-WIS test and 61 and 75 percent respectively were positive to the CF-HILL test. Under these conditions it appears that the CF test (either method), lacks the necessary precision to discriminate between culture positive and culture negative animals. Although the experimental design of this investigation places considerable limitations on the evaluation of 45/20 bacterin as a diagnostic method, it is obvious that overcondemnation is a factor using either CF method. The fact that a substantial portion of the animals, which were later proved to be infected, were not detected could be due to recent exposure, exposure to brucella at a later date or failure of 45/20 bacterin as a diagnostic method.

The serologic responses observed following the second dose of 45/20 bacterin are considerably different than those observed in a previous investigation involving mature calfhood vaccinated cattle given 45/20 bacterin\[4\]. In that investigation, only 4 of 24 cows disclosed significant CF titers 10 weeks after the second dose of 45/20 bacterin and at 20-weeks postvaccination only 2 animals retained significant CF titers. Also in that trial, all animals were negative to the RIV test by the 10th week. Seven animals were positive to the card test on the 10th week but only 3 animals were still classed as suspicious on the SST. This contrasts strongly with the 16 animals classed as reactors and 5 suspects to the SST at 10 weeks in this study.

The complication of active herd infection during this period undoubtedly influenced the development and retention of postvaccinal responses. The simultaneous occurrence of both 45/20 bacterin administration and probable exposure to virulent brucella as mechanisms for stimulating antibody production increases the problem of distinguishing the nature and reason for the observed response. The absence of similar titer responses in the Strain 19 group and in the control group, however, indicates that the magnitude of the antibody stimulation among the 45/20 bacterin group was
not solely a result of exposure to infected animals and their discharges.

Persistent reactions to the various tests 20 weeks after the second dose of 45/20 bacterin are not fully understood but may be evidence of reinforcement of antibody synthesis by multiple and continuing exposure after 45/20 bacterin administration.

The order of appearance of newly detected reactors is indicated in Figure 2 as well as the total number of reactors disclosed on each herd test. Animals with positive reactions to any test were listed as new reactors provided all of the tests were negative on the immediately preceding test date. A few animals with fluctuating titers were classed as new reactors more than once. In July 1972, 10 reactors were slaughtered which accounts for the rapid decrease in reactors on that date. Two serologically positive animals died earlier; one in November 1971 and the second in June 1972.

After the first 26 reactors were disclosed in April, May and June 1971, only 1 additional reactor was detected over the next 7 months and there were no calvings or abortions during the next 5 months. The appearance of a second peak in serologic responses in February to May 1972 in Figure 2 correlates well with the calvings and abortions shown in Figure 1. The interval between the abortions and calvings of infected cows during December 1971 and January 1972 and the appearance of reactors in the spring of 1972 is consistent with the epidemiology of brucellosis.

Summary

Under the conditions of this experiment, the animals inoculated with two doses of 45/20 bacterin had a significantly higher rate of infection than the controls or the Strain 19 calfhood vaccinated cattle. In this investigation, 25 of 27 animals given two doses of 45/20 bacterin became serologically positive whereas only 3 out of 13 control and 2 of 7 Strain 19 vaccinated cattle were serologically positive during the same period.

The administration of 45/20 bacterin did not provide significant protection against subsequent abortion. Six animals aborted at least 5 months after becoming serologically positive and 9 months after receiving the first dose of bacterin.

Most of the serologically positive animals were detected within 2 months following the second dose of 45/20 bacterin. However, a second peak of serologically positive animals was detected 9 to 14 months after the second dose was given. It is assumed that this second group of reactors resulted from exposure to infected cattle and their discharges during the calving season of December 1971 to April 1972.

The CF test as a diagnostic tool used 6 weeks after the first dose would have classified 11 animals as infected by the CF-HILL method and only 3 by the CF-WIS method. If the diagnostic tests were deferred until the 12th week after the first dose, the CF-HILL would have identified 16 animals and the CF-WIS would have identified 10 animals as infected; however, both tests would have condemned cattle which were not proved to be infected.

The lack of apparent protection provided by 45/20 bacterin under these conditions and the significant differences observed on antibody stimulation between this and previous reports indicate that further investigations on the
use of 45/20 bacterin in infected herds are needed. It is obvious from the serologic patterns observed for infected animals in the 45/20 bacterin group that great difficulty could be anticipated when interpreting serologic titers under field program conditions.

REFERENCES


DISTRIBUTION OF CALVINGS AND ABORTIONS AMONG 47 COWS DURING 18 MONTH EVALUATION PERIOD

STATUS OF ANIMAL

- NEGATIVE
- INFECTED
- ABORTIONS


FIGURE 1
APPEARANCE OF NEW REACTORS AND TOTAL NUMBER OF REACTORS FOR ALL GROUPS BY TEST DATE*

TOTAL ALL REACTORS
TOTAL 45/20 REACTOR

NUMBER OF REACTOR CATTLE

1971

TEST DATES

1972

*FIRST 18 MO. PERIOD OF PROJECT.

FIGURE 2
TABLE 1
BACTERIOLOGIC AND SEROLOGIC RESULTS OF 47 ADULT CATTLE IN A 45/20 BACTERIN INVESTIGATION

<table>
<thead>
<tr>
<th>INTERPRETATION OF ANIMAL STATUS</th>
<th>VACCINATION HISTORY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45/20 BACTERIN</td>
</tr>
<tr>
<td>SERO-POSITIVE ANIMALS</td>
<td></td>
</tr>
<tr>
<td>TOTAL—DURING ENTIRE INVESTIGATION</td>
<td>25</td>
</tr>
<tr>
<td>—ON OR BEFORE 8-8-72</td>
<td>25</td>
</tr>
<tr>
<td>BRUCELLA ISOLATION BY 8-8-72—ATTEMPTS*</td>
<td>16</td>
</tr>
<tr>
<td>—POSITIVE*</td>
<td>13</td>
</tr>
<tr>
<td>BRUCELLA ISOLATION DURING INVESTIGATION</td>
<td>24</td>
</tr>
<tr>
<td>—ATTEMPTS</td>
<td>18</td>
</tr>
<tr>
<td>SERO-NEGATIVE ANIMALS</td>
<td></td>
</tr>
<tr>
<td>TOTAL DURING ENTIRE INVESTIGATION</td>
<td>2</td>
</tr>
<tr>
<td>BRUCELLA ISOLATION DURING INVESTIGATION</td>
<td>2</td>
</tr>
<tr>
<td>—ATTEMPTS</td>
<td>0</td>
</tr>
<tr>
<td>—POSITIVE</td>
<td>0</td>
</tr>
</tbody>
</table>

*Refers to number of animals from which specimens were collected and cultured for brucella
<table>
<thead>
<tr>
<th>SEROLOGIC RESULTS ON OR AFTER 10TH WEEK FOLLOWING SECOND DOSE</th>
<th>BRUCELLA ISOLATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POSITIVE</td>
</tr>
<tr>
<td>SUSTAINED SERO POSITIVES</td>
<td>9</td>
</tr>
<tr>
<td>IRREGULAR SERO POSITIVES</td>
<td>6</td>
</tr>
<tr>
<td>DELAYED SERO POSITIVES</td>
<td>3</td>
</tr>
<tr>
<td>SERO NEGATIVES</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL (27)</td>
<td>18</td>
</tr>
</tbody>
</table>

*ONE ANIMAL (#38) WAS NOT SUBJECTED TO BRUCELLA ISOLATION ATTEMPTS.
### TABLE 3
SEROLOGIC RESULTS FOR SELECTED DATES FOLLOWING ADMINISTRATION OF 45/20 BACTERIN

**ANIMAL NUMBER 7 (CULTURE POSITIVE)**

<table>
<thead>
<tr>
<th>DATE</th>
<th>STT</th>
<th>CARD</th>
<th>ME</th>
<th>RIV</th>
<th>CF-HILL</th>
<th>CF-WIS</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-23-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N.D.1</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>5-18-71</td>
<td>+25</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>6-29-71</td>
<td>1200</td>
<td>POS</td>
<td>1200</td>
<td>+50</td>
<td>4+50</td>
<td>1+80</td>
<td>POSITIVE 10-22-71 (MILK)</td>
</tr>
<tr>
<td>11-2-71</td>
<td>+100</td>
<td>POS</td>
<td>+100</td>
<td>125</td>
<td>4+50</td>
<td>2+40</td>
<td></td>
</tr>
<tr>
<td>11-30-71</td>
<td>1100</td>
<td>POS</td>
<td>+25</td>
<td>125</td>
<td>4+50</td>
<td>2+40^2</td>
<td></td>
</tr>
<tr>
<td>3-21-72</td>
<td>+50</td>
<td>POS</td>
<td>+50</td>
<td>150</td>
<td>4+50</td>
<td>2+20</td>
<td></td>
</tr>
<tr>
<td>6-13-72</td>
<td>+50</td>
<td>POS</td>
<td>+50</td>
<td>25</td>
<td>4+50</td>
<td>2+40^2</td>
<td></td>
</tr>
<tr>
<td>9-5-72</td>
<td>150</td>
<td>N^4</td>
<td>100</td>
<td>N</td>
<td>N</td>
<td>1+20^2</td>
<td></td>
</tr>
<tr>
<td>10-31-72</td>
<td>N</td>
<td>POS</td>
<td>+25</td>
<td>125</td>
<td>4+50</td>
<td>3+40</td>
<td></td>
</tr>
<tr>
<td>12-26-72</td>
<td>150</td>
<td>POS</td>
<td>1100</td>
<td>N</td>
<td>2+50^3</td>
<td>4+20</td>
<td></td>
</tr>
<tr>
<td>1-23-73</td>
<td>+25</td>
<td>POS</td>
<td>+50</td>
<td>+25</td>
<td>4+50^3</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>2-20-73</td>
<td>1100</td>
<td>POS</td>
<td>+25</td>
<td>+25</td>
<td>N.D.</td>
<td>3+80</td>
<td>POSITIVE 4-16-73 (TISSUES)</td>
</tr>
</tbody>
</table>

**ANIMAL NUMBER 10 (CULTURE POSITIVE)**

<table>
<thead>
<tr>
<th>DATE</th>
<th>STT</th>
<th>CARD</th>
<th>ME</th>
<th>RIV</th>
<th>CF-HILL</th>
<th>CF-WIS</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-23-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>6-29-71</td>
<td>1200</td>
<td>POS</td>
<td>1100</td>
<td>+50</td>
<td>1+20</td>
<td>N.D.</td>
<td>AC^5</td>
</tr>
<tr>
<td>7-26-71</td>
<td>1100</td>
<td>POS</td>
<td>+50</td>
<td>150</td>
<td>N.D.</td>
<td>AC</td>
<td></td>
</tr>
<tr>
<td>9-7-71</td>
<td>150</td>
<td>N</td>
<td>150</td>
<td>+50</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>1-25-72</td>
<td>150</td>
<td>N</td>
<td>N</td>
<td>150</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>2-22-72</td>
<td>+200</td>
<td>POS</td>
<td>+200</td>
<td>+200</td>
<td>N</td>
<td>1+80^2</td>
<td></td>
</tr>
<tr>
<td>3-21-72</td>
<td>+200</td>
<td>POS</td>
<td>+200</td>
<td>+200</td>
<td>2+10</td>
<td>2+80</td>
<td></td>
</tr>
<tr>
<td>4-18-72</td>
<td>+200</td>
<td>POS</td>
<td>+200</td>
<td>+200</td>
<td>N</td>
<td>1+40^2</td>
<td></td>
</tr>
<tr>
<td>10-3-72</td>
<td>+200</td>
<td>POS</td>
<td>+200</td>
<td>+200</td>
<td>2+10</td>
<td>4+80</td>
<td></td>
</tr>
<tr>
<td>6-12-73</td>
<td>+200</td>
<td>POS</td>
<td>+200</td>
<td>+200</td>
<td>N</td>
<td>1+80</td>
<td>POSITIVE 6-14-73 (TISSUES)</td>
</tr>
</tbody>
</table>

**ANIMAL NUMBER 22 (CULTURE POSITIVE)**

<table>
<thead>
<tr>
<th>DATE</th>
<th>STT</th>
<th>CARD</th>
<th>ME</th>
<th>RIV</th>
<th>CF-HILL</th>
<th>CF-WIS</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-23-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>4-6-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>4+10</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>5-18-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>2+10</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>5-29-71</td>
<td>1100</td>
<td>POS</td>
<td>1100</td>
<td>+25</td>
<td>4+50</td>
<td>3+40</td>
<td></td>
</tr>
<tr>
<td>7-26-71</td>
<td>150</td>
<td>N^4</td>
<td>125</td>
<td>N</td>
<td>4+20</td>
<td>3+20</td>
<td></td>
</tr>
<tr>
<td>10-5-71</td>
<td>150</td>
<td>N</td>
<td>125</td>
<td>N</td>
<td>4+50</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>11-2-71</td>
<td>N</td>
<td>N^4</td>
<td>N</td>
<td>N</td>
<td>4+10</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>12-28-71</td>
<td>+25</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>4-18-72</td>
<td>1100</td>
<td>POS</td>
<td>+100</td>
<td>150</td>
<td>4+50</td>
<td>1+40</td>
<td>POSITIVE 1-25-73 (TISSUES)</td>
</tr>
<tr>
<td>5-16-72</td>
<td>+200</td>
<td>POS</td>
<td>+200</td>
<td>+100</td>
<td>4+50</td>
<td>4+80</td>
<td></td>
</tr>
<tr>
<td>3-13-72</td>
<td>+200</td>
<td>POS</td>
<td>+200</td>
<td>+200</td>
<td>4+50</td>
<td>4+80</td>
<td></td>
</tr>
<tr>
<td>1-23-73</td>
<td>+200</td>
<td>POS</td>
<td>+200</td>
<td>+200</td>
<td>4+50</td>
<td>4+80</td>
<td></td>
</tr>
</tbody>
</table>
(TABLE 3 CONTINUED)

<table>
<thead>
<tr>
<th>DATE</th>
<th>ANIMAL NUMBER</th>
<th>CARD</th>
<th>ME</th>
<th>RIV</th>
<th>CF-HILL</th>
<th>CF-WIS</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-23-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>3-16-71</td>
<td>+25</td>
<td>N</td>
<td>N</td>
<td>+25</td>
<td>N</td>
<td>3+20</td>
<td></td>
</tr>
<tr>
<td>4-6-71</td>
<td>+200 POS</td>
<td>+100</td>
<td>+50</td>
<td>4+50</td>
<td>4+40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-18-71</td>
<td>100 N⁴</td>
<td>+50</td>
<td>+25</td>
<td>1+50</td>
<td>3+40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-29-71</td>
<td>+100 POS</td>
<td>+100</td>
<td>+25</td>
<td>4+50</td>
<td>1+80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-7-71</td>
<td>+50 POS⁶</td>
<td>+50</td>
<td>+25</td>
<td>4+20</td>
<td>1+10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-18-72</td>
<td>+50 N⁴</td>
<td>150</td>
<td>125</td>
<td>4+20</td>
<td>1+20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-16-72</td>
<td>N</td>
<td>N</td>
<td>+50</td>
<td>N</td>
<td>4+20</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

DIED 5-23-72

<table>
<thead>
<tr>
<th>DATE</th>
<th>ANIMAL NUMBER</th>
<th>CARD</th>
<th>ME</th>
<th>RIV</th>
<th>CF-HILL</th>
<th>CF-WIS</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-23-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>3-16-71</td>
<td>+25 N</td>
<td>125</td>
<td>QNS</td>
<td>QNS</td>
<td>QNS</td>
<td>QNS</td>
<td></td>
</tr>
<tr>
<td>4-6-71</td>
<td>150 POS</td>
<td>+50</td>
<td>N</td>
<td>4+20</td>
<td>1+10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-18-71</td>
<td>125 N⁴</td>
<td>+25</td>
<td>N</td>
<td>4+10</td>
<td>2+10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-29-71</td>
<td>1200 POS</td>
<td>1200</td>
<td>+100</td>
<td>4+50³</td>
<td>2+40²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-26-71</td>
<td>+100 POS</td>
<td>1100</td>
<td>150</td>
<td>3+50³</td>
<td>1+40²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-5-71</td>
<td>+50 POS⁶</td>
<td>+50</td>
<td>N</td>
<td>4+20³</td>
<td>1+10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOT ATTEMPTED DIED 11-2-71

<table>
<thead>
<tr>
<th>DATE</th>
<th>ANIMAL NUMBER</th>
<th>CARD</th>
<th>ME</th>
<th>RIV</th>
<th>CF-HILL</th>
<th>CF-WIS</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-23-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>3-16-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>5-18-71</td>
<td>125</td>
<td>+25</td>
<td>N</td>
<td>N</td>
<td>2+10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-29-71</td>
<td>1100 POS</td>
<td>1100</td>
<td>150</td>
<td>N</td>
<td>1+20²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-26-71</td>
<td>1100 POS</td>
<td>1100</td>
<td>+25</td>
<td>N</td>
<td>2+20²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-7-71</td>
<td>1100 POS</td>
<td>1100</td>
<td>N</td>
<td>2+20³</td>
<td>2+20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-5-71</td>
<td>1100 POS</td>
<td>+100</td>
<td>N</td>
<td>N</td>
<td>1+10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-30-71</td>
<td>+100 POS</td>
<td>+50</td>
<td>125</td>
<td>3+10</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-21-72</td>
<td>1200 POS</td>
<td>+100</td>
<td>+200</td>
<td>N</td>
<td>1+80²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-31-72</td>
<td>1200 POS</td>
<td>+200</td>
<td>1200</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-18-73</td>
<td>1200 POS</td>
<td>+100</td>
<td>+100</td>
<td>N</td>
<td>1+10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-11-73</td>
<td>+50 POS</td>
<td>+25</td>
<td>1+10</td>
<td>4+10</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-5-73</td>
<td>+25 N⁴</td>
<td>150</td>
<td>N</td>
<td>N</td>
<td>4+10</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

NEGATIVE 7-3-73 (MILK) NEGATIVE 9-11-73 (TISSUES)

<table>
<thead>
<tr>
<th>DATE</th>
<th>ANIMAL NUMBER</th>
<th>CARD</th>
<th>ME</th>
<th>RIV</th>
<th>CF-HILL</th>
<th>CF-WIS</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-23-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>3-16-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>5-18-71</td>
<td>+100 POS</td>
<td>+100</td>
<td>1100</td>
<td>4+50</td>
<td>4+80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-26-71</td>
<td>1200 POS</td>
<td>1100</td>
<td>1100</td>
<td>4+50</td>
<td>3+40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-5-71</td>
<td>150 POS</td>
<td>+50</td>
<td>N</td>
<td>4+20</td>
<td>2+20²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-30-71</td>
<td>150 POS</td>
<td>+25</td>
<td>N</td>
<td>4+60</td>
<td>3+20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-28-71</td>
<td>1100 POS</td>
<td>150</td>
<td>+100</td>
<td>4+50</td>
<td>3+40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-18-72</td>
<td>+25 N</td>
<td>+25</td>
<td>125</td>
<td>3+20</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-16-72</td>
<td>N</td>
<td>N⁴</td>
<td>+25</td>
<td>N</td>
<td>2+20</td>
<td>1+10</td>
<td></td>
</tr>
</tbody>
</table>

NEGATIVE 9-12-73 (TISSUE)
### ANIMAL NUMBER 48 (CULTURE POSITIVE)

<table>
<thead>
<tr>
<th>DATE</th>
<th>STT</th>
<th>CARD</th>
<th>ME</th>
<th>RIV</th>
<th>CF-HILL</th>
<th>CF-WIS</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-23-71</td>
<td>+25</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>4-6-71</td>
<td>150</td>
<td>POS</td>
<td>+25</td>
<td>N</td>
<td>4+20</td>
<td>2+20</td>
<td></td>
</tr>
<tr>
<td>5-18-71</td>
<td>150</td>
<td>POS</td>
<td>150</td>
<td>N</td>
<td>4+20</td>
<td>3+20</td>
<td></td>
</tr>
<tr>
<td>6-29-71</td>
<td>1200</td>
<td>POS</td>
<td>1200</td>
<td>+100</td>
<td>N</td>
<td>3+40²</td>
<td></td>
</tr>
<tr>
<td>7-26-71</td>
<td>1100</td>
<td>POS</td>
<td>1100</td>
<td>4+20²</td>
<td>2+20²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-7-71</td>
<td>+50</td>
<td>POS</td>
<td>+50</td>
<td>150</td>
<td>4+20</td>
<td>1+10</td>
<td></td>
</tr>
<tr>
<td>11-30-71</td>
<td>+50</td>
<td>POS</td>
<td>125</td>
<td>125</td>
<td>4+20</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>12-28-71</td>
<td>+50</td>
<td>N</td>
<td>+25</td>
<td>+25</td>
<td>2+10</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>3-21-72</td>
<td>150</td>
<td>POS</td>
<td>+50</td>
<td>150</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>5-16-72</td>
<td>150</td>
<td>POS</td>
<td>+50</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>8-8-72</td>
<td>N</td>
<td>POS</td>
<td>+50</td>
<td>+25</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>10-3-72</td>
<td>+50</td>
<td>POS</td>
<td>+50</td>
<td>2+20</td>
<td>N</td>
<td>2+10</td>
<td>N</td>
</tr>
<tr>
<td>10-31-72</td>
<td>+100</td>
<td>POS</td>
<td>+100</td>
<td>+50</td>
<td>4+50⁴</td>
<td>4+40</td>
<td></td>
</tr>
<tr>
<td>11-28-72</td>
<td>+200</td>
<td>POS</td>
<td>+200</td>
<td>1200</td>
<td>4+50</td>
<td>4+80</td>
<td>POSITIVE 7-3-73 (MILK)</td>
</tr>
<tr>
<td>7-10-73</td>
<td>1200</td>
<td>POS</td>
<td>+100</td>
<td>+200</td>
<td>4+50</td>
<td>4+80</td>
<td>POSITIVE 8-10-73 (TISSUE)</td>
</tr>
</tbody>
</table>

### ANIMAL NUMBER 49 (CULTURE POSITIVE)

<table>
<thead>
<tr>
<th>DATE</th>
<th>STT</th>
<th>CARD</th>
<th>ME</th>
<th>RIV</th>
<th>CF-HILL</th>
<th>CF-WIS</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-23-71</td>
<td>+25</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>5-18-71</td>
<td>125</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>6-29-71</td>
<td>+50</td>
<td>POS</td>
<td>+50</td>
<td>N</td>
<td>3+20</td>
<td>1+10</td>
<td></td>
</tr>
<tr>
<td>7-26-71</td>
<td>1200</td>
<td>POS</td>
<td>+100</td>
<td>1200</td>
<td>4+50</td>
<td>4+40</td>
<td></td>
</tr>
<tr>
<td>10-5-71</td>
<td>1100</td>
<td>POS</td>
<td>+100</td>
<td>+25</td>
<td>1+50</td>
<td>1+20</td>
<td></td>
</tr>
<tr>
<td>11-30-71</td>
<td>1200</td>
<td>POS</td>
<td>+100</td>
<td>+25</td>
<td>4+50</td>
<td>3+20</td>
<td>POSITIVE 10-22-71 (MILK)</td>
</tr>
<tr>
<td>3-21-72</td>
<td>+100</td>
<td>POS</td>
<td>+100</td>
<td>+100</td>
<td>4+50</td>
<td>2+80</td>
<td></td>
</tr>
<tr>
<td>6-13-72</td>
<td>1200</td>
<td>POS</td>
<td>+100</td>
<td>+100</td>
<td>4+50</td>
<td>4+20</td>
<td></td>
</tr>
<tr>
<td>8-9-72</td>
<td>1100</td>
<td>POS</td>
<td>+25</td>
<td>125</td>
<td>4+20</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>9-5-72</td>
<td>1100</td>
<td>POS</td>
<td>+50</td>
<td>N</td>
<td>N</td>
<td>1+20²</td>
<td></td>
</tr>
<tr>
<td>10-31-72</td>
<td>1200</td>
<td>POS</td>
<td>+100</td>
<td>+50</td>
<td>4+50²</td>
<td>3+40</td>
<td></td>
</tr>
<tr>
<td>12-26-72</td>
<td>1200</td>
<td>POS</td>
<td>+100</td>
<td>+25</td>
<td>4+50</td>
<td>4+20</td>
<td></td>
</tr>
<tr>
<td>1-23-73</td>
<td>150</td>
<td>POS</td>
<td>+25</td>
<td>N</td>
<td>2+20</td>
<td>4+80</td>
<td></td>
</tr>
<tr>
<td>2-20-73</td>
<td>N</td>
<td>N</td>
<td>+25</td>
<td>N</td>
<td>N.D.</td>
<td>3+10</td>
<td>NEGATIVE 3-19-73 (MILK &amp; TISSUES)</td>
</tr>
</tbody>
</table>

### ANIMAL NUMBER 50 (CULTURE POSITIVE)

<table>
<thead>
<tr>
<th>DATE</th>
<th>STT</th>
<th>CARD</th>
<th>ME</th>
<th>RIV</th>
<th>CF-HILL</th>
<th>CF-WIS</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-29-71</td>
<td>1200</td>
<td>POS</td>
<td>+200</td>
<td>1200</td>
<td>4+50²</td>
<td>3+80</td>
<td></td>
</tr>
<tr>
<td>9-7-71</td>
<td>1100</td>
<td>POS</td>
<td>+100</td>
<td>150</td>
<td>2+50²</td>
<td>1+10</td>
<td></td>
</tr>
<tr>
<td>10-5-71</td>
<td>+50</td>
<td>POS</td>
<td>+25</td>
<td>N</td>
<td>3+20</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>11-2-71</td>
<td>150</td>
<td>POS</td>
<td>+25</td>
<td>N</td>
<td>4+10</td>
<td>1+10</td>
<td></td>
</tr>
<tr>
<td>2-22-72</td>
<td>1200</td>
<td>POS</td>
<td>+200</td>
<td>1200</td>
<td>4+50</td>
<td>4+80</td>
<td></td>
</tr>
<tr>
<td>3-21-72</td>
<td>+200</td>
<td>POS</td>
<td>+200</td>
<td>2+200</td>
<td>N</td>
<td>2+80²</td>
<td></td>
</tr>
<tr>
<td>5-16-72</td>
<td>1200</td>
<td>POS</td>
<td>+200</td>
<td>200</td>
<td>2+50²</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>7-11-72</td>
<td>+200</td>
<td>POS</td>
<td>+200</td>
<td>2+200</td>
<td>2+50²</td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

---

N D = not done
1Incompletely fixed at all lower dilutions
2Trace agglutination observed but interpreted as negative test
3AC = anticomplementary serum
4Trace agglutination observed but interpreted as positive test
5QNS = Quantitative (of serum) not sufficient for test
6Incompletely fixed at all lower dilutions
7Trace agglutination observed but interpreted as negative test
8QNS = Quantity (of serum) not sufficient for test
## Table 4

### Serologic Response of 26 Cows Following Administration of 45/20 Bacterin

<table>
<thead>
<tr>
<th>Culture Results</th>
<th>Weeks</th>
<th>Number of Cows with Positive Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>STT</strong></td>
</tr>
<tr>
<td><strong>POSITIVE</strong></td>
<td></td>
<td>Reactor</td>
</tr>
<tr>
<td>(18 Animals)</td>
<td>6 *</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10 **</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td><strong>NEGATIVE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(8 Animals)</td>
<td>6 *</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10 **</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
</table>

*WEEKS AFTER 1ST DOSE OF 45/20

**WEEKS AFTER 2ND DOSE OF 45/20
PROGRESS OF THE STATE-FEDERAL BRUCELLOSIS ERADICATION PROGRAM

Gerald J. Fichtner, D.V.M.¹

In reports to this Association in 1972, 1973, and in recent reports at national and regional assemblies of livestock and regulatory representatives, warnings have been repeatedly raised that unless brucellosis program activities were expanded to an effective level the critical day of reevaluating program goals would soon be upon us. Those inadequate program activities repeatedly emphasized as precluding progress toward eradication include surveillance approaching 100 percent effectiveness, incorporation of minimum standards of the UMR in each state with no exceptions or compromises, maximum utilization of available resources including both State and Federal manpower and money, and a commitment from all sections of the affected livestock industry including regulatory officials to the eradication goal with a corresponding acceptance of the added inconveniences associated with eradication. The livestock industry must be confident that a successful end to the brucellosis eradication program can be realized. The figures which they will see today will not build such confidence. The crossroads for determining program goals is NOW.

BLOOD TESTING CATTLE
(Figure 1)

The total number of cattle blood tested in FY 1974 was over 14.6 million. This is a 7 percent increase over the previous year and includes an additional 420,000 cattle tested on farms or ranches and 529,000 more under the MCI Program. However, this increase was offset by a 24 percent rise in brucellosis reactors to 196,000. This includes 133,000 reactors (28 percent increase) disclosed on the farm and 63,000 MCI reactors (17% increase). The upward trend in the overall reactor rate continued in FY 1974 with 1.34 reactors per 100 blood tests compared to 1.16 in 1973 and 1.05 in 1972. It must be concluded that the rate of spread of brucellosis exceeds the effect of program activities to detect and contain the disease. In reality, the disease is only being controlled — but at a higher national infection rate during each of the past 3 years.

MARKET CATTLE IDENTIFICATION PROGRAM
(Figure 2)

The 8.9 million tests conducted under the MCI surveillance program in FY 1974 continued the upward trend since 1970. The 529,000 increase

¹. Chief Staff Veterinarian, Brucellosis Eradication, Cattle Diseases, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, Hyattsville, Maryland.
includes an additional 13,000 animals tested at packing plants and a
516,000 increase at livestock markets. Again, however, this 6 percent
increase was accompanied by a sharper rise in the number of MCI
reactors. The 63,000 MCI reactors is 17 percent greater than the
previous year. The upward trend in the reactor rate established in 1973
continued with an increase from 0.46 in 1972, 0.63 in 1973, and 0.70
in 1974.

Fifteen thousand nine hundred and twelve (15,912) herds of origin
were identified and tested from tracing these reactors. Additional
infection was disclosed in 5,607 of these herds (35%). The animal
infection rate in infected herds was 14 percent—the same rate identi-
fied during the previous year.

MILK RING TESTS RESULTS
(Figure 3)

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

The 2,597 dairy herds which reacted to the BRT in FY 1974 repre-
sents 0.28 percent of the herds sampled, an increase over the BRT
positive rate of 0.23 percent in 1973 and the highest rate in the past
4 years. Of the 2,192 herds blood tested as a result of the BRT positive
test, infection was disclosed in 497 (23 percent). In those herds where
brucellosis was detected, 4.0 percent of the animals tested were reactors
on the initial herd test compared to 4.4 percent the previous year.

BRUCELLOSIS INFECTED HERDS
(Figures 4 & 5)

The 14,207 infected herds identified in the 50 States in FY 1974
represent a 12 percent increase over the previous year. This compares
unfavorably with the 8 percent increase in 1973 over the previous year
and should be cause for deep concern. Not only have the total number of
infected herds detected increased during each of the past 2 years but
the rate of increase has also magnified. Five hundred and fifty (550)
infected herds were identified in the 30 certified-free States compared to
435 in the same States the previous year. There were 13,657 infected
herds in the remaining 20 modified certified States, an increase of some
1,700 in these same States in 1973.

Brucellosis remains predominantly in the central and southern re-
gions of the United States. Thirty-four and eight tenths (34.8) percent
of the infected herds (4,953) were identified in Texas. Although this is an
increase of approximately 1,600 over 1973, it reflects the recent drama-
tic strengthening of program activities in Texas. Continuing increases
in the number of infected herds should be expected in Texas in the near
future. Twenty-eight and six tenths (28.6) percent of the Nation's
infection was identified in Louisiana, Mississippi, and Oklahoma. Each of these three States has reported a relatively constant number of infected herds (between 1,000 and 1,700) each year during the previous five-year period in spite of efforts nationally to strengthen program activities. Seven States reported infected herds within the 300 to 1,000 range and collectively account for 25.9 percent of the total. These include Alabama (1974-688; 1970-490), Arkansas (1974-556; 1970-237), Florida (1974-378; 1970-600), Georgia (1974-437; 1970-334), Kentucky (1974-409; 1970-386), Missouri (1974-326; 1970-407), and Tennessee (1974-898; 1970-492).

Not one of the 11 States which collectively reported 89 percent of the infected herds in FY 1974 has significantly reduced the level of infection in their respective States during the past three years.

Twelve (12) States and Puerto Rico had 8.7 percent of the infected herds and are in the range of 30-300 infected herds reported. Of these States, only Nebraska and South Dakota showed a decrease from the previous year. Twenty-eight States reported less than 30 infected herds. No infection was detected in six States and the Virgin Islands.

CERTIFICATION STATUS — JUNE 30, 1974
(Figure 6)

Only 32 counties achieved Certified Brucellosis-Free 'status during the year. The total of 1,978 such counties represents 63 percent of the Nation's counties and includes 53 percent of the adult cows. No States qualified for statewide-free status. One thousand one hundred and sixty-nine (1,169) counties in 20 States and Puerto Rico held Modified Certified Area status at the end of FY 1974. One county in Oklahoma, one in Missouri, and three in Texas were listed as non-certified areas because of program deficiencies. Action to remove certification status from 27 additional counties for similar program deficiencies was required in 1974.

CALFHOOD VACCINATION
(Figure 7)

The previous downward trend in the number of calves vaccinated annually was halted in FY 1974 with 3.8 million vaccinations - the same number as reported in 1973. Fifty percent of the calves vaccinated were in the 30 Certified Brucellosis-Free States. This is approximately 27 percent of the eligible calves in these free areas. Sixteen percent of the eligible calves in the 20 modified certified States received Strain 19.

SWINE BRUCELLOSIS
(Figure 8)

During FY 1974 the swine brucellosis program continued the accelerated pace of the preceding year and substantial progress was made in all measurable aspects of the program.
The number of swine blood tested during the year increased to 2.3 million animals, up 85 percent from FY 1973. This total includes 362,000 tested on farms and 1.9 million sows, boars, and stags tested at livestock markets and at slaughter under the Market Swine Testing Program (MST). The latter figure represents over 40 percent of the eligible swine slaughtered nationally during this period.

The majority of the MST samples were collected under contract arrangements at 29 federally inspected slaughter plants located in nine States having over 75 percent of the Nation's swine population.

The animal infection rate was 0.13 compared to 0.17 in FY 1973. The MST reactor rate in FY 1974 was 0.08, slightly less than the previous year.

Four States—Arkansas, Oregon, Wisconsin, and Wyoming attained validated status during the year joining Arizona, California, Montana, Nevada, Utah, and the Virgin Islands on the list of Validated Brucellosis-Free States or territories. (Figure 9).

The number of Validated Brucellosis-Free counties increased from 275 to 496 during the year. In addition to all counties in the validated States, there were free counties in Hawaii - 3, Maryland - 11, Massachusetts - 1, Michigan - 13, New Mexico - 1, South Dakota - 12, and Puerto Rico - 61.

There was a 19 percent increase in the number of Validated Brucellosis-Free herds during the year from 3,668 to 4,355. (Figure 10). A significant part of this increase was due to recent regulations in North Carolina which required that swine sold for breeding purposes must originate in validated herds.

Two States—North Dakota and South Dakota—adopted regulations during the year that require the identification of swine. These States, along with Minnesota, Nebraska, and Wisconsin bring to five the number of States with similar identification requirements.

SUMMARY

There are several statement regarding the progress of the program as reflected by this data and concerning eradication efforts in general that I wish I could make today.

1. I wish I could say that the increased suspicion of infection (MCI and BRT) is caused by improved surveillance systems and not be increased infection, and that it's not important to reach 100 percent efficiency in detection methods—but I can't.

2. I wish I could tell you that the increase in infected herds is simply the result of detecting existing foci of infection and that brucellosis is not spreading faster than we are finding it—but I can't.

3. I wish I could tell you that attention to program details wasn't important, that success could be attained by test and slaughter alone—but it won't be.

4. I wish I could tell you that the disease will go away once it reaches a certain low level — but it won't.

5. I wish I could tell you that additional resources are easy to obtain
and that increased funding alone will cure much of the ills in the pro-
gram—but I can’t.

6. I wish I could tell you that the minimum standards of the Univer-
seal Methods and Rules can be compromised, ignored, or given lip service,
and we will still make progress—but I can’t.

7. I wish I could tell you that you need not be committed to this
effort to make it succeed, you don’t have to solve your individual people
problems because they will go away—but they won’t.

I can promise you, however, that unless we act forthrightly to solve
our problems regarding resources, spread of disease, inadequate
surveillance and lack of epidemiology, then the spiral of compromise,
nonconcern, program interruption, increased infection rates, increasing
undulant fever, lack of producer confidence, and more compromise
will lead us to defeat in this eradication effort.

The repeated question arises, “Can brucellosis be eradicated?”
The answer is still “Yes.” The factors preventing early attainment of this
goal are not “cattle problems,” but rather “people problems!” Such
factors were expressed by R. R. Birch, DVM, in his presentation on the
control of brucellosis in cattle at the 1943 annual meeting of the United
States Livestock Sanitary Association when he said, “... that given
reasonable release from artificial obstacles, the control (and eradica-
tion) of brucellosis in cattle is easy. I am saying that of the men who
founded this association, the few who looked south from the Mason
and Dixon Line across the areas that eventually were to be freed from
the Texas fever tick were facing a bleak prospect involving difficulties
far greater than those confronting us in the control (and eradication)
of brucellosis today. If we can not control (and eradicate) brucellosis
with the combined and judicious use of the proved weapons and trained
personnel now at our disposal, it is only because we have become a
lesser breed of men.”

I do not believe that we are a lesser breed of men — we must become
committed, resolve the problems, and move forward with the challenge of
brucellosis eradication — and IT MUST BE NOW!!!
**Fig. 1.**

**Brucellosis Eradication**

**BLOOD TESTING: CATTLE**

<table>
<thead>
<tr>
<th>FISCAL YEAR</th>
<th>FARM OR RANCH</th>
<th>MCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1968</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>1969</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>1970</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td>1971</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>1972</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>1973</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>1974</td>
<td>14.6</td>
<td></td>
</tr>
</tbody>
</table>

**THOUS. REACTORS FOUND**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>149</td>
<td>130</td>
<td>119</td>
<td>116</td>
<td>124</td>
<td>158</td>
<td>168</td>
</tr>
</tbody>
</table>

**MARKET CATTLE TESTING PROGRAM**

<table>
<thead>
<tr>
<th>FISCAL YEAR</th>
<th>AT PACKING PLANTS</th>
<th>OTHER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1967</td>
<td>58.0%</td>
<td>42.0%</td>
</tr>
<tr>
<td>1968</td>
<td>59.0%</td>
<td>41.0%</td>
</tr>
<tr>
<td>1969</td>
<td>59.6%</td>
<td>40.4%</td>
</tr>
<tr>
<td>1970</td>
<td>58.2%</td>
<td>41.8%</td>
</tr>
<tr>
<td>1971</td>
<td>58.4%</td>
<td>41.6%</td>
</tr>
<tr>
<td>1972</td>
<td>62.2%</td>
<td>37.8%</td>
</tr>
<tr>
<td>1973</td>
<td>63.3%</td>
<td>36.7%</td>
</tr>
<tr>
<td>1974</td>
<td>60.6%</td>
<td>39.4%</td>
</tr>
</tbody>
</table>

**MILLIONS CATTLE TESTED**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**US DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE**
MILK RING TEST RESULTS
(BRT)

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


Fig. 3

BRUCELLOSIS INFECTED HERDS FOUND
In Noncertified, Modified Certified, and Certified-Free States

NUMBER INFECTED HERDS
35,000

Fig. 4

STATES WHERE INFECTED HERDS FOUND
Certified-Free
Modified Certified
Noncertified

FISCAL YEAR  NUMBER STATES

1966  12  29  9
1967  11  29 10
1968  8  29 13
1969  6  29 15
1970  4  27 19
1971  1  27 22
1972  1  22 27
1973  0  20 30
1974  0  20 30

U.S. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE
DISTRIBUTION OF BRUCELLOSIS REACTOR HERDS

Percent of Total Reactor Herds Found

- 28 STATES, <30 HERDS: 34.8%
- 13 STATES, 30<300 HERDS: 28.6%
- 7 STATES, 300<1,000 HERDS: 25.9%
- 3 STATES, 1,000<3,000 HERDS: 8.7%
- 1 STATE, >3,000 HERDS: 2.0%

FISCAL YEAR 1974

Fig. 5

Cooperative State-Federal

BRUCELLOSIS ERADICATION PROGRAM

JUNE 30, 1974

Fig. 6
Fig. 7

Brucellosis Eradication

CALVES VACCINATED

MILLION CALVES VACCINATED

FISCAL YEAR

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

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

Fig. 8

SWINE BRUCELLOSIS
Animals Blood Tested

THOUSAND ANIMALS

1,400 1,200 1,000 800 600 400 200 0

FISCAL YEAR

1972 1973 1974

INFECTION RATE

TOTAL TESTS ON FARM MST

FISCAL YEAR

1972 1973 1974

U.S. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE
DR. CARROLL K. MINGLE
AWARDEE OF THE HONORARY DIPLOMA
AMERICAN VETERINARY EPIDEMIOLOGY SOCIETY
October 17, 1974
Roanoke, Virginia

Dr. Carroll K. Mingle has long been recognized for his contribution to advancement of animal health programs and improvements in veterinary education. He received the degree of D.V.M. from Ohio State University in 1930 and a M. Sc from Ohio State in 1934. He was on the faculty of the College of Veterinary Medicine, Ohio State University, from 1934-1936. He subsequently entered service with the Bureau of Animal Industry, U.S. Department of Agriculture. After a brief tour of duty in tuberculosis eradication, he was assigned to the Pathological Division, Beltsville, Maryland, where he served until 1947. During this period, he assisted the British Government in establishing a laboratory to produce Brucella abortus Strain 19 vaccine.

Dr. Mingle was reassigned to the headquarters staff for eradication of brucellosis and tuberculosis in 1947. In this assignment, and for the remainder of his career, he was very active in developing and leading the effort to eradicate brucellosis. He has been recognized internationally as an authority on brucellosis and its eradication as evidenced by his participation in many committees and programs throughout the world. In recognition of these many contributions, this award is presented which reads as follows:

The American Veterinary Epidemiology Society is proud to present the Honorary Diploma to Carroll K. Mingle for his distinguished service and contribution to the progress of public health. His efforts and vision, compassion and understanding, desire and energy, have advanced Veterinary Public Health, and research, his counsel and advice have been of great value to his country.

Your colleagues are honored to have you accept this diploma.

Respectfully,
James H. Steele, President
K. F. Meyer, Honorary President

October 16, 1974
The American Veterinary Epidemiology Society is proud to present the Honorary Diploma to Carroll K. Mingle for his Distinguished Service and Contribution to the progress of public health. His efforts and vision, compassion and understanding, desire and energy, have advanced veterinary Public Health, and research. His counsel and advice have been of great value to his country.

Your colleagues are honored to have you accept this Diploma.

Respectfully,

[Signature]

October 16, 1974

Honorary President
BRUCELLOSIS COMMITTEE REPORT

Chairman: Bob Laramore, Gillette, Wyo.
Co-Chairman: G. J. Fichtner, Dayton, Md.


The Brucellosis Committee met in open session on Monday, October 14, 1974. All interested persons were given the opportunity to introduce resolutions and comment on the various aspects or program activities and standards.

On Tuesday, October 15, 1974 an executive meeting of the Brucellosis Committee was held with all resolutions previously submitted given consideration. The following recommendations were favorably passed:

1. That an executive meeting of the Brucellosis Committee be held on December 4, 1974, at Chicago, Illinois, for the sole purpose of considering action on the Florida resolution concerning adult vaccination with Strain 19 in select dairy herds. This will allow all committee members ample time to obtain and review all evidence concerning this problem so that a more valid and equitable decision can be made.

2. That the following procedures for revalidating on a statewide basis be incorporated into the UMR:
   a. Test 90% of all sows, boars and stags at slaughter during each year of the 3-year testing period and
   b. When reactors are found the herd of origin is sent to slaughter within 30 days or a plan for eradicating brucellosis from the herd is put into effect.

3. That the USDA be urged to promulgate a regulation requiring the identification of all sows and boars going to slaughter, and that such identification be a requirement for participation in swine brucellosis eradication program activities.
4. That the definition of HERD TEST in the UMR be amended by adding the following sentence: “herd test decisions under this definition must be based on sound epidemiological evidence”.

5. That the definition of OFFICIAL VACCINATE in the UMR be amended as follows:
   a. Substitute the following sentence where appropriate: “A female bovine animal in a beef herd vaccinated against brucellosis with an approved brucella vaccine while from 2 to 10 months (60 to 299 days) of age”.
   b. Omit the following sentence: The Brucellosis Committee of the USAHA has recommended that beginning January 1, 1975, the vaccination age for both beef and dairy breeds be 3 to 6 months of age”.

6. That the UMR be amended regarding “Services to Owner” by addition of the following sentence: “Within 3 days of an initial herd test, an owner shall be allowed to request an additional test on reactors at his own request and expense provided such request is based on sound epidemiological evidence and provided all animals remain under herd quarantine”.

7. That the minimum standards of the UMR apply similarly to bison as to cattle.

8. That the UMR be amended to allow additions to Certified Brucellosis Free Herds from herds in Modified Certified Areas as follows: Animals from herds not under quarantine must be tested negative for brucellosis within 30 days prior to the date of movement and must be retested negative between 60 and 120 days after being moved. Animals added under this provision shall not receive new herd status for sale purposes until they have been members of the herd for at least 30 days and are included in a complete herd test.

9. That the UMR be amended to include the following Recommended Procedure:
   “Brucellosis Problem Herds - Herds which do not progress favorably under routine testing procedures may be considered for enrollment in a program under the direct supervision of a trained brucellosis epidemiologist. Such enrollment must be for the enhancement of brucellosis from the herd and may include altered testing schedules, management practices, supplemental testing, etc., as approved by the owner and officials in charge of program activities in the state.

10. That the UMR be amended by inclusion of the following: “The semen plasma test for brucellosis in bulls used for artificial insemination be designated as an official test in addition to the standard diagnostic sero-agglutination tests.”

11. That the UMR be amended as concerns the addition of semen to validated herds and areas: “Swine semen for artificial insemination may enter validated herds and areas as follows:
   a. Purebred herds from which semen is collected must be validated on the basis of herd blood tests.
   b. Commercial boar studs from which semen is collected must be validated on the basis of herd blood tests and herd semen
agglutination tests.

12. That the Brucellosis Committee of USAHA supports the goal of brucellosis eradication, provided additional funding is made available to conduct program activities at an effective level during fiscal year 1975 and for each succeeding year. The Executive Committee of USAHA is requested to advise the Secretary of Agriculture of this support in personal audience within 30 days.

13. That the definition of "Herd Quarantine" in the UMR be amended as follows: "All cattle, except steers and spayed heifers in infected herds must be confined to the premises until freed of brucellosis or sold for slaughter under permit".

The remainder of the definition to remain intact.

14. The USDA is urged to cooperate with the Department of Interior on a range research program to conduct transmissibility studies of Brucellosis from bison to cattle.

Several resolutions concerning "Brucellosis Critical Areas", "Brucellosis Eradication Areas", and further amendments were considered but not passed.

USAHA Brucellosis Committee Meeting
Chicago O'Hare International Airport
Chicago, Illinois
December 4, 1974

This special meeting of the Brucellosis Committee, USAHA, was held for the purpose of acting on the previously tabled Florida proposal concerning adult vaccination with Strain 19. A preliminary open meeting was held for the purpose of allowing invited speakers to present their views concerning the problem of brucellosis in Florida dairy herds and possible solutions to this problem. The enclosed agenda was followed by a discussion of each presentation.

In the Executive Meeting, three resolutions were presented as follows:

1. That adult vaccination with Strain 19 be approved as a standard program procedure for use in problem dairy herds consistent with the Blue Ribbon Committee report. Motion defeated.

2. The Brucellosis Committee of USAHA recommends that USDA (APHIS), the Florida Department of Agriculture, and the dairy herd owner enter into an agreement to conduct such field studies or research that will result in the eradication of brucellosis in selected problem dairy herds in Florida. This project is to begin on January 1, 1975. Motion passed.

3. The BRT be authorized for 30-day interval testing procedures in large dairy herds, as recommended by Drs. D. E. Pietz and B. L. Deyoe, subject to the approval of State and Federal supervising veterinarians. Motion passed.

The meeting adjourned at 4:00 p.m., with no unfinished business pending.

This was duly passed by majority of the Executive Committee by mail ballot and now becomes a part of the brucellosis report. (Florida abstained)
ISOLATION OF LEPTOSPIRAL SEROTYPE SZWAJIZAK FROM DAIRY CATTLE IN OREGON

James W. Glosser, D.V.M.*
Cathering R. Sulzer, B.S.**
Guy C. Reynolds, D.V.M.***
Douglas K. Whitsett, D.V.M.****

Introduction

Leptospiral agglutinins for serotypes belonging to the Hebdomadis serogroup have been reported in United States cattle since 1951. Initially, serotype sejroe was considered responsible for the many positive serologic reactions in cattle sera tested for leptospiral agglutinins. In 1960, Roth reported the first isolation of serotype hardjo in the United States and suggested that it was probably responsible for the positive sejroe reactions previously reported. Alexander and Evans confirmed that sejroe titers were the result of hardjo infections in 1962. Since then, isolation of hardjo from aborting cattle has been reported in Nebraska and Illinois. Moreover, Robertson et al in Canada also isolated hardjo from a cow following an abortion.

Since 1967, the United States Animal Health Association (USAHA) Leptospirosis Committee reports have considered hardjo infection in cattle to be a major economic threat. Also, the committee has repeatedly encouraged the screening of all domestic animal sera submitted to diagnostic laboratories with hardjo antigen to determine the prevalence and geographic distribution of hardjo infections in cattle.

The purposes of this report are to present the first cultural evidence of serotype szwajizak in the United States and discuss the clinical and epidemiological features noted in dairy cattle infected with this serotype.

Materials and Methods

Liquid and semisolid bovine albumin polysorbate medium was used for cultivation of leptospires from the urine of infected animals. Semi-solid BAP medium (5ml/tube) was sent to Dr. Whitsett to inoculate urine from cattle suspected of having leptospirosis. Midstream urine was collected aseptically and approximately .03ml (1 drop from a 20 gauge needle) of undiluted urine was inoculated into a tube of medium (3 cultures/cow). The cultures were forwarded to the Center for Disease Control and incubated at 29°C for 8 weeks. A small amount (approximately .03ml) of each culture was checked by darkfield microscopy at weekly intervals to determine the presence of leptospires.

*Chief Deputy State Veterinarian, Animal Health Division, Montana Department of Livestock, Capitol Station, Helena, Montana 59601.
**Bacterial Immunology Branch, Bacteriology Division, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia 30333.
***Extension Veterinarian, Department of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97330.
****Private Practitioner, Klamath Animal Hospital, Klamath Falls, Oregon 97601.
All isolates were definitely identified based on cross agglutinin-absorption tests by Mrs. Sulzer at the Center for Disease Control Leptospiral Reference Laboratory.

Sera collected by Dr. Whitsett from cattle on the infected premise were tested with the microscopic agglutination (MA) test. In October 1972, 14 cows (4 percent of the milking herd) were bled and their sera submitted for leptospiral serology. In June 1973, another random sample of 39 cattle (11 percent) were tested for the presence of leptospiral agglutinins. The microscopic agglutination test was conducted according to the procedure described by Cole et al.\textsuperscript{10} utilizing an antigen battery of 14 serotypes: ballum, canicola, icterohaemorrhagiae, bataviae, grippotyphosa, pyrogenes, autumnalis, pomona, wolfi, hardjo, australis, tarassovi, georgia and szwajizak.

**Herd History**

The infected herd has had a history of infertility and abortion problems for the past ten years. However, the problem increased in magnitude in 1970 after the addition of a dairy herd from the Astoria, Oregon area. Prior to that time the herd was closed with the milking herd consisting of approximately 40 cows in 1952; 80 in 1955; 200 in 1960; 250 in 1965; and 350 in 1970. The herd additions and replacements were home raised cattle except for those in 1970.

Detailed histories were available on 42 head of affected cows from the milking herd in June 1973. The prominent signs in order of decreasing frequency (in percent) were: metritis 71; gave birth to a weak calf which died within 2 weeks 36; repeat breeders 31; anestrus (duration greater than 3 months) 25; abortion 22; stillbirth 19; mastitis 17; keratitis and/or conjunctivitis 10; retained placenta 5; hemoglobinuria 2.5; and encephalitis (calf) 2.5. In addition to mastitis, agalactia has been a problem in this herd (Table 1).

The mortality rates experienced in 1973 were 10 percent for cows and 23 percent for calves two weeks of age or younger. Routine necropsies performed on cows revealed chronic uterine and mammary gland infections with an occasional traumatic reticulitis. In calf necropsies, the most striking feature was the lack of gross lesions which could explain the cause of death. The only significant finding routinely encountered was a polyarthritis primarily affecting the tibial-tarsal joint.

No seasonal distribution for cow losses was noted as deaths occurred throughout the year. In calves, deaths occurred throughout the year but losses have been as high as 100 percent in the months of February and March. A direct correlation existed between calf loss and the existence of peak incidence of metritis and fertility problems in the cow herd.

Since 1970, many specimens from cows and calves have been sent to several diagnostic laboratories, none of which reported isolation of any agent that could be considered as a primary pathogen. However, none of the specimens were cultured for leptospires. Common bacterial isolations reported were: \textit{Bacillus sp.}; \textit{Proteus sp.}; \textit{Pseudomonas sp.};
Corynebacterium sp.; Staphylococcus sp.; Streptococcus sp.; Moraxella sp. and Escherichia coli.

The management of this Grade A dairy has remained constant for years. Artificial insemination is practiced for the milking herd, whereas the 2 year old replacement stock is bred naturally. The milking strings are concentrated in small lots where they are fed free choice alfalfa hay, a liquid molasses-mineral-vitamin solution and approximately 30 pounds of a 14-16 percent protein dairy supplement. Heated water tanks in the lots serve as the cows' sole source of water. Urinary contamination of the water and supplement tanks is a problem. Annual herd vaccination against pomona has been practiced since 1967.

Of the 14 cows tested in October 1972 for MA antibodies, 9 (65 percent) had significant titers to pomona, whereas 1 (7 percent) had a significant Hebdomadis titer. In June 1973, the MA test results revealed 10 cows (26 percent) with titers of 1:100 or greater to pomona and 13 (33 percent) with Hebdomadis titers (Table 11).

All urine cultures (18) were heavily contaminated with bacteria at the time of arrival at the Center for Disease Control in October 1972. However, leptospires were demonstrated by darkfield microscopy from 3 of the 6 cows sampled within 30 days of incubation despite the contaminated cultures. All cultures (9) from the 3 infected cows supported the growth of leptospires. Decontamination of the cultures proved to be a major task as it required approximately 4 months to rid the cultures of secondary bacteria. The cultures were decontaminated by serial dilutions and rapid subculture techniques into 50ml amounts of liquid BAP medium.

The clinical, cultural and serological data from the 6 cows in which isolation attempts were performed are summarized in Table III.

Cow 12 was negative both serologically and culturally. Subsequent to calving on March 6, 1972, she developed a metritis that responded to antibiotic therapy followed by anestrus of at least 15 months duration. The calf died within 2 weeks of birth.

Cow 22 had a 1:400 Hebdomadis titer in October 1972, and szwajiazk was isolated from her urine. She went off feed, became listless and developed a metritis and mastitis after calving. After a long convalescent period she was sold for slaughter.

Cow 638 had an initial pomona titer of 1:200, negative to hardjo, but, szwajizak was isolated in October 1972 and she seroconverted to Hebdomadis antigens in June 1973. She gave birth to a stillborn calf in September 1972, and developed a metritis, mastitis and agalactia of 3 weeks duration.

Cow 814 was seronegative on both tests but szwajizak was isolated in October 1972. She aborted a 7 month old fetus on September 23, 1972 and developed a metritis and anestrus of several months duration.

Cow 826 had an initial Pomona titer of 1:12,800, however, no leptospires were isolated from her urine but seroconverted to 1:3200 for Hebdomadis and had a Pomona titer of 1:6400 in June 1973. She calved on March 13, 1972 and developed a hemoglobinuria, metritis and anestrus of 15 months.
Cow 884 was serologically and culturally negative for leptospirosis. Subsequent to calving on August 12, 1972, she developed a low grade fever, diarrhea and metritis. She conceived 6 months postcalving.

Hyperimmune rabbit sera of all three cultures were prepared subsequent to the purification of the isolates. The hyperimmune serum was used to positively identify the isolates as *szwajizak* based on cross agglutinin-absorption studies.

As a result of the isolation of *szwajizak*, the entire herd was vaccinated in November 1973 with the bivalent *canicola-icterohaemorrhagiae* bacterin and the monovalent *hardjo* bacterin using two doses at 14 days. In addition, the herd was vaccinated in April 1974 with an experimental *szwajizak* bacterin and boostered one month later with an additional dose.

The overall mortality rate (cows and calves) has declined from 10 and 23 percent to approximately 2 percent subsequent to the administration of the different bacterins. Moreover, the conception rate (non-return after first service) has increased from 71 to 85 percent as of this time.

**Discussion**

The diagnosis of leptospirosis in animals and man is almost entirely dependent on laboratory findings. The most frequently used diagnostic tool is the serologic demonstration of antibodies in serum specimens. However, the findings in this study vividly demonstrate the limitation and danger involved when serologic data alone are used to diagnose leptospirosis in either individual animals or herds.

The most significant serotype in this herd based on seropositivity rates was *pomona*, since more than 25 percent of the cows tested had significant titers. However, it is noteworthy that leptospires belonging to the Pomona serogroup were not isolated. This was somewhat surprising since leptospires of the Pomona group are relatively easier to isolate than those of the Hebdomadis group. This is particularly true with direct culture into artificial medium of clinical specimens.

Of particular interest and importance is cow 814. This cow was seronegative on both tests to all serotypes used as antigens, including the leptospires isolated from her urine. Seronegative but culturally positive cows have been reported, but the incidence of this problem is unknown. Certainly, the laboratory data from this cow reinforces the recommendation that a proportionate number of animals must be tested to establish a diagnosis of leptospirosis in a herd.

The isolation of serotype *szwajizak* constitutes the first cultural evidence of Hebdomadis infection other than *hardjo* in the United States cattle. Infections with *szwajizak* in man and animals in Israel have been described in detail by van der Hoeden. Dr. Michael Torten summarized the status of *szwajizak* infections in Israel at the Leptospirosis Research Workers Conference in December, 1973: (1) *szwajizak* was the leading serotype causing leptospirosis in dairy cattle; (2) it also was the leading serotype responsible for human leptospirosis; (3) it is possible that the *hardjo* bacterin may not protect cattle against
szwajizak infections and (4) serotypes hebdomadis, wofffi and hardjo are better antigens to detect szwajizak infections in the microscopic agglutination test than szwajizak itself. This last point was confirmed at the Center for Disease Control in the serologic studies of this infected herd.

In Israel, van der Hoeden determined the hedgehog to be the reservoir for szwajizak. In his thorough study of this serotype, he commented on the benefit of culturing brains of wild animals to recover leptospires in epidemiologic studies. The occurrence of an occasional meningio-encephalitis and keratitis in calves from this herd suggests the possibility of brain infections. Brain infection in hamsters, rats and turtles from experimental and field investigations at the Center for Disease Control have been documented.

From an epidemiologic point of view, two questions must be answered to achieve adequate control of leptospirosis in cattle. What is the incidence of szwajizak infections and what is its geographical distribution in the United States? It is possible that some of the herds diagnosed as hardjo infections on the basis of only serologic data could be szwajizak instead. This is of vital importance since szwajizak infections are readily detected by the use of hardjo antigen. The answer to this question lies in the need to conduct cultural studies to isolate the infecting leptospire once serologic evidence of leptospirosis is available.

The second question that needs to be answered is the efficacy of the hardjo bacterin in preventing clinical disease in szwajizak infected herds. Laboratory and field research should be performed to evaluate the efficacy of the hardjo bacterin in szwajizak infected animals. Preliminary evidence in hamsters suggests that the degree of protection is lower in hardjo vaccinated hamsters challenged with szwajizak than hamsters vaccinated with szwajizak. Field evaluation of the hardjo bacterin in naturally infected szwajizak is of prime importance.

The clinical picture of infertility problems encountered in this szwajizak infected herd closely parallels that seen in herds infected with hardjo. There is increasing evidence that cattle leptospirosis due to leptospires of the Hebdomadis group is manifested principally as an infertility problem in contrast to an abortion problem in cattle infected with Pomona.

It is impossible to speculate if the total disease picture noted in this herd was due to infection with szwajizak at this time. Two factors suggest that leptospirosis is the primary factory in this disease complex. First, two additional dairy herds in the area are experiencing the same clinical problem. Of interest, neither herd had the problem until they purchased 2 year old replacement heifers from the infected dairy herd two years ago. Since then, the same clinical picture, morbidity and mortality have been experienced in these herds. Second, the vaccination of the herd with the various leptospiral bacterins with concurrent reduction in morbidity and mortality also supports the fact that leptospirosis is a primary factor in the disease syndrome. As a result, the two
additional herds were also vaccinated with the various leptospiral bacterins based on clinical observations only. The same reduction in morbidity and mortality was noted.

It was most unfortunate that a valid field evaluation of these bacterins could not be implemented using appropriate controls. With imperical information such as reported here, it is impossible to answer the question of efficacy of the individual bacterins in the control of this disease problem.

Summary

In October 1972, the first cultural evidence of Leptospira szwajizak in the United States was obtained from a dairy herd in Klamath Falls, Oregon. Leptospires were isolated from the urine of three of six cows sampled. The limited leptospiral serology performed on this herd suggested that the leptospiral infections were due to serotypes pomona and hardjo instead of szwajizak. The limitations of utilizing only serologic methods of diagnosing leptospirosis are discussed.

The predominant clinical feature noted in this infected herd was one of an infertility problem in contrast to the abortion problem usually associated with bovine leptospirosis.

<table>
<thead>
<tr>
<th>SIGN</th>
<th>NUMBER OF COWS AFFECTED</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metritis</td>
<td>30</td>
<td>71</td>
</tr>
<tr>
<td>Gave birth to a weak calf that died within 2 weeks</td>
<td>15</td>
<td>36</td>
</tr>
<tr>
<td>Repeat breeders</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td>Anestrus (duration ≥ 3 months)</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td>Abortion</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>Mastitis</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Keratitis (conjunctivitis)</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Retained placenta</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Hemoglobinuria</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Encephalitis (calf)</td>
<td>1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

1-42 head of cows with clinical histories.

<table>
<thead>
<tr>
<th>DATE</th>
<th>SEROGRUP</th>
<th>Pomona²</th>
<th>Hebdomadis³</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 1972</td>
<td>Pomona²</td>
<td>9/14(65)</td>
<td>1/14(7)</td>
</tr>
<tr>
<td>June 1973</td>
<td>Hebdomadis³</td>
<td>10/39(26)</td>
<td>13/39(33)</td>
</tr>
</tbody>
</table>

1. Number of positive sera with a titer of 1:100 or greater/total sera submitted (percent).
2. Includes serotype pomona as antigen.
3. Includes serotypes hardjo, wolffi, georgia and szwajizak as antigens.
# ISOLATION OF LEPTOSPIRAL SWZAJIZAK

## TABLE III. CLINICAL, CULTURAL AND SEROLOGIC DATA FROM SIX COWS IN A DAIRY HERD INFECTED WITH SEROTYPE SWZAJIZAK - OREGON - 1973

<table>
<thead>
<tr>
<th>COW</th>
<th>MA TITER RESULTS&lt;sup&gt;1&lt;/sup&gt;</th>
<th>LEPTOSPIRES ISOLATED</th>
<th>MAJOR CLINICAL SIGNS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>October 1972</td>
<td>June 1973</td>
<td>October 1972</td>
</tr>
<tr>
<td>12</td>
<td>Negative</td>
<td>Negative</td>
<td>No</td>
</tr>
<tr>
<td>222</td>
<td>400 Hebdomadis</td>
<td>Not Tested</td>
<td>Yes</td>
</tr>
<tr>
<td>638</td>
<td>200 Pomona</td>
<td>50 Pomona 100 Hebdomadis</td>
<td>Yes</td>
</tr>
<tr>
<td>814</td>
<td>Negative</td>
<td>Negative</td>
<td>Yes</td>
</tr>
<tr>
<td>826</td>
<td>12,800 Pomona</td>
<td>6400 Pomona 3200 Hebdomadis</td>
<td>No</td>
</tr>
<tr>
<td>884</td>
<td>Negative</td>
<td>Negative</td>
<td>No</td>
</tr>
</tbody>
</table>

<sup>1</sup>-Reciprocal of the highest dilution where complete agglutination occurred.

## REFERENCES

THE CONTROL OF OUTBREAKS OF LEPTOSPIROSIS IN BEEF CATTLE BY SIMULTANEOUS VACCINATION AND TREATMENT WITH DIHYDROSTREPTOMYCIN

P. J. South, D.V.M.*, and H. G. Stoenner, D.V.M.**

Among herds of beef cattle affected by leptospirosis the primary economic loss is due to aborted fetuses. The abortion rate is known to vary, and it is influenced by factors such as virulence of the organism, rate of spread, and stage of pregnancy at the time the organism is introduced. Because 20 to 40 percent of pregnant cows infected in the last trimester may abort, interruption of the epizootic to prevent further losses should be of primary concern to the attending veterinarian. This report deals with the termination of epizootics in 5 naturally infected herds after simultaneous vaccination and chemotherapy.

*Box 87, Salmon, Idaho 83467.

Herd Histories, Treatment, and Results

Since 1969, I (P.T.S.) have controlled outbreaks of leptospirosis by simultaneously vaccinating all animals in the herd and treating pregnant cows with one injection of 25 mg dihydrostreptomycin/kg of body weight. Most herds were moved to clean pastures or enclosures after treatment. In most herds so managed, the disease was diagnosed during the early phase of the epizootic. Diagnosis was based on results of plate tests with formalin-killed antigens prepared from 10 serotypes. Abortions occurred in one herd in late December, in 3 in early January, and in one in late February. These herds were seasonally bred for calving to begin in early March, and none had been vaccinated with any leptospira serotype vaccine. All 5 herds were free of brucellosis. The following are brief descriptions of the herds and clinical histories prior to diagnosis.

V.E. — This herd of 250, 2-year-old Hereford heifers had been assembled through purchases from various sources in Idaho and Wyoming during the 4 months preceding the outbreak. It was maintained in a 120-acre pasture in valley bottom ground and watered from irrigation ditches fed from a natural stream. Six abortions occurred during the 3 days before blood samples were obtained 18 January 1969. At that time, 10 of 14 cows bled were seropositive against the hardjo serotype. During the following week 9 more cows aborted. After treatment with dihydrostreptomycin and vaccination with pomona serotype vaccine on 25 January 1969, no more abortions were observed. The herd was treated primarily to terminate the abortion storm, and pomona serotype vaccine was given because the herd was not protected against the pomona serotype. Hardjo serotype vaccine was not yet available.

Q.S. — This herd of 425 head of grade Hereford cows was maintained in a 600-acre pasture in valley bottom ground and watered from a natural
CONTROL OF OUTBREAKS OF LEPTOSPIROSIS

Stream. Brood cows in this herd were examined annually for pregnancy. During the 2 months preceding serologic tests made 14 January 1971, 20 abortions were observed. Five of the 20 aborted cows were tested and all were seropositive against the pomona serotype, 4 in high titer. Only one abortion occurred after the herd was treated 16 January 1971 with dihydrostreptomycin and vaccinated with pomona serotype vaccine. Pomona vaccine has been administered annually ever since.

S.C. — This herd of 200 mixed Santa Gertrudis and Herefords was maintained in small pastures in the foothills and watered from a natural stream. Immediately after an abortion was observed on 25 December 1973, the aborting cow and 4 others were bled. This cow and one other were seropositive in high titer against the pomona serotype; the remaining 3 were seronegative. No further abortions occurred after treatment and vaccination 1 day later.

D.S. — This herd of 160 grade Hereford cows was maintained in a 100-acre pasture in valley bottom ground and watered from a natural stream. On 7 January 1974, 4 cows, including 2 cows that had aborted 2 to 4 days before, were bled; one aborted cow was seropositive (1:160) against the hardjo serotype and the other 3 were negative. To further evaluate the serologic status of the herd, blood samples were obtained 10 January 1974 from 11 additional cows; of these, 7 were seronegative and 4 were seropositive against the hardjo serotype. No further abortions occurred after chemotherapy and vaccination of the herd with hardjo serotype vaccine on 21 January 1974.

S.M. — This herd of 500, 2-year-old heifers of mixed breed, chiefly Herefords, Angus, and crossbreeds, was maintained in a 200-acre pasture in valley bottom ground and watered from a natural stream. During 12 days preceding sampling of the herd on 26 February 1974, 11 cows aborted. Seven of these, 6 pregnant cows, and one with a mummified fetus were bled; the 7 aborted cows were seropositive, 6 at high titer, against pomona serotype and all pregnant cows were seronegative (Table 1). Two additional abortions occurred before the herd was treated on 8 March 1974 and none thereafter.

Discussion

In herds in which a diagnosis of leptospirosis is made early in an epizootic, the rationale for combined vaccination and treatment with dihydrostreptomycin is sound. This leptospirocidal drug destroys the organism in tissues of both the dam and fetus and effectively terminates the urinary shedder state. During the period the herd is protected chemotherapeutically, active immunity is stimulated by the vaccine.

The dramatic cessation of abortions in these herds after treatment would have more significance if the treatment regime had been controlled. However, securing the owner's permission to establish valid controls for this type of study is difficult, especially in light of the known efficacy of dihydrostreptomycin for leptospirosis. Alternate herds left untreated would not represent valid controls unless the stage of the epizootic at the time of diagnosis was comparable in both principal and control herds. Leaving one-half of the pregnant animals untreated likewise would not be suitable be-
cause cows vaccinated and treated would continue to be exposed to leptospi- 
spiras not present in a completely treated herd. Certainly in the present 
study, additional abortions would have been expected in herds SM, 
SC, and TS, because only about one-third of the animals samples from 
these herds were seropositive at treatment.

The value derived from controlling an outbreak depends chiefly on how 
many calves are saved. Based on current costs of dihydrostreptomycin, 
the cost of carrying a brood cow for one year, and the value of day-old 
calves, the salvage of 3 calves for every 100 cows treated would be eco-
nomical. Further benefits would be derived from promptly eliminating 
the disease from the herd and thereby protecting the subsequent calf crop 
from infection. One owner reported that his losses from scours among 
calves born after the brood cows were treated were nil, whereas the problem 
had regularly occurred in preceding years. Vaccination without treatment 
with dihydrostreptomycin of a herd undergoing an epizootic of leptospirosis 
is of questionable merit, because the procedures of assembling, confining, 
and vaccinating cattle increase their exposure to urine of infected animals. 
Such procedures are often followed by abortion storms 2 to 3 weeks later.

Obviously the attending veterinarian should make every effort to es-
tablish the extent to which the disease has spread before recommending 
chemotherapy and vaccination. In this regard, it is important that enough 
pregnant and aborted cows be sampled to determine the serologic status of 
the herd. Established guidelines for this purpose should be followed.6
CONTROL OF OUTBREAKS OF LEPTOSPIROSIS

Table 1. Clinical and serologic findings in Herd SM, which are typical of pomona leptospirosis in recently infected herds.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Condition</th>
<th>Serotypes*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pomona</td>
</tr>
<tr>
<td>1</td>
<td>aborted</td>
<td>160**</td>
</tr>
<tr>
<td>2</td>
<td>aborted</td>
<td>640</td>
</tr>
<tr>
<td>3</td>
<td>aborted</td>
<td>640&gt;</td>
</tr>
<tr>
<td>4</td>
<td>aborted</td>
<td>640</td>
</tr>
<tr>
<td>5</td>
<td>aborted</td>
<td>640&gt;</td>
</tr>
<tr>
<td>6</td>
<td>aborted</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>mummified</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>fetus</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>pregnant</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>pregnant</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>pregnant</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>pregnant</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>pregnant</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>pregnant</td>
<td>0</td>
</tr>
</tbody>
</table>

* Serums free of agglutinins against ballum, hardjo, hebdomadis, grippotyphosa and bataviae serotypes.

** Reciprocal of dilution positive on plate test.
REFERENCES

HOST ANIMAL EFFICACY STUDIES
USING A MULTIVALENT LEPTOSPIRA BACTERIN

Presented before the Leptospirosis Committee on Oct. 17th

Henry L. Strother

I. PURPOSE OF STUDY
A. The study was designed and performed by Affiliated Laboratories to investigate the potency and efficacy of a multivalent leptospira bacterin containing *L. pomona, hardjo and grippotyphosa* in cattle and swine. This included investigation of leptospiremia and the renal shedding status of the vaccinated and non-vaccinated control animals after challenge with *L. pomona, hardjo and grippotyphosa* challenge cultures.
B. The study was also performed as partial fulfillment to meet the requirements for licensure for production and sale of the bacterin.

II. MATERIALS AND METHODS
A. BACTERIN
The *L. pomona* fraction of the bacterin which was used to vaccinate the animals was tested and passed the current federal Standard Requirements for that product. There is no Standard of Potency for the *L. hardjo* and *L. grippotyphosa* fractions.

The recommended dose is 2cc injected subcutaneously or intramuscularly. Revaccination is recommended at 4 to 6 weeks and annually thereafter.

B. ANIMALS
The average weight of the cattle used in the study was 400 lbs with a range of 300-500 lbs. The average weight of the swin was 40 lbs.

C. VACCINATION
Twelve seronegative cattle and twelve seronegative swine were vaccinated at the beginning of the study, then revaccinated four weeks later as recommended. These animals were then bled monthly and individual animal serums assayed for agglutinating antibody by the macroscopic plate agglutination test up until the time of challenge.

D. NONVACCINATED CONTROLS
A short time prior to challenge, fifteen nonvaccinated control cattle and fifteen nonvaccinated control swine were screened for seronegativity against the three leptospira serotypes.

E. CHALLENGE
Six months following the first vaccination, a total of 54 animals were challenged. These animals were separated into groups corresponding to the challenge serotype which they received. Each group consisted of 4 vaccinated cattle, 4 vaccinated swine, 5 nonvaccinated control cattle, and 5 nonvaccinated control swine.

*Trade name: Leptomune-GHP*
The *L. pomona* group was challenged with *L. pomona* strain 11002-31A which had been obtained from the National Animal Disease Laboratories in Ames, Iowa. It had a previous history of being pathogenic in calves. An inoculum of 48 billion organisms was given to each of the cattle in a volume of 16 cc. Each of the swine received 18 billion organisms contained in 6 cc. The *L. pomona* inoculum was determined to be 4.74 hamster LD50 per cc.

The *L. grippotyphosa* group was challenged with *L. grippotyphosa* F4397 which was obtained from Dr. Lyle Hanson at the University of Illinois. An inoculum of 6 cc. containing approximately 49.2 billion organisms was administered to the cattle and 2 cc. containing approximately 16.4 billion organisms was administered as the swine challenge. The hamster LD 50 of the *L. grippotyphosa* challenge inoculum was 3.16 hamster LD50 per cc.

The third group was challenged with *L. hardjo* strain 836 which was also obtained from Dr. Hanson. An inoculum of 30 cc containing approximately 54 billion organisms was given to each of the cattle and 12 cc. containing approximately 21.6 billion organisms was given to each of the swine.

All challenges were given by the intravenous route.

**F. POST-CHALLENGE COLLECTION AND ASSAY OF SERUM SAMPLES**

Serum samples were collected from all animals immediately prior to challenge, then 15, 33 and 60 past post-challenge. All serum samples were individually assayed for agglutinating antibody by the macroscopic plate agglutination method. We realized that the microscopic agglutination test is more sensitive than the macroscopic test but because of the volume of samples, the application of the microscopic test would have been a nearly impossible task.

**G. BLOOD CULTURES**

Heparinized blood samples were collected from the challenged cattle and swine for leptospiremia determination from day 2 through day 13 after challenge. 0.25 cc of each blood sample was cultured in 5 cc of Ellinghausen medium, incubated at 30° C. and examined weekly for a total of 6 weeks or until leptospira were found.

**H. URINE COLLECTION AND ASSAY**

Urine samples were collected from the challenged animals for lepto spiruria determinations from day 16 post-challenge through day 60. 0.25 cc of urine was cultured immediately after collection in 5 cc of Ellinghausen medium. During this period a total of 11-12 samples were collected from each animal usually on every 3rd to 4th day.

**I. KIDNEY TISSUE COLLECTION**

Kidney tissue was obtained from each animal by an expert surgical team from the University of Illinois. Half of the animals were biopsied on day 45 post-challenge and the other half on day 55. Approximately 3 to 4 grams of tissue were obtained from each animal. A portion of each sample was homogenized and cultured in Ellinghausen medium for detection of leptospira and a second portion was presented for histopathological examination.
J. **CLINICAL OBSERVATIONS**

Daily observations for signs or symptoms of disease as a result of challenge were made. Temperatures were recorded daily and the animals were observed for signs of being off-feed or depressed.

III. **RESULTS**

A. **BLOOD ISOLATIONS (LEPTOSPIREMIA) Table A**

As can be seen, isolation of leptospira are fairly consistent in the controls of the *L. pomona* and *L. grippotyphosa* groups while the vaccinates of these groups were negative. We failed to culture *L. hardjo* from any of the controls or vaccinates.

B. **KIDNEY TISSUE ISOLATIONS — Table B**

As can be seen in this table, the kidneys of the controls were positive for leptospira fairly consistently, except for the *L. hardjo* group while the vaccinates were negative.

C. **HISTOPATHOLOGICAL EXAMINATIONS**

No significant histological changes in the cortex of the kidney which was attributable to leptospirosis were observed in vaccinated or non-vaccinated animals.

D. **SEROLOGICAL STUDIES**

1. **Pre-challenge Macroscopic Plate Agglutination Titers of Vaccinates**

All of the vaccinated animals gave a serological response to vaccination except there was no detectable seroconversion for *L. hardjo* in swine.

2. **Post-challenge Macroscopic Plate Agglutination Titers of Vaccinates and Controls**

The vaccinates and controls both had a high titer at 15 days post-challenge with the controls generally higher than the vaccinates. Then at 33 days, both the vaccinates and controls showed a drop in titer with the controls generally higher than vaccinates. At 60 days both vaccinates and controls had a sharp drop in titer with the vaccinates generally higher.

E. **CLINICAL OBSERVATIONS**

No significant manifestations indicative of disease caused by leptospiral serotypes could be observed in the vaccinates or controls after challenge.

IV. **CONCLUSION**

This study shows that the *L. pomona* and *L. grippotyphosa* fractions of the bacterins provided protection against leptospiremia and kidney infection in the vaccinates while viable leptospires were isolated from controls. The fact that no isolations of *L. hardjo* were made in the control animals indicates that the *L. hardjo* challenge culture had a low order of pathogenicity for cattle and swine and, therefore, the leptospiremia and kidney infection tests on the *L. hardjo* group of animals were inconclusive.

There was seroconversion in the vaccinates which was measurable by the macroscopic plate agglutination test in all the vaccinates except for
an *L. hardjo* seroconversion in swine. This may be explained by the fact that the test which was used for detection was not sensitive enough to detect the low titers which may have been detectable by the microscopic test. Further serological studies are being completed using a growth inhibition test as an indicator of protective antibody in the serum and will be reported at some future date.

Research & Development Department
Affiliated Laboratories Division
Whitmoyer Laboratories, Inc.
White Hall, Illinois 62092

9/30/74

Table A

<table>
<thead>
<tr>
<th>Animal</th>
<th>Challenge Serotype</th>
<th>Vaccinated Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td><em>L. pomona</em></td>
<td>0/4</td>
<td>2/5*</td>
</tr>
<tr>
<td>Cattle</td>
<td><em>L. grippotyphosa</em></td>
<td>0/4</td>
<td>3/5</td>
</tr>
<tr>
<td>Cattle</td>
<td><em>L. hardjo</em></td>
<td>0/4</td>
<td>0/5</td>
</tr>
<tr>
<td>Swine</td>
<td><em>L. pomona</em></td>
<td>0/4</td>
<td>3/5</td>
</tr>
<tr>
<td>Swine</td>
<td><em>L. grippotyphosa</em></td>
<td>0/4</td>
<td>1/5</td>
</tr>
<tr>
<td>Swine</td>
<td><em>L. hardjo</em></td>
<td>0/4</td>
<td>0/5</td>
</tr>
</tbody>
</table>

*No. of animals with leptospiremia/total No. in the group.*
### Table B

**Isolation of Viable Leptospires**  
*From Renal Tissue of Vaccinated and Control Cattle and Swine Following Challenge Exposure*

<table>
<thead>
<tr>
<th>Animal</th>
<th>Challenge Serotype</th>
<th>Vaccinated Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>L. pomona</td>
<td>0/3</td>
<td>3/5*</td>
</tr>
<tr>
<td>Cattle</td>
<td>L. grippotyphosa</td>
<td>0/4</td>
<td>3/5</td>
</tr>
<tr>
<td>Cattle</td>
<td>L. hardjo</td>
<td>0/4</td>
<td>0/5</td>
</tr>
<tr>
<td>Swine</td>
<td>L. pomona</td>
<td>0/4</td>
<td>3/4</td>
</tr>
<tr>
<td>Swine</td>
<td>L. grippotyphosa</td>
<td>0/4</td>
<td>1/4</td>
</tr>
<tr>
<td>Swine</td>
<td>L. hardjo</td>
<td>0/4</td>
<td>0/5</td>
</tr>
</tbody>
</table>

*No. of animals with renotropic infection/total No. in the group.*
Chairman: L. E. Hanson, Urbana, Illinois
Co-Chairman: H. G. Stoenner, Hamilton, Montana
S. L. Diesch, St. Paul, MN; R. L. Morter, Lafayette, IN; W. E. Lyle, Madison, WI; C. S. Roberts, Auburn, AL; S. F. Rosner, Jefferson City, MO; J. W. Glosser, Helena, MT; L. P. Thomas, Charleston, WVA; R. E. Smith, Amherst, MA; G. B. Smith, Kansas City, MO; J. R. Ragan, Nashville, TN; Larry Schaffer, O'Neill, NB; Rodney Larson, Fruitdale, SD; R. R. Smith, Cincinnati, Ohio; Bruce Walker, Jackson-ville, FL

The Committee reviewed developments in leptospirosis as presented in reports on diagnostic control and research activities of the previous year. The meeting agenda included: (1) the results of serologic testing of various species for various serotypes; (2) the significance of serologic titers; (3) reports of recent outbreaks from newly recognized serotypes; (4) the status of new bacterins; (5) control of leptospiral outbreaks in cattle, and (6) revision of the 1957 USDA special report on leptospirosis.

The summary of a leptospiral serologic survey was obtained from data from 20 diagnostic laboratories located in 18 states. The results were obtained with the microscopic agglutination (MA) test in 10 laboratories and with the macroscopic agglutination (Plate) test in 10 laboratories. Table 1 summarizes the compiled information on cattle, swine, horse and dog sera. The data on cattle and swine involved reports from all laboratories, the equine test reports were from 8 laboratories, and the dog test reports from 10 laboratories. An analysis of the summary of the test results indicates the predominant serotypes in cattle were pomona, hardjo, and grippotyphosa, in swine pomona, grippotyphosa, and icterohaemorrhagiae, in horses all the five serotypes except hardjo, and in dogs canicola, icterohaemorrhagiae, and grippotyphosa. Some regional variations were apparent as hardjo reactions were not reported from the two laboratories from the northeastern region.

The Committee discussed various problems related to serodiagnosis of leptospirosis. Inadequate sampling has been a problem in confirming the clinical diagnosis of leptospirosis in cattle and swine herds. Frequently field sampling has been limited to serum samples from only one or two animals that have aborted or shown other clinical signs associated with leptospirosis. The committee recommends that when leptospirosis is suspected as a herd problem the practicing veterinarian should submit serums from at least 10 animals or if greater, from 10 percent of the heard. The sampling should include serum from both affected and normal animals so that the extent and duration of the epizootic can be estimated. Samples should be accompanied by clinical histories from each animal tested.

Diagnostic laboratories are encouraged to use serotypes pomona, hardjo, canicola, icterohaemorrhagiae, and grippotyphosa in their test
systems. Those having the technical competence to safely cultivate leptospires and use living antigens are encouraged to use the microscopic agglutination test and the microtiter system which enables the more efficient application of the test. The long retrospect of the microscopic agglutination test should be considered in relating serologic findings to clinical diseases.

The proper interpretation of the significance of antibody titers was emphasized. Antibody titers of at least 1:40 on the plate test (Stoeuner antigen) or 1:100 on the microscopic agglutination test indicate past exposure to leptospires and the height of antibody titer bears little or no relationship to the shedder state. Some infected animals fail to develop antibodies, and some shed organisms longer than they remain seropositive. Hence, certification of individual animals as free of leptospirosis for interstate or international shipment without knowledge of the serologic status of the rest of the herd cannot be recognized as a sound control procedure.

An outbreak of leptospirosis due to serotype mini-szwajizak in an Oregon dairy herd was reported to the committee. This is the first cultural evidence of the existence of this serotype in the United States. Mini-szwajizak is the major serotype causing leptospirosis in man and dairy cattle in Israel. The major signs noted in this herd were infertility, stillborn or weak calves that died within two weeks and mastitis. Abortions were also noted, but they were not considered to be the major problem. The clinical problems and mortality rate were reduced subsequent to vaccination of the entire herd with hardjo and mini-szwajizak bacterins. Therefore the efficacy of the individual bacterins could not be determined. The same clinical problems are also occurring in two additional epidemiologically related herds. However, laboratory studies have not been attempted.

Status of new bacterins was discussed. In September, 1974 a one year license was granted to one pharmaceutical company to manufacture for sale a triple bacterin containing the pomona, grippotyphosis, and hardjo serotypes. A special report entitled “Host Animal Efficacy Studies Using a Multivalent Leptospira Bacterin” was presented by H. L. Strother and is published elsewhere in this proceeding. Since August, 1973 single hardjo and grippotyphosis bacterins have been available and utilized for prevention and control. These two single bacterins will now be replaced by the triple bacterin product. The product is only available from one veterinary biologic company.

Bacterins containing the icterohaemorrhagiae and canicola serotypes are licensed by several companies for use in cattle and swine. The pomona bacterin is available as a single antigen from several companies.

Utilization of bacterins is dependent upon identification of serogroup in the geographical area of herds under consideration. A report on the use of combined chemotherapy and vaccination for the control of outbreaks of leptospiral abortion in beef cattle was received by the committee. Brood cows in five herds in Idaho were vaccinated with leptospiral vaccine and simultaneously treated with 25 mg. dihydro-
streptonycin per kilo body weight. In every herd abortions ceased to occur shortly after treatment. The authors emphasized that this treatment regime is best applied to herds in which the disease is diagnosed during the early phase of an epizootic.

Although the committee recognized many of the previous goals in leptospirosis research have been accomplished, the following areas are in need of further research: (1) as serologic data indicate leptospirosis is an important disease of horses, and a more intensive investigation of the effects of the disease on reproduction in horses and determination of the efficacy of bacterins in the control of equine leptospirosis; (2) further evaluation of the efficacy of leptospiral bacterins utilizing available testing procedures; (3) further determination of the distribution of the serotypes tarrasovi, autumnalis and szwajizak in the United States and the pathogenesis in cattle, swine, and horses and (4) long term evaluation of dihydrostreptonycin therapy in cattle and swine.

The committee commends the US Department of Agriculture for the decision to revise the information release on leptospirosis. The committee has accepted the responsibility for assistance in the preparation of the new paper.
### TABLE I

**ALL SPECIES**  
**MICROSCOPIC AND MACROSCOPIC**  
**AGGULUTINATION TITERS FOR LEPTOSPIROSIS**  
**1974**

<table>
<thead>
<tr>
<th>Species</th>
<th>No. Sera Tested</th>
<th>hardjo</th>
<th>pomona</th>
<th>grippotyphosa</th>
<th>canicola</th>
<th>icterohaemorrhagiae</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>66,522</td>
<td>7.2</td>
<td>6.5</td>
<td>1.4</td>
<td>0.7</td>
<td>0.8</td>
<td>16.4</td>
</tr>
<tr>
<td>Swine</td>
<td>13,458</td>
<td>0.4</td>
<td>5.6</td>
<td>0.9</td>
<td>0.2</td>
<td>0.8</td>
<td>7.9</td>
</tr>
<tr>
<td>Horses</td>
<td>2,121</td>
<td>0.4</td>
<td>4.9</td>
<td>2.2</td>
<td>2.8</td>
<td>4.3</td>
<td>14.6</td>
</tr>
<tr>
<td>Dogs</td>
<td>296</td>
<td>0</td>
<td>0.7</td>
<td>1.7</td>
<td>7.7</td>
<td>4.4</td>
<td>14.5</td>
</tr>
</tbody>
</table>

* Most were conducted in 1973 but some diagnostic laboratories reported July to June tests for the 1973-1974 year.*
MOBILIZING EXTENSION FOR MASTITIS CONTROL

R. P. Natzke

I would like to begin this talk by giving you a little background in some of the work that has been conducted at Cornell University in mastitis control, then take a look at some of the reasons for going the extension route to get the information out and finally to describe what we are doing presently in Cooperative Extension. Beginning some eight years ago a cooperative project was started between the National Institute for Dairying, Reading, England, the Veterinary College and Animal Science Department at Cornell. This project was to be a follow up of earlier field studies conducted at the NIRD. Twenty-seven commercial New York dairy herds were selected for our portion of the study and 32 herds were used in the British study. One-half the herds were placed on a partial hygiene system which included wearing rubber gloves at each milking, dipping gloved hands in a sanitizing solution before moving from cow to cow, using individual towels for washing udders, using a sanitizing solution in the water, dipping all teats in a 4% chlorine solution immediately after milking and treating all quarters of all cows at the time of drying off with a million units of penicillin and 1 gram of dihydrostreptomycin in a slow release base. The remaining herds dipped teats using the same teat dip solution and treated all quarters of all cows at the time of drying off with the same dry treatment product. A rigid bacteriological sampling regime was followed, however, the results were never given to a dairyman. As a result, quarters were never treated on the basis of the infection present which allows us now to make recommendations to dairymen suggesting that they should be able to get equal results without the benefit of bacteriological testing. The average infection level of the herds in our study was 28.1 percent of the quarters infected at the start of the experiment and this was reduced to 7.1 percent quarters infected at the end. Cases of clinical mastitis were also reduced by 40 percent. The program worked equally well in reducing infection in the parlor as well as the stall barn milked herds. The herds on each of the hygiene systems had similar decreases in infection level. This indicates that although organisms are undoubtedly transferred by the operator's hand and the common rag or udder sponge, the teat dip is effective in killing the organisms which are transferred during the milking process and therefore no difference in infection rate occurs. All herds selected for the study were required to be on the New York DHIA milk recording system so that we would be able to register any changes in milk production. After comparing the change in milk production in the control herds to the all-New York State DHIA average and adjusting this figure for initial differences, we found that the project herds had an average increase of 1,051 lbs. of milk per cow over the three year period. With the price of milk at $8.50 per hundredweight and the cost of teat dip dry cow therapy and labor used for the program add about $8 per cow per year, one can quickly calculate that the dairyman receives an added income of about $80 per cow per year. We recognize that added feed was necessary to get this extra production, however, it must be kept in mind that other economic benefits also occurred such as less antibiotics were used, less
discarded milk, less veterinary costs, and less cow losses which are not figured into the $80 figure. It is obvious that the total benefit of the program is far in excess of the $80 per cow.

To insure that no long term detrimental effects would occur due to the project, some of the herds were continued for an additional three years. From this portion of the study it can be concluded that the infection level remained at approximately the level that it was at the end of the three year period. There was no increase in new coloform infection or in clinicals caused by them. Two herds discontinued the dry cow therapy - teat dip procedure and within two years had more infections than they started with. This points out the need for a continuous program.

We now had a program which was highly successful which worked in all herds and we were now at the point where the information had to get to the dairyman or it was of no value. There were several approaches of which we could have taken to try and make use of the information. The first of these would be to use it through the normal New York State mastitis control program. Many states have similar services and I have selected New York since I am more familiar with it. This program has been operating for 30 years in New York State and has four regional laboratories with a staff for collecting bacteriological samples identifying the infecting organisms, examining herd management problems and making recommendations for changes. While this staff is highly competent and makes dramatic improvements in problem herds it has made only modest inroads into solving the New York State mastitis problem. I suggest there are two reasons for this.

1. Only 300 to 800 new herds are contacted each year, the remainder of the work is used to resurvey herds already on the program or those in the process of eliminating Streptococcus agalactiae.

2. In the past the program was designed primarily as a Streptococcus agalactiae eradication program. The elimination of S. agalactiae from highly infected herds gives very dramatic improvement and in spite of warnings by the staff, the dairyman gets the impression that once the S. agalactiae is gone his mastitis problem is solved. It is therefore very difficult to get him motivated to use the teat dip-dry cow therapy procedures.

A simple calculation will illustrate why an extension program is probably the one of choice to get the work out to our dairymen.

Let us assume that through the control program we continue to contact 600 new herds a year and that the infection level in these herds is reduced by 75%. Let us also assume that an all out extension effort will get 50% of dairymen and that due to less individual consultation infection is only reduced 50%, then we come up with the following figures.

With the control program the average percent infected quarters for the "state herd" would drop less than 1% per year. However, with the extension program, the average percent quarters infected would drop by more than 6% or in other words, it would be more than 10 times as effective. Don't misunderstand me, diagnostic programs are essential but they are not the answer to getting information out to the masses.

We noticed that during the 3-year project many of the neighbors of the dairymen on the trial started to use the recommended procedures. A field demonstration project was designed to determine what percentage of dairy-
men are presently using the recommended procedures of teat dipping and dry cow therapy. All dairy agents were asked to interview 10% of the herds in their county with a minimum of 10 per county. The results of that survey showed that just under 50% of our dairymen are using the recommended procedures. This is much higher than figures found in other states. This difference is due to a strong extension effort over the last five years and the publicity which came from the original project. The second phase of the project will involve veterinarians, health department personnel, dairy plant fieldmen and the county dairy agent, so that all will see the results and be convinced of the value of the mastitis control procedure. One-half of the counties will be included in the demonstration project. This will allow us to determine by the end of the project if this technique is more successful in motivating dairymen than the traditional extension meetings.

Four-hundred herds will be individually interviewed and enrolled in the program if they are willing to begin using routine dry cow therapy and an effective teat dip. One hundred dairymen who are unwilling to use the procedure will be used as controls. The project will be carried out over a two-year period. The dairy plant will collect bulk milk samples for leukocyte counts every other month to determine the effect of the program in reducing udder irritation. The plant fieldman will record the number of cows and milk weights to enable measurement of any change in milk production per cow. The local dairy agent will visit each herd in his county to encourage the dairymen and to keep in touch with the progress. The veterinarians who service these dairymen will be supplied with recent research information on the comparative value of some antibiotic preparations for dry cow therapy. This information will be helpful to them in consulting with the project herds. The routine bulk milk samples will be used by the health department to determine if any change in the amount of antibiotic contaminated milk occurs over the two-year period.

Throughout the project the county dairy agent will be supplied with short articles outlining the program and giving information on the various facets of the control procedures. He will use this information for his local newsletter. Toward the end of the first year some dairymen enrolled in the project should be seeing some positive results. The dairy agent will then go out to the farm, take some pictures and collect information for "public interest" stories for his county newsletter. With this sort of coverage, we should be able to build up a large amount of local interest among the dairymen.

Upon completion of the project, a second survey will be taken to determine if there was an increase in the number of dairymen using the recommended procedures, and to see if this increase was greater in the counties which are involved in the demonstration project.

All data will be analyzed to determine the effect of the control procedures with limited supervision, on change in milk production and bulk milk cell counts.

We feel through this program we will get maximum motivation of dairymen with limited funds. It will give us an opportunity to make maximum use of the "multiplier effect" of one person telling the next.
THE ROLE OF DELAYED-TYPE HYPERSENSITIVITY AND CELL-MEDIATED IMMUNITY IN BOVINE STAPHYLOCOCCAL MASTITIS

Robert L. Jones, D.V.M.

Delayed-type hypersensitivity in Staphylococcal infection has been proven in mice, guinea pigs, and rabbits according to the criteria for such a reaction which are: 1) in vivo tests by intradermal injection of antigen causing an inflammatory response with onset near or after 3 hours and maximizing at 24 to 48 hours, 2) passive transfer of the reaction to nonsensitized animals by transfer of viable lymphocytes but not serum, 3) in vivo tests demonstrating the phenomena of macrophage migration inhibition. This reaction is presumed to occur in humans because of the response to skin test and the response to therapy.

Studies of Staphylococcal skin infections have shown that infection predisposes to reinfection. If an animal is infected with Staphylococci, subsequent inoculations do not have to be as large to produce infection. The initial infecting does not have to be large enough to produce a grossly observable lesion.

It has been shown that a delayed-type hypersensitivity reaction can develop to Staphylococcus and indeed does develop with a high incidence in many populations. This reaction can help explain why infection predisposes to reinfection. The initial infection sensitizes the animal to Staphylococcus and the upon subsequent contact with the Staphylococcal organism this delayed-type hypersensitivity reaction can occur. setting up on area of acute inflammation and allowing for Staphylococci to become established easier. The same predisposition to reinfection may account for the repeated flare-ups of Staphylococcal mastitis and the chronicity of the infection if delayed-type hypersensitivity does occur in the bovine.

Preliminary evidence for this delayed-type hypersensitivity reaction occurring in Staphylococcal mastitis is present. Viable leukocytes have been transferred from the udder of one cow to the udder of another cow and have produced a typical mononuclear cell reaction that occurs in chronic Staphylococcal mastitis and cell-mediated immune responses. The bacterial flora of the udders in the experiment was not identified. However, it was stated that 80% of the udders in the herd shed Staphylococci, so one would tend to assume that all udders were exposed, if not in fact infected.

The inflammatory response in chronic Staphylococcal mastitis is typical of a cell-mediated immune response. The Staphylococcus organism does not necessarily invade the tissue of the udder. The active disease process may be limited to necrosis of epithelium by the exotoxins. However, the host inflammatory response does involve the stroma of the udder where a very characteristic mononuclear cell infiltrate of the interalveolar tissue occurs. These mononuclear cells are mostly proliferating reticuloendothelial cells, but numerous pyroninophilic lymphocytes and plasma cells may also be found.

This inflammatory response has a unique resemblance to the normal involuting process of the nonlactating mammary gland. It appears that
it could be a reversible reaction resulting in little impairment of glandular function.

This report is concerned with the response to intradermal inoculation of Staphylococcal antigen and presents a therapeutic regimen presuming that delayed-type hypersensitivity is an important part of the pathogenesis of Staphylococcal mastitis.

The antigens used in this study were an autogenous heat-killed *Staphylococcus aureus* bacterin, an autogenous formalin inactivated *Staphylococcus aureus* bacterin, a Staphylococcal phage lysate*, and a commercial Staphylococcal bacterin-toxoid** containing whole cultures inactivated with formalin and adsorbed on aluminum hydroxide.

The heat-killed autogenous Staphylococcal bacterin produced nearly identical areas of inflammation in 14 calves and 10 cows. The formalin inactivated autogenous Staphylococcal bacterin and the Staphylococcal phage lysate did not produce grossly detectable inflammation in any of approximately 12 cows tested.

Fourteen 3 to 4 month-old Holstein calves were inoculated intradermally (ID) in the mid-cervical area with 0.1 ml. of Staphylococcal bacterin-toxoid. These calves have been fed raw whole milk, occasionally including mastitic milk. When examined 48 hours post inoculation, the folded skin thickness was increased approximately 2-3 mm. in all calves.

Twenty-five lactating cows were inoculated ID in the mid-cervical area with 0.1 ml. of Staphylococcal bacterin-toxoid. The inoculation site was examined at 48 hours post inoculation and the increased thickness in the folded skin was tabulated in Table 1. These cows were grade Holsteins in various stages of lactation and of varying ages. Mastitis has not been a severe problem in the herd and they have never been vaccinated with any type of Staphylococcal antigen.

Composite samples of milk from all quarters of each cow inoculated were cultured for bacterial growth and identification by standard microbiologic laboratory techniques.

Because of the uniformity of the reaction in the calves and a large number of cows, this data seems to indicate that a reaction less than 5mm. may be due to nonspecific irritation from the inoculation or the calves may already be sensitized because of the ubiquitousness of Staphylococcal organisms. A reaction from 5mm. to 10 mm. begins to show some evidence of a delayed-type hypersensitivity. Reactions greater than 10 mm. are undoubtedly indicators of delayed-type hypersensitivity.

Cows showing a smaller skin test reaction, yet positive on bacteriological culture may not have developed a delayed-type hypersensitivity since this response may not occur in all infected individuals. Some infected individuals may have an overwhelming infection that has rendered them anergic due to the massive antigenic stimulus that is constantly present.

For nearly 50 years, it has been considered that in some Staphylococcal

---

*Staphage Lysate-Types I and III, Delmont Laboratories, Inc., Swarthmore, Pennsylvania 19081

**Staphoid A-B, Jensen-Salsbery Laboratories, Division of Richardson-Merrell, Inc., Kansas City, Missouri 64141
infections there is an allergic reaction occurring and the patient must be desensitized\textsuperscript{19}. Attempts to pursue this course of therapy using an autogenous bacterin have met with varying results. There has been good success in many cases when a specific schedule of vaccine inoculation that was based on skin test reaction was followed\textsuperscript{20}. Even though this therapy frequently produced the desired clinical results, many clinicians have shown a reluctance to use it because of a lack of a satisfactory explanation for its action. It was not a desensitization of an immunoglobulin-mediated hypersensitivity as thought, but its effectiveness occurred when the bacterin was given in the proper dosage to elicit cell-mediated immunity.

There is no known method at present of desensitizing an animal with delayed-type hypersensitivity\textsuperscript{25}. Hyposensitization or blocking of the reaction causing temporary anergy may occur when the animal is persistently challenged with an overwhelming amount of the antigen. Delayed-type hypersensitivity cannot be abolished because it depends on sensitized T-lymphocytes which live for a relatively long time and pass the sensitized condition on to subsequent cells when they divide.

Therefore if cell-mediated immunity cannot be destroyed, we should attempt to make use of it. Repeated studies in mice and rabbits have established that delayed-type hypersensitivity, indicating the presence of cell-mediated immunity, occurs frequently in \textit{Staphylococcus aureus} infections. It has been shown that appropriate elicitation of this immunity with Staphylococcal antigens activates the reticuloendothelial system causing increased microbicidal activity in the macrophages providing an enhanced defense against \textit{Staphylococcus aureus}\textsuperscript{8,17}. Induction and elicitation with \textit{Staphylococcus aureus} has also been used for nonspecific protection in mice against vaccinia virus and mouse adapted swine influenza virus infections\textsuperscript{11}. There seems to be little doubt as to whether or not this process functions as described here. It seems that it definitely has value in therapy.

Based on these examples, elicitation with Staphylococcal antigen is being advocated in human therapy of surface \textit{Staphylococcus} infections\textsuperscript{2,10,11,12,13,25}. Although clinical efficacy has not been proven through controlled studies and demonstration of activation of the reticuloendothelial system, it seems reasonable to assume that it is working by this mechanism. Clinical results have been reported as 73\% excellent, 18\% improvement and 9\% failure\textsuperscript{10} and 80\% excellent, 18\% improved and 2\% unchanged in another report\textsuperscript{15}.

It seems that activation of the reticuloendothelial system could afford valuable defense against \textit{Staphylococcal} infection in domestic animals. Where other measures have failed to work in chronic staphylococcal mastitis this may be a new approach worth careful consideration. The cellular inflammatory response, passive transfer of the reaction with cells and the skin reaction strongly suggest the presence of sensitized T-lymphocytes. If elicitation could be effected, valuable results might be obtained.

The exact technique of elicitation and an understanding of how it is carried about have not been established. Proper stimulation of sensitized T-lymphocytes with antigen so that activation of macrophages results is called elicitation. The T-lymphocytes cause the activation of macrophages through mediators called lymphokines. Activated macrophages undergo morphologic and metabolic changes resulting in enhanced microbicidal
activity. Elicitation does appear to be dependent on four factors: 1) the antigen, 2) route administered, 3) dosage, and 4) timing of eliciting doses.

It has been found that the antigen responsible for delayed-type hypersensitivity is the peptidoglycan fraction of the cell wall which seems to be common to most Staphylococci. All that is necessary for an eliciting antigen is that it be homologous to the antigen to which some of the T-Lymphocytes have previously been sensitized. Therefore, almost any Staphylococcal bacterin containing cell wall material could be used for elicitation once a proper dosage schedule has been established.

While various routes of administration seem to be effective for elicitation and activation, preference should probably be given to the subcutaneous and intramuscular routes.

Dosage and timing are somewhat interrelated factors to consider in elicitation. If a sufficiently large dose of antigen to which the animal exhibits delayed-type hypersensitivity is given, the animal will become lymphopenic, hyperthermic and anergic. The fever begins in 1-2 hours and diminishes at about 10 hours. It may elevate as much as 5-9°C. The lymphopenia is transient and normal levels will return in the next few days. The complete anergy will be present for a few days and then be limited only to an anergic reaction to the antigen which caused this reaction. In spite of the lymphopenia and anergy that follows, this dose of antigen will have effected activation of the reticuloendothelial system which lasts only a few days. It must then be restimulated by elicitation. If the animal has been rendered anergic by the first dose of antigen, it will not respond to the second eliciting dose. Apparently a factor (or factors) necessary for the cell-mediated reaction have been depleted. This is probably related to the lymphopenia which occurred. Therefore, it is important not to give too large an eliciting challenge and that it not be given too frequently. There is a minimum dose of antigen that is required to cause elicitation. This minimum threshold is not met in the natural infection so artificial elicitation must be given. This eliciting antigen must be given at frequent enough intervals to maintain an activated population of macrophages. As the elicitation process progresses over a period of weeks, the dosage should be increased. This will not result in blocking because the sensitized lymphocytes have been proliferating so there are more of them that can be elicited thereby providing more activation of the reticuloendothelial system.

As already discussed, activation of cellular immunity has proven to be valuable in Staphylococcal infections in rabbits and mice and clinical results indicate value in humans. There is one report of clinical improvement in cats and dogs even though the mechanism was attributed to increased immunoglobulin production. In an earlier experiment in this study 4 cows with chronic Staphylococcal mastitis were challenged twice weekly for 6 weeks with an autogenous formalin inactivated Staphylococcus aureus bacterin. Clinical improvement occurred as evidenced by less udder inflammation, lower CMT scores and a temporary reduction of the culturally positive state.

Elicitation of Staphylococcal cell-mediated immunity should be considered as an integral part of any therapeutic program for chronic Staphylococcal mastitis in dairy cows.
A formula or standard for establishing an elicitation program has not been established. The inoculation schedule in Table 2 is presented as a possible program that can be modified as experience and increased clinical data dictate.

If the cow develops either local, focal, or generalized reaction to the inoculation, the dosage must be decreased. Cows having reacting exceeding 2 cm. increased skin fold thickness when tested with 0.1 ml. antigen ID should be inoculated with one-half the amount recommended in Table 2. Cows should be given an eliciting dose of antigen twice a week for 6 weeks. Thereafter a single weekly elicitation maintenance dose may be given in special problem cases, but will probably not be routinely necessary.

Unless there is a problem of repeated clinical flare-ups of mastitis during lactation, this therapy should be used during the dry period for two reasons. Since the normal involuting udder already has a mononuclear cell infiltrate in the interalveolar tissue\textsuperscript{16,21}, perhaps a more efficient function of these cells could be achieved through activation. Secondly, antibiotics must be infused into the udder at the same time and will necessitate withholding the lactating cow's milk from market. Since macrophages cannot migrate into the lumen of the udder to control bacteria growing in the milk, antibiotics should be used to control them and prevent them from re-establishing infection. The antibiotic must be one that the Staphylococci are sensitive to, therefore antibiograms are essential. It probably should be administered at the last milking before drying off, and again two weeks after the start of elicitation. Corticosteroids should not be used because they suppress cell-mediated immunity and cellular immunity\textsuperscript{26}.

This course of therapy should enable the host to overcome the infection, but it will not provide permanent protection from reinfection. This therapy is not a desensitization even though it may cause temporary hyposensitization. The population of T-lymphocytes continues to live bearing cell-mediated immunity. The presence of delayed-type hypersensitivity may actually predispose the animal to reinfection, so good milking hygiene must still be employed to combat reinfection.

<table>
<thead>
<tr>
<th>Increased folded skin thickness 48 hrs. postinoculation</th>
<th>Number of Cows</th>
<th>Number of positive S. aureus cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5 mm.</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>5-10 mm.</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>&gt;10 mm.</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

TABLE 1.

Results of ID inoculation of 0.1 ml. of Staphylococcal bacterin-toxoid and milk bacteriological examination.
TABLE 2.

Elicitation dosage and schedule of inoculations with Staphylococcal bacterin-toxoid.

<table>
<thead>
<tr>
<th>Week No.</th>
<th>ml. given SC or IM twice during week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>0.75</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>1.25</td>
</tr>
<tr>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>6</td>
<td>1.75</td>
</tr>
</tbody>
</table>

Maintenance dose 2.0 ml. given once per week if required

REFERENCES

REPORT OF THE MASTITIS COMMITTEE OF
THE UNITED STATES ANIMAL HEALTH ASSOCIATION

Chairman: N. B. Haynes, Ithaca, N.Y.
Co-Chairman: R. B. Bushnell, Davis, Calif.
B. R. McCallon, Adelphi, Md.; Rufus Weidner, Chicago, Ill.; D. N.
Stern, Amherst, Mass.; L. F. Williams, Storrs, Conn.; D. A. Kirkbride,
Brookings, S. Dak.; C. B. Dearborn, Concord, N.H.; E. L. Henkel, Sil-
verton, Oreg.; K. J. Peterson, Corvallis, Oreg.; J. S. McDonald, Ames,
Iowa, K. M. Weinland, Lafayette, Ind.; J. D. Kornder, Atlanta, Ga.;
R. A. Lilly, Austin, Tex.

The annual open meeting of the Committee convened at 1:30 p.m. on
October 15, 1974. Twenty-three guests and nine members were present.
In accordance with action recommended at the 1973 meeting the Chairman
reported contact with Food and Drug Administration regarding the poten-
tial contamination hazard with multiple dose infusion product containers
and with the U.S. Public Health Service regarding a possible link between
bovine and human infections with Streptococcus agalactiae. A response was
received from Dr. C. D. Van Houwling, Director, B.V.M., F.D.A., asking for
documentation of the multiple dose infusion product contamination hazard.
Documentation was provided.

MASTITIS CONTROL ACTIVITY

Reports on mastitis control activities in California, Connecticut, New
York, Oregon, West Virginia, and Wisconsin were given by members of the
Committee from those States. From this sampling it is apparent that State
programs range from those that are intensive and well coordinated to no pro-
gram per se except limited consultative service for herds with a serious mas-
titis problem. Data from New York where upward of 160,000 milk samples
are cultured annually points to a very close correlation between Strepto-
coccus agalactiae infection and abnormal milk.

UDDER INFUSION PRODUCTS

The Chairman reported briefly on the deliberation of the Infusion Prod-
uct Review Committee of the National Mastitis Council at its first meeting
in September. That Committee was concerned about the increased use of
home-formulated udder infusion products brought about in part by the
withdrawal of many proprietary products from the market. The efficacy,
safety and withdrawal times of such preparations is unknown. Should their
use result in antibiotic contamination of milk or meat the user would be lia-
tle to prosecution. From the ensuing discussion it was obvious that your
Committee shares that concern and it recommends that dairymen and vet-
erinarians be cautioned against using such products.

In this regard the Wisconsin Veterinary Medical Association makes
available to veterinarians backtags to be placed on cows given antibiotics
that can be labeled with the appropriate withdrawal time for milk or meat.
The California association uses a standard form for that purpose which is
given to the dairymen when antibiotics are used.
TEAT DIPS

The Committee then turned its attention to teat dips. Post-milking dipping of teats in an effective residual germicidal solution coupled with dry cow therapy has been proven to be an effective means of mastitis control. Many products are now sold as teat dips. Some are highly effective; others are ineffective and have, in fact, contributed to herd mastitis problems. At present, their safety and efficacy is not regulated. A Committee of the National Mastitis Council has established criteria for effective teat dips. A resolution reflecting the view of the Mastitis Committee with regard to teat dips follows at the end of this report.

STREPTOCOCCUS AGALACTIAE INFECTION

In terms of production loss and milk quality as measured by somatic cell count mastitis may be the most costly disease facing the dairy industry. One controlled study involving 27 herds and over 1600 cows showed that the reduction of mastitis through dry cow therapy and teat dipping alone resulted in a production increase of over 1,000 lbs. per cow annually. Mastitis is a complex disease with a multiple etiology. However, states where extensive culture work is done have found *Streptococcus agalactiae* to be the leading pathogen involved. This organism is amenable to detection, treatment and, ultimately, eradication. For five consecutive years your Mastitis Committee has recommended that the States undertake control of the unrestricted movement of cows infected with this organism. To date no discernible progress has been made and herds that have achieved *Streptococcus agalactiae* free status are in constant jeopardy of reinfection.

RESOLUTIONS

As a result of its deliberations the Mastitis Committee unanimously adopted the following Resolutions:

Be it resolved that the U.S. Animal Health Association recommends:

1. That the Bureau of Veterinary Medicine, F.D.A., in consultation with the National Mastitis Council and other appropriate authorities establish without delay criteria for effective teat dips and institute measures whereby teat dips shall be proven efficacious and safe prior to marketing.

2. Adoption, by each State, of a program for the eradication of *Streptococcus agalactiae* infection in dairy herds; and

3. That each State prohibit importation of pre-parturient and lactating dairy cattle unless such cattle are shown to be free of *Streptococcus agalactiae* infection.

This constitutes the report of the Committee on Mastitis. On behalf of the Committee I respectfully submit the report for approval by the Executive Committee.
THE IMPACT OF ELECTRONIC IDENTIFICATION AND DISEASE DETECTION ON THE ROLE OF THE VETERINARIAN

J. C. Hensley,* L. S. Cram,** G. C. Saunders,** and D. M. Holm**

SUMMARY
An interagency developmental activity at the Los Alamos Scientific Laboratory seeks application of physical sciences technology to disease detection and surveillance. High speed and sensitive serological methods and unique methods for virus differentiation by study of single cell effects promise rapid and automated disease screening. Electronic surveillance methods for temperature sensing and identification can offer disease control procedures now impossible. Application of this technology, much of which was not available as few as five years ago, can bring about revolutionary conceptual changes in disease detection and surveillance effecting the role of the veterinarian in animal health and regulatory veterinary medicine.

INTRODUCTION
As the world need for increased animal protein resources develops, so the need for preservation of our poultry and animal populations becomes imperative. Preservation of these resources can generally be accomplished by effective measures applied to two broad areas: disease control and eradication in animal and poultry populations, and development of new protein resources as animal feeds. Many research and development activities confront the latter objective and in recent years there have been developed alternative food resources for livestock from materials considered inedible.1,2 As we approach the "world food crisis," we find that resource depletion is not so much the problem, as development of alternative sources with which to produce animal and poultry protein resources for an ever increasing world population.

The need for increased productivity brings emphasis to the need for concentration of animal resources through "confinement feeding practices," new technology that has been most beneficial to the industry. This concentration furthers the need for improved disease control and detection and demands application of new and old technology.

The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), realizing the above; has sought out new resources from which to glean this technology. Through interagency agreements with the United States Atomic Energy Commission (AEC), disease surveillance and animal identification problems are approached through application of biological and physical sciences of the Los Alamos Scientific Laboratory (LASL). Through scientific interaction of USDA scientists and LASL scientists, and cooperation with other federal

**The Los Alamos Scientific Laboratory, Los Alamos, N.Mex.
agencies and industry; disease surveillance and animal identification have been approached on three broad fronts: cell/virus effect studies, serological test systems development, and remote electronic temperature monitoring and identification.

Although application of such technology offers no panacea to current disease control activities; each of these aforementioned areas of development stands alone as a breakthrough as tools-of-the-trade. They are presented here as individual activities, allowing the reader to speculate as to how they may function together in a practical manner to provide an integrated system for disease control and eradication in animal and poultry populations.

I. APPLICATION OF CELL ANALYSIS AND SORTING TECHNIQUES TO DISEASE DETECTION — (L. S. Cram)

The measurement of biological properties of cells in suspension through a technique known as “Flow Microfluorometry” (FMF) has been an ongoing activity at LASL for many years. Initial investigation centered about quantitative examination of cell suspensions for DNA cell-cycle changes. In cooperation with the USDA, APHIS, studies were undertaken to develop the technique as a diagnostic method for hog cholera during the 1970 hog cholera emergency. A method for quantitation of fluorescent conjugates evolved and has been used in practice for some time.

Light scatter measurements were made on HCV infected cells in an effort to delineate early markers indicative of viral infection. At large angles (20°), PK-15 cells infected with HCV were found to scatter two times more light than control or noninfected cells. LASL scientists were able to define light scatter parameters of HCV infected cells in an effort to better understand cell-virus relationships. With the onset of Velogenic Newcastle Disease Virus (NDV) outbreaks in California, attention turned to examination of NDV cell fusion as a measure of virus virulence as reported by Poste. The resulting application of the flow microfluorometry technique is published by Cram and provides a significant step forward in disease differentiation methodology. The technique has been developed to accelerate the differentiation of NDV strains. Current techniques require two weeks or longer for relatively good NDV strain differentiation while the LASL technique requires about 3-4 days. It is expected that a “cell fusion index” (CFI) will evolve which is directly related to virus virulence. The CFI is expected to assign cell fusion values which correspond to presently accepted lentogenic, mesogenic and velogenic differentiations.

A detailed protocol for assay of the degree of cell fusion is given by Cram in a previous publication and this protocol is presented graphically in Figure 1. The protocol is described as a simple easily controlled system for determining the virulence of NDV strains.

FIGURE 1

The sensitivity of the method is best described in introduction of actual data derived during technique development. B1 (lentogenic), and Roakin (mesogenic) plaque-purified reference strains were obtained from Veterin-
ary Services Laboratories (VSL) at Ames, Iowa, and were used to fuse cells. The results are illustrated in Figure 2. The degree of cell fusion produced by the two lentogenic strains is easily distinguishable and the reference Roakin strain (mesogenic) produced less fusion than did either of the two lentogenic strains. Although biochemical methods exist for distinguishing the two lentogenic strains, they are not suitable for field use. The response of the Roakin strain (less fusion although mesogenic) is understandable in that this reference strain has lost its pathogenicity for poultry through multiple isolations. The change in virulence of a vaccine or field strain can thus be determined by cell fusion assay, providing a valuable epidemiologic tool for the field.

FIGURE 2

Further examination of several field strains of velogenic virulence has provided confirming evidence of the reliability of the method. In Figure 3, four strains are presented as compared to controls (noninfected CEF), demonstrating the ease of virulence comparison for well known strains of the velogenic type. The large number of cells measured with the FMF method provides a sensitivity which allows for differentiation of very similar virus strains. The relative virulence of field strains can be determined, thus providing information on an increase or decrease of virulence during a given outbreak in a natural strain or mutant vaccine strain.

FIGURE 3

Although this particular technique has been developed in response to the Newcastle disease emergency, it will certainly evolve as a useful technology in other diseases where cell effects of this type are realized (i.e., rinderpest). As research reveals other cell-virus effects, new parameters will be of value in distinguishing virulent agents and sorting out the complexities of viral and perhaps bacterial disease epidemiology.

The technological tools exist, therefore, for a better understanding of virulence of an agent. Application of the technology calls for a concerted effort on the part of many scientists to bring about significant data collection, pointing the way to rapid diagnosis, agent differentiation, and epidemiological prediction.

II. DISEASE SCREENING THROUGH SEROLOGICAL TESTING
— (G. C. Saunders)

The detection of humoral antibody in screening sera for disease is a well accepted technique which employs a broad and diversified group of test systems. Many existing systems, though relatively successful, suffer from difficulties in both sensitivity and specificity. Therefore, a multiplicity of test systems have evolved, many capable of detecting response to a single antibody producing disease.

The *desirata* of the USDA/LASL interaction has been the development of a rapid, singular test system with adequate specificity and sensitivity for the detection of antibodies to a multiplicity of diseases; any disease which
elicit host/humoral immune responses. Sensitivity desired of such a system is that seen in radioimmunoassay systems without the inherent radiologic hazards of same. Reagents for such a test must be inexpensive, extremely stable, and simple to use. Further, the test should have the capacity for screening any given serum (less than 1 ml) for the multiplicity of diseases in a time period less than slaughter processing times. Also desired is a reagent system capable of screening all sera of a given species of animal with specificity and test sensitivity to not only infectious agents, but to a multiplicity of toxins, drugs, and metabolic by-products of toxic exposure. The method must be amenable to automation and high level output providing for individual testing of each carcass. The system must also possess characteristics such that a simple and reliable field test can be devised (card test). Certainly the objectives of such a program are euphoristic. Just as certainly, these goals are now to be realized in the evolution of a system known as the Enzyme-labeled Antibody test (ELA).

The Horseradish Peroxidase (HP) system is well known to those familiar with the field of electron-microscopy. Many quantitative serological tests have been developed utilizing the principle of HP conjugates. The ELA test seems the only HP system of record with the simplicity and characteristics required for a routine test.

The technique is similar to the indirect Soluble Antigen Fluorescent Antibody test (SAFA), a fairly well established system. Details of test protocol are available in previous publications. The protocol is simply outlined in Figures 4 and 5. Test results are easily interpreted as is illustrated in Figure 6 and present a literal “black and white” result on automated readout.

FIGURES 4, 5, 6

The test thus far has been successful in the detection of parasitic (trichinosis) disease, bacterial (brucellosis and tuberculosis) disease, and viral (hog cholera) disease. It has shown promise in detection of toxic metabolites (Staph enterotoxin) and has been used successfully in screens for multiple diseases on a single reaction filter disk (trichinosis, brucellosis, tuberculosis). The success with tuberculosis is considered particularly significant in that no serological test thus far has been recognized of value for TB testing. Although work thus far has been primarily in swine, there is reason to believe that similar success is to be expected in all species, including poultry.

Comparisons of sensitivity and specificity continue to show reliability in comparison to currently acceptable methods. Thus, we can now provide two elements of a comprehensive system for disease detection and differentiation. Although both are developmental, current data indicates that application of the technology is that lacking element to usefulness for animal disease control. The detection/differentiation system could be implemented as follows:

A. Eventual total screening of .3 ml blood samples from each animal slaughtered.

B. Where disease (viral, particularly) detection is difficult and differentiation is required, samples are collected for virus culture and single-cell differentiation.
C. All data logged by on-line computer terminal with readout. Continued development of the automated system can provide immediate computer logging and reporting, thus providing an easily retrievable immunological data bank for epidemiological evaluation. Specific virulence data in diseases such as Newcastle can be added to this data bank for provision of high probability automated epidemiological predictions. The same, or other serological system, would also be available for on site "card testing" for field diagnosticians and the data resulting could be added to a continually updated epidemiological survey by on-line telephone computer terminals. The lacking element to a complete system is that of disease alert without high error rate. This detection could be provided through utilization of an available technology. That, then, is the subject of the following section.

III. ELECTRONIC IDENTIFICATION AND TEMPERATURE MONITORING — (D. M. Holm)

The use of biotelemetry using battery powered sources is certainly not a novel art. The Agricultural Research Service’s (ARS), Southeastern Poultry Research Laboratory in Athens, Georgia, has pioneered unique developments in transmission of pertinent physiological data. Puckett, et al. have used such telemetry for controlled feeding of dairy cattle.

A relatively new area of activity utilizing passive (no batteries) transponders (receive and transmit) evolved during the latter stages of World War II. Radar impulses, beamed at a device likened to a mirror, return and collect information sensed by the device from its environment.

Thorough examination of the problem in animal identification revealed a yet unused potential of electronic microcircuitry, a product of the aerospace age. Scientists at LASL with assistance from APHIS staff, have developed a unique microcircuitry which provides both identification and temperature data remotely from a miniature passive transponder.

In simple terms, a radar-like beam is generated from a device called an interrogator/receiver. The beam is translated by the transponder such that the return (mirror reflection) beam contains information regarding identifying numbers and temperature of the environment of the transponder. Basic characteristics of the system are shown in Figure 7. Detailed explanations of the system at present and proposed utilization have been related by Baldwin, et al. Experiments currently under way seek to define the usefulness of the temperature monitoring capability in specific disease detection and identification in livestock. The temperature sensing element provides accuracy to ±1°Cs. It is expected that these types of data will be useful to husbandry and disease control.

FIGURE 7

In routine or emergency disease control, diagnostic information on which to base epidemiological forecasts is vital. The advance indications of herd disease development through constant monitoring of livestock at the time of slaughter is now accepted as a primary route. If disease is detected at slaughter or market points, the necessity for speed in traceback to a point of origin is vital. All of the above depends on the availability of an
adequate system for identifying animal populations. What then are the industrial requirements of a highly desirable animal identification system?

1. The system should provide desired information without a requirement for animal handling and accompanying stress.
2. The system should be accurate and collect computer-compatible data.
3. The system should be high speed, or less than one second for readout at meaningful ranges.
4. The system should handle a virtually unlimited number of digits and with capability for monitoring certain physiological data (temperature).
5. The system should be inexpensive, reliable, and passive (no batteries or wires).
6. The identifier should be a permanent unit, requiring for placement, handling of the animal but once in its lifetime.

Let us now consider the uses of such a system.

A. Sentinel Animal Disease Detection

With such a device implanted on each animal in a feedlot or confinement feeding situation, constant surveillance of the group for erratic temperature changes could be maintained. The interrogation system could be preset such that only temperatures in excess of "normal" for the species would be recorded along with an identifying number for the animal. This recorded information could alert an operator who could then segregate the diseased animal from the rest of the herd. Once separated, continued interrogation would provide a time record of temperature response. Data resulting could then be fed into a computer terminal (FM telephone) and thus to a national data bank. This data would then be logged, interpreted, and a disease prediction, recommended therapy, and other information returned to the feedlot operator or his consulting veterinarian. If the predicted disease is a regulatory or "foreign disease" significance, the data would be automatically transmitted to the appropriate epidemiological authority. In this manner, actual disease prediction data could be used to "forecast" an outbreak and provide a continual picture of that outbreak if required.

B. Market Animal Identification and Disease Detection

The system has many practical applications at the market points (stock yards, collection points, sales barns, etc.). If Farmer Jones decides to sell 12 head of cattle, sheep, or swine, the process can be virtually automatic. On reaching the unloading dock, temperature and digital information is automatically relayed to the national data center. Information is monitored by an inspector for immediate use, with a printout copy (and tape recorded copy) of data regarding that shipment. A copy is given to Farmer Jones and one retained by the sale barn officials. Should disease prediction result from this activity, the barn is notified by teletype and animals are confined and retained awaiting final disposition (i.e., slaughter only, test for disease, etc.). The sale proceeds; animals are monitored entering the sale ring, for instance, and monitored on exit as sold or retained. Using a buyer's credit card system, the new buyer (or slaughter establishment) is computer terminal identified, changing ownership automatically with necessary significant data on location, estimated time of arrival, etc. The seller is paid
based on computer printout and the buyer is billed. All functions, including commissions, can be completely automatic. The above is but one example of the system in operation. Although many complexities may result from improper ownership, collection buyers, etc., it is designed to protect all parties involved, the seller as well as the buyer. It would, of course, discourage irregular ownership transfer, theft and rebranding, etc.

C. Slaughter Establishment Inventory and Disease Detection

If we may continue our conjecture on the disposition of Farmer Jones's livestock, we would follow the several animals purchased by the slaughter buyer into the facility by computer terminal, thus completing the relationship with sales information. After monitoring for the required preslaughter period (in a manner similar to feedlot alert monitoring), if these animals have met prediction criteria for disease possibilities and are deemed “disease free,” the animal enters into slaughter with but cursory physical inspection and thus to the food market chain. Identification of the carcass and subsequent owner payment can be accomplished automatically.

D. Ownership Transfer

In the case of sale to a new owner through sale barns, etc., ownership transfer would be accomplished automatically through telephone terminals, as previously described. The one time implant would remain with the animal with information exchange only at a national data computer center. The use of 15 digits is envisioned (figure 8), which would provide all necessary data on a particular animal. The improper ownership transfer (now a problem) would be discouraged, as automatic billing and payment procedures would cause payment to the registered owners, not the actual improper owner.

FIGURE 8

IV. DISCUSSION

With little imagination, one can relate automatic disease detection methods (serological, cellular, or electronic), to a national system of identification.

Suffice it to say that we now have a system in development which can meet the detection criteria. Assuming detection of one or more diseases from sera taken at slaughter of Farmer Jones's livestock, we can immediately through the national data bank, identify the cattle as having originated on Farmer Jones's ranch and dispatch appropriate regulatory diagnostic personnel to that farm; issuing quarantines to prevent movement and identifying the sales market, collection points, feedlots, or other sites which were exposed to Farmer Jones's cattle. We have thus accomplished “epidemiological traceback.” In the case of highly infectious or “foreign disease” detection, the speed of this traceback is of essence. The design and implementation of a national system, integrated at all points and computer programmed to provide desired functions will require organization and planning, as well as total cooperation among stockmen, veterinarians, and state-federal regulatory personnel. We can thus visualize a system of dis-
ease control and eradication, population surveys, and market predictions of great value today. The technology exists and must now be methodically applied. Such a system will require:

1. Individual animal identification with a code of value to the industry (Figure 8).

2. Computer terminals at all points through which domestic livestock may pass.

3. A central computer facility linked to the above, which can handle such massive data.

4. Completion and fabrication of automated disease screening instrumentation linked to the above at all slaughter facilities and selected collection points.

5. Programming which will allow for ultimate use of the system on an international basis.

The veterinary profession can now look forward to a level of sophistication and technical professionalism heretofore impossible. The benefits of remote detection of temperature changes in animals are numerous and could, in time, extend to even the small animal practice. How then, can these new tools be utilized by the profession?

1. **Constant herd monitoring for disease detection:**
   a. Continued monitoring of temperature profiles in herds can provide data to computer banks which can eventually predict the onset of disease in a given herd considerably in advance of clinical signs.
   b. With the onset of disease, computer management of data provided by the veterinarian would assist in speedy diagnosis and reporting.

2. **Preslaughter disease prediction:**
   A group of animals (feedlot, etc.) monitored constantly over a two to three-week period prior to slaughter and exhibiting no significant temperature profile changes, could thus be taken to slaughter with little but cursory inspection, both antemortem and postmortem. Cost benefits to the industry are incalculable.

3. **Brood animal breeding programs:**
   The added capability of such a system to detect an animal when ready to breed (slight temperature decrease at ovulation) or the onset of labor (periodically marked temperature increase) could not only improve breeding results through time accurate insemination, but increase newborn survival to a degree that multiple benefits to both stockmen and veterinarians are realized.

4. **Dairy management practices:**
   Existing methods of dairy management by computer\textsuperscript{20} could be immeasurably improved through combinations of electronic identification and temperature monitoring methods. Automatic feeding of appropriate feed mixtures based on computer log predictions, day-to-day disease control, breeding, herd improvement,\textsuperscript{21} all are realistic applications which would save the dairyman, as well as the dairy-oriented veterinarian.
These are but a few of the potential applications of such a system and benefits to the industry, of which the veterinarian is a part. Applications in small animal medicine are equally numerous and can be equally advantageous. A new era in veterinary medicine is emerging of which we have described but a small part. The technology exists and must now be applied to bring the animal industry and veterinary medicine abreast of the aerospace age.

A CORRELATION HAS BEEN FOUND BY LASL SCIENTISTS BETWEEN DIFFERENT STRAINS OF NEWCASTLE VIRUS (A DISEASE IN CHICKENS) AND CULTURE CELL FUSION. THIS IS AN IMPORTANT LIVESTOCK DISEASE AND THE TECHNIQUES WILL LIKELY BE APPLICABLE TO OTHER DISEASES IN MAN AND ANIMALS. THE CONCEPT OF CELL FUSION IS ILLUSTRATED BELOW.

CELL FUSION VIRUS ASSAY

ADD LIQUID FROM SUSPECT ANIMAL TO CELL CULTURE AND INCUBATE

IF CERTAIN VIRUS IS PRESENT MANY CELLS FUSE TO FORM GIANT CELLS CONTAINING MULTIPLE NUCLEI

MAKE A SINGLE-CELL SUSPENSION AND STAIN THE NUCLEI WITH A FLUORESCENT DYE

READ FLUORESCENCE OF EACH CELL WITH FLOW MICROFLUOROMETER TO MEASURE THE AMOUNT OF CELL FUSION THAT HAS TAKEN PLACE.*

*FUSED CELLS WILL HAVE EXTRA NUCLEI AND WILL FLUORESC BRIGHTER THAN NORMAL CELLS

Fig 1.
Fig. 2. Channel Number (PROPORTIONAL TO CELL FLUORESCENCE)

- LaSota
- B₁
- Roakin
- C E F · 2 (3-36)
Different strains of the same virus cause different amounts of cell fusion in a particular cell culture. The pattern of cell fusion is varied with the type of cell culture (FL vs CEF).

These facts should make it possible to identify a particular strain of virus with high certainty. Since the government only pays indemnity for destruction of chickens infected with the Fontanna strain, it is very important to identify the strain. It also helps to trace the source of infection (epidemiology).

**Fig. 3.**

**LASL MULTIPLE DISEASE SCREENING SYSTEM**  
(Proposed)

**Fig. 4.**
1. A CONJUGATE IS A MATERIAL THAT WILL ADHERE TO AN ANTIGEN-ANTIBODY COMPLEX AND HAS "TAG" OF A DYE, AND ENZYME OR RADIOACTIVE ELEMENT ASSOCIATED WITH IT.

2. THE SUBSTRATE IS A MATERIAL THAT UNDERGOES A COLOR CHANGE IN THE PRESENCE OF THE ENZYME.

Fig. 5
A SMALL PRINTED CIRCUIT (TRANSPONDER) WITH DIGITAL CODING IS PLACED IN A SECURE POSITION ON THE ANIMAL. AN INTERROGATOR ENERGIZES THE TRANSPONDER WHICH THEN TRANSMITS THE IDENTIFICATION AND TEMPERATURE BACK TO THE RECEIVER. THE SYSTEM HAS THE FOLLOWING PROPERTIES:

1. PASSIVE (NO BATTERIES OR WIRES)
2. NEARLY UNLIMITED NUMBER OF DIGITS
3. INEXPENSIVE
4. HIGH SPEED (MUCH LESS THAN ONE SECOND TO READOUT)
5. ACCURATE (REQUIRES THE SAME INFORMATION TWICE IN A ROW TO REGISTER)
6. LONG RANGE (CAN EASILY READ AT 30 FT. DISTANCES)
7. COMPUTER COMPATIBLE (FOR RECORD KEEPING AND QUICK ACCESS)
8. RELIABLE (SAME ELEMENTS AS IN MODERN COMPUTERS)

ANIMAL IDENTIFICATION
SUGGESTED ANIMAL IDENTIFICATION CODE

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>OWNER OR HERD</th>
<th>STATE OF ORIGIN</th>
<th>ANIMAL</th>
<th>YEAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>999</td>
<td>999999999</td>
<td>9999</td>
<td>9</td>
</tr>
</tbody>
</table>

Fig. 8.

REFERENCES


16. J. C. Hensley, "Observations on the Physiological and Pathological Effects of FAE Warfare Exposure to Sub-human Primate Subjects; both Behaviorally Trained and Untrained; a Study in Depth." Report to Naval Weapons Center, Pasadena, CA-GSR1, (February 1970).


REPORT OF THE COMMITTEE ON ANIMAL IDENTIFICATION

Chairman: S. H. Flora, Brownsville, Tex.
Co-Chairman: J. Ralph Bishop, Tipton, Ind.


An active meeting of the Animal Identification Committee was held Wednesday, October 16, 1974, in the Shenandoah Room of the Hotel Roanoke, Roanoke, Virginia.

Dr. Ralph C. Knowles presented a resolution forwarded by the Committee on Infectious Diseases of Horses to implement horse identification on a State basis; after discussion and slight modification the resolution passed.

Horse Identification Resolution

Inasmuch as large numbers of horses move interstate, intrastate and internationally, and it is desirable that a voluntary uniform system of identification be established to assist in the control of intrastate and interstate and international movement of horses relative to infectious diseases; to help minimize theft of horses; and further to minimize the promiscuous substitution of similarly marked horses in exhibitions, sales and at horse race tracks; be it resolved that the U. S. Animal Health Association recommend to all States that a system of horse identification similar to the New Mexico passport be instituted.

Such a system should operate in a manner similar to that presently used for automobiles in the United States. The Passport description of an individual horse should include sex, height, weight, body color, color markings, size and location of chestnuts and location and number of whorls. Also, if marked by brands or scars, these should be included. This passport should be kept with the horse. In the case of sale or transfer of ownership, a new passport should be issued. It is recommended that such passports have reciprocity among States comparable to automobile registrations.

The Committee heard a report on progress from Los Alamos Scientific Laboratory presented by Dr. J. C. Hensley and Dr. D. M. Holm relating to ongoing experiments to develop electronic identification for animals.

Dr. E. C. Roukema of USDA, APHIS, reported that there are indications that we may be advancing to a higher level of surveillance and traceback capability. July reports indicate 15 percent over-all increase over July 1973 in brucellosis sample collection under the MCI program. However, while the
over-all number of animals identified with backtags at slaughter increased, the ratio of backtagged cattle versus non-backtagged cattle sampled decreased. This points up the need to enforce the backtagging requirements both for interstate and intrastate movement to slaughter. States are encouraged to support this effort not only through assistance in enforcement of Federal regulations, but through the enforcement of similar requirements for intrastate movement through existing authorities or where necessary acquiring such authority. Where basic authority exists, as it does in many States, implementing regulations or guide lines may be necessary. Records are, of course, essential to an effective identification system. Frequently, the initial movement is from farm of origin to a dealer within a single State. Dealer licensing laws requiring that records be maintained covering the identification and source of all animals handled are extremely important.

Swine Identification

The Committee voted to support the Brucellosis Committee’s Resolution to urge the USDA to promulgate a regulation requiring identification of all sows, boars and stags going to slaughter. The Committee voted to reaffirm their belief in the need for swine identification indicated by their resolution directed to the Secretary of the United States Department of Agriculture to carry out the further research and development needed to assist in the eradication of tuberculosis, brucellosis, hog cholera, and trichinosis as well as other diseases in swine. Veterinary Services, USDA, require all swine moving in interstate commerce be uniformly identified as to herd of origin at or before the time they reach the first concentration point. “Trash”, “Junk”, or cull swine that might be moved in commerce to further feeding shall be so marked that they are clearly identified and moved to immediate slaughter or destroyed. Each state is urged to take companion action to identify swine in intrastate commerce.

Respectfully submitted,

Lee S. Garner
Chairman

J. W. Bishop
Co-Chairman
FOOT-AND-MOUTH DISEASE IN WHITE-TAILED DEER: CLINICAL SIGNS AND TRANSMISSION IN THE LABORATORY


From the Plum Island Animal Disease Center, Northeastern Region, Agricultural Research Service, United States Department of Agriculture, Greenport, New York 11944.

"Mention of a trademark or proprietary product does not constitute a guarantee or warantee of the product by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable."

Acknowledgements

The authors are grateful to Dr. Frank A. Hayes, Director, Southeastern Cooperative Wildlife Disease Study, Athens, Georgia who supplied the deer: to Drs. L. F. Jennings and C. J. Issel for development of management techniques; to Mr. William Parrish and Mr. Daniel Zaveski for their valuable technical assistance; and to Mr. H. Mazzaferro, Jr. and the entire group of animal caretakers for the special effort required for this project. The authors are further indebted to Mr. Howard A. Baldwin, Sensory Systems Laboratory, Los Alamos, New Mexico; Dr. Bailey W. Mitchell, USDA, ARS, Southeast Poultry Research Laboratory, Athens, Georgia; and Mr. Joseph L. Riley, USDA, ARS, National Animal Disease Center, Ames, Iowa, for their valuable counsel and assistance in establishing the radiotelemetry systems.

Summary

White-tailed deer \textit{(Odocoileus virginianus)} were shown to be susceptible to infection with FMD virus type O. The clinical syndrome was intermediate in severity between that seen in cattle and that in sheep and goats similarly exposed. The disease was transmitted by contact from deer to other deer, from deer to cattle, and from cattle to deer. One deer remained a carrier of the virus for 11 weeks after infection.

Introduction

Control of an extensive epizootic of foot-and-mouth disease (FMD) in California between 1924 and 1926 was complicated by the extension of the infection into the deer herd of the Stanislaus National Forest. The disease was first diagnosed in the deer in July 1924 and by September 1925 22,000 deer had been slaughtered, 10\% of which showed lesions typical of FMD. Susceptibility of many species of deer to FMD has been well documented, but there are no reports on the clinical syndrome and transmissibility of FMD in native North American deer. We report here on these aspects in white-tailed deer \textit{(Odocoileus virginianus)} under laboratory conditions.

Materials and Methods

Test Animals. The test group consisted of six 2- to 3-year old adults.
and four 6- to 10-month-old fawns. All were housed in isolation units described elsewhere. Feed consisted of commercial calf starter and 16% dairy ration plus free access to hay and alfalfa pellets. Trace mineral salt was available at all times.

The deer were always given zylazine* intramuscularly before they were handled. The dose used was subject to individual variation but in general ranged from 0.35 to 0.75 mg/kg. Within this dose range the animals usually became recumbant within 2 to 3 minutes and could be examined and sampled with relative ease. Most animals regained their feet in 1 to 3 hours and showed no effects of the drug by the next morning.

_Virus._ FMD virus type O, subtype 1, strain CANEFA-2 of bovine origin was used in all experiments as the sixth passage in primary bovine kidney (BK) cell cultures.

_Sampling Procedures._ Samples of oesophageal-pharyngeal fluid (OPF) were taken with a probang as described for use in sheep and goats. The OPF was rinsed from the instrument with tissue culture fluid containing antibiotics** and 2% normal equine serum. Blood was taken from the jugular vein and heparin was added to that part reserved for virus assay. The rest was allowed to clot at room temperature, and the serum was aspirated. Epithelium from vesicular lesions was suspended in the same tissue culture medium as that used as a diluent for the OPF. Samples of OPF, heparinized blood, and epithelium were stored at -20°C until assayed.

_Virus Assay._ Samples were assayed for virus infectivity in secondary BK cell cultures; infectivity titers were reported as plaque-forming units (pfu) per sample for OPF and epithelial material and pfu/ml for blood.

_Serum Assay._ The neutralizing activity of serums was determined by means of a plaque reduction neutralization test as previously described.

_Temperature Telemetry._ Whenever possible, body temperatures of the unrestrained deer were monitored continuously by means of radiotelemetry. The temperature near the tympanic membrane was sensed by a thermister placed deep in the ear canal, and the information obtained was transmitted outside of the isolation unit by a small radio transmitter attached to the animal. The signal was received on commercially available equipment, transformed into meaningful form and plotted. Temperature was sensed, transmitted, and transformed by a variety of methods the details of which will be found in publications by Howard A. Baldwin, Bailey W. Mitchell, and Joseph L. Riley. Certain minor modifications were made because different combinations of equipment were used, but the basic principles remained the same.

_Experimental and Observations_ During these experiments, 2 deer (1 adult and 1 fawn), which had been reserved for later exposure to agents of other exotic diseases, died suddenly having had no virus exposure. Necropsies revealed these animals to have, in addition to other gross pathology, oral lesions that were indistinguishable from those seen in deer known to be infected with FMD virus. No agent was

---

*Rompun, Chemagro, Kansas City, Mo 64120

**2,000 u sodium penicillin G, 2 mg dihydrostreptomycin, and 25 u nystatin (Mycostatin, Squibb and Sons, 745 Fifth Ave., New York, N.Y.) per ml.
Figure 1. Body temperature of an adult white-tailed deer (Deer No. 1) after the intranasal instillation of $10^3$ plaque-forming units of foot-and-mouth disease virus type O.
Figure 2. Body temperature of a white-tailed fawn (Fawn A) after exposure to a steer infected with foot-and-mouth disease.
Figure 3. Body temperature of a steer after exposure to four white-tailed deer (2 adults and 2 fawns) infected with foot-and-mouth disease.
REFERENCES

APPLICATION OF IMMUNO-PEROXIDASE TECHNIQUES FOR THE DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS ANTIGENS

P. Sutmoller* and K. M. Cowan

From the Plum Island Animal Disease Center, Northeastern Region, Agricultural Research Service, United States Department of Agriculture, Greenport, New York 11044.

"Mention of a trademark or proprietary product does not constitute a guarantee or warantee of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable."

As yet immuno-peroxidase (PO) techniques have not been extensively used in veterinary diagnostics or in veterinary research even though their application has certain advantages over immuno-fluorescence (FA) techniques. For instance, PO staining is permanent and can be observed with a regular light microscope. Counter staining of PO stained preparations is possible by common histological techniques and may greatly facilitate the location of antigen in the cells.

Immuno-peroxidase techniques can be utilized much the same way as the FA techniques in either direct or indirect staining. At the Plum Island Animal Disease Center (PIADC) we have successfully applied both techniques to locate foot-and-mouth disease virus (FMDV) in infected cell cultures. For the direct technique, antivirus antibody is labeled with horseradish PO and reacted with virus antigens. With the indirect technique, virus antigens are reacted with unlabeled antibody followed by a reaction of the virus antibody complex with PO labeled anti-immunoglobulin against the species from which the anti-virus antiserum originated. In both cases, the PO is observed as a brown reaction product resulting from the enzymatic action of PO on peroxide and 3,3' diaminobenzidine. A third immuno-PO technique was described by Dougherty et al which is a modification of the technique of Sternberger et al. With this technique, soluble peroxidase anti-peroxidase complexes are linked to immunoglobulin bound to virus antigens through an anti-immunoglobulin serum. This technique is sensitive and highly specific. Moreover, only one anti-immunoglobulin serum and one PAP preparation are needed to test different antigens provided that antiserums against virus can be obtained from the same species as used for the preparation of the PAP.

Techniques

Direct conjugation of antibody with PO: Globulin fractions were obtained by the precipitation of serum by ammonium sulfate and labeled with horseradish PO as described by Kawaoi and Nakane who developed an

*Present address:
Pan American Foot-and-Mouth Disease Center
Caixa Postal 589 ZC 00, 20.000 - Rio de Janeiro,
GB - Guanabara, Brasil
improved method of labeling antibody with peroxidase. Basically they oxidized the carbohydrate moiety of the peroxidase resulting in peroxidase-aldehyde which then was reacted with the globulin. With this method approximately 95% of the globulin could be PO labeled.

Preparation of PAP: The PAP complex was prepared essentially as described by Sternberger et al. and Dougherty et al. Anti-peroxidase globulins obtained from guinea pigs were precipitated with PO at equivalence. The equivalence region was determined in the following manner: Equal amounts of undiluted serum and of buffer with decreasing amounts of PO were reacted overnight at 4°C as outlined in Table 1. As shown, maximum precipitation appeared in Tube 4. A further check for equivalence was made by Ouchterlony Analysis using the supernates of the tubes (Table 1) in the outer wells and 100 μg/ml of PO in the center well (Fig. 1). The result can be interpreted as follows: Antigen excess in Tubes 1, 2, 3, slight antigen excess in Tube 4, and antibody excess in Tubes 6 and 7. Equivalence was obtained in Tube 5. The serum control was Tube 9. On the basis of these results, 2 mg of PO in 1 ml was added to 6.7 ml of heat inactivated and clarified serum to give approximately 300 μg PO/ml. This mixture was reacted overnight at 4°C and then centrifuged at 10,000 rpm for 45 minutes in a Sorval RC-2B centrifuge. The supernatant fluid was removed, and shown to be free of either antibody or antigen excess. The precipitate was resuspended in 12 ml of phosphate buffered saline (PBS) at 4°C and centrifuged as above. This washing procedure was repeated a total of 3 times. The washed precipitate was resuspended in 1 ml of PBS and then dissolved by mixing with a large excess of PO (2 mg PO in 3 ml). This mixture was left overnight at 4°C and most of the precipitate went back into solution. In order to improve the homogeneity of the solution, the pH was adjusted to 2.4 with approximately 10 drops of 1 N HCl and immediately to 7.1 by the addition of 1 ml of PBS (0.2 M, pH 8.0) and about 30 drops of 0.1 N NaOH. This mixture was clarified by centrifugation for 10 minutes at 5,000 rpm. The supernatant fluid was collected and PBS added to make 7 ml. This crude PAP preparation was mixed with 7 ml of chilled saturated (NH₄)₂SO₄, left in an ice bath for 1 hour and centrifuged for 30 minutes at 7,000 rpm. The supernatant fluid was removed and discarded. The precipitate was resuspended in 25 ml of chilled one-half saturated (NH₄)₂SO₄ and centrifuged as above. The precipitate was dissolved in 5 ml of saline (0.15 M NaCl) and dialized overnight at 4°C against 4 liters of saline. Dialysis was continued for another day against 4 liters of new PBS (0.15M NaCl, 0.01 M phosphate, pH 7.6). Precipitate formed during dialysis was removed by centrifugation for 30 minutes at 5,000 rpm. The resulting “purified” PAP was stored in small aliquots at -70°C.

Applications

Cell cultures stained 3 hours and 24 hours after infection with FMDV respectively, will be used to illustrate the use of the PAP technique. Three different cell systems were used: Secondary bovine kidney (BK) cells; a line of pig kidney (PK) cells; and, a baby hamster kidney (BHK-21) cell line. These cells were grown in plastic plates with 6 wells of 35 mm diameter. (Linbro Chemical Company, New Haven, CT 06511). Some cultures were
given 10^7 pfu of FMDV type 0 (5 pfu/cell) and others were infected with approximately 50 pfu/well (about 1 pfu/40,000 cells). The plates were held in a CO incubator at 37°C for 30 minutes. The cultures were then overlayed with 0.6% gum tragacanth in culture medium and further incubated at 37°C. The cells were fixed with absolute ethyl alcohol after removal of the overlay and rinsing with PBS. The cultures with high multiplicity of infection were fixed after 3 hours incubation and those with a low multiplicity after 24 hours. The PO staining of FMD viral antigens has been described earlier. Briefly, fixed cultures after rinsing with PBS were flooded with antiserum specific for virus coat protein or for virus-infection-associated (VIA) antigen. The antiserums were used as dilutions in PBS of 1/200 and 1/40 respectively. Next, rabbit anti-guinea pig globulin serum in a 1/25 dilution was added followed by PAP of guinea pig origin in a 1/50 dilution. The culture dishes were shaken every 5 minutes for 20 minutes to redistribute the reagent over the cell sheet. Between each reaction the cultures were rinsed by 4 changes of PBS of 3 minutes each. The final step was the addition of freshly prepared PO indicator consisting of 10 mg of 3, 3' diaminobenzidine tetrahydrochloride in 10 ml of 0.01% hydrogen peroxide in PBS. The whole staining procedure was done at room temperature. When the staining was fully developed, usually within 10 minutes, the culture dishes were submerged in a 2% formalin solution. The cultures were then dehydrated with ethyl alcohol and if desired, counterstained with 0.5% methylene blue and covered with a film of immersion oil to facilitate observation with a light microscope. For each cell system, 2 cultures were stained with a crystal violet solution for plaque counts. There were 40-50 plaques of 2-3 mm in the BK cell cultures but only 2-3 small plaques in the PK cell cultures. In the BHK cell cultures there was evidence of approximately 10 plaques per culture; however, macroscopically those plaques were very indistinct.

Figures 2, 3, and 4 illustrate than many PK and BHK cells produce VIA antigen and virus protein 3 hours after the infection in spite of poor plaque formation. This phenomenon was also observed in infected serially fixed BK cultures stained by the PAP technique (manuscript in preparation). These figures also show that particularly with the PK and the BHK cells, more cells stained for VIA antigen than for Virus protein. Figure 5 illustrates the differences in cell involvement with the different cell systems as they relate to plaque morphology. Plaques of the BK cultures (5 A) had complete destruction of the cell sheet in the center. This area was surrounded by a zone of cells in various stages of virus development. The plaques in the BHK culture (5 B) had some loss of cells in the center but characteristically many virus containing cells were intermingled with unstained normal looking cells causing the fuzzy appearance of the BHK plaques. The PK culture (5 C) shows a plaque with a small center of rounded cells. Quite distant from this little defect in the cell sheet there were several virus containing cells surrounded by normal appearing unstained cells.

The PAP staining technique also can be used to determine whether an antigen is intra- or extracellular. Fixing of cell cultures with formalin or gluteraldehyde was reported to interfere with the penetration of the staining reagents through the cell membrane. Dougherty, Marucci and Distefano showed that the reagents used in the PAP technique did not penetrate the
live unfixed cells. They used this observation for differentiation between extra- and intracellular antigen.

The PAP method was chosen to illustrate the application of immunotechnique but both the direct and the indirect method have been used successfully in this laboratory. The modification of labeling antibody with PO as described by Kawaoi and Nakana was found to be very satisfactory.

Other workers have used PO techniques for the localization of viral antigens in animal tissues. However, the natural PO activity of some cells requires cautious interpretation of the results. A further illustration of the usefulness of the immunoperoxidase technique is the application of PO techniques for the measurement of hog cholera antibody by Saunders and Wilder.

Fig. 1. Determination of equivalence by Ouchterlony Analysis using the supernatant of the tubes of Table 1 in the outer wells and a dilution of peroxidase in the center well.

Fig. 2. Pig kidney cell cultures 3 hours after infection with FMDV, stained with the PAP technique. Left: anti-VIA antigen serum. Right: antivirus-protein serum.
Fig. 3. Baby hamster kidney cell cultures 3 hours after infection with FMDV, stained with the PAP technique. Left: anti-VIA antigen serum. Right: antivirus-protein serum.

Fig. 4. Bovine kidney cell cultures 3 hours after infection with FMDV, stained with the PAP technique. Left: anti-VIA antigen serum. Right: antivirus-protein serum.
Fig. 5 Plaques in a bovine kidney cell culture (A), a baby hamster kidney cell culture (B), and a pig kidney culture (C) after staining with the PAP technique. The pig kidney cells were counterstained with methylene blue.

Table 1. Determination of the Approximate Equivalence of Guinea Pig Anti-peroxidase globulin and Peroxidase.

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td>Buffer</td>
<td>-</td>
<td>0.02</td>
<td>0.04</td>
<td>0.06</td>
<td>0.08</td>
<td>0.09</td>
<td>0.095</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>PO,1 mg/ml</td>
<td>0.1</td>
<td>0.08</td>
<td>0.06</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Volume in ml.
**TABLE 2. Detection of Foot-and-Mouth Disease (FMD) Virus in the Oesophageal-Pharyngeal Fluid (OPF) of White-Tailed Deer Infected with FMD Virus**

<table>
<thead>
<tr>
<th>Test Animal</th>
<th>Weeks after inoculation or exposure</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deer 1*</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Deer 2*</td>
<td>Died</td>
<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>Deer 3**</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Deer 4**</td>
<td>Died</td>
<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>Fawn A**</td>
<td></td>
<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>Fawn B**</td>
<td></td>
<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>Deer 5***</td>
<td>Died</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>DIED</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deer 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>
<td></td>
</tr>
<tr>
<td>Fawn C***</td>
<td>Died</td>
<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>Fawn D***</td>
<td></td>
<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>

*Intranasal inoculation

**Contact exposure with infected deer

***Contact exposure with infected steer

+ = Virus infectivity in untreated OPF; ± = virus infectivity only in samples of OPF treated with fluorocarbon; - = no infectivity detected.
TABLE I. Viremia Studies of White-Tailed Deer Infected with Foot-and-Mouth Disease (FMD) Virus

<table>
<thead>
<tr>
<th>Test Animal</th>
<th>Days after inoculation or exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 4  5  6  7  8  9  10</td>
</tr>
<tr>
<td>Deer 1*</td>
<td>4.7  &lt;0.4</td>
</tr>
<tr>
<td>Deer 2*</td>
<td>6.8***</td>
</tr>
<tr>
<td>Deer 3**</td>
<td>4.0  5.8  &lt;0.4  &lt;0.4</td>
</tr>
<tr>
<td>Deer 4**</td>
<td>&lt;0.4  4.6  5.7  &lt;0.4</td>
</tr>
<tr>
<td>Fawn A**</td>
<td>4.5  &lt;0.4  &lt;0.4</td>
</tr>
<tr>
<td>Fawn B**</td>
<td>6.0  &lt;0.4  &lt;0.4</td>
</tr>
<tr>
<td>Deer 5***</td>
<td>5.3</td>
</tr>
<tr>
<td>Deer 6***</td>
<td>5.6</td>
</tr>
<tr>
<td>Fawn C***</td>
<td>4.6</td>
</tr>
<tr>
<td>Fawn D***</td>
<td>5.7</td>
</tr>
</tbody>
</table>

*Intranasal inoculation
**Contact exposure with infected deer
***Contact exposure with infected steer
****Log_{10} plaque-forming units/ml of blood
sheep and goats exposed and housed under similar conditions. The oral lesions closely resembled those seen in the last 2 species. Foot lesions were quite uniform in appearance and different from those that we have seen in either cattle, sheep or goats; being characterized by involvement of the leathery pad which covers the bulb of the heel. Initially the skin around the edge of the heel pad blanched. Separations and oozing of vesicular fluid soon followed, giving a characteristic wet appearance to the heel. At this time, gentle traction allowed the entire distal end of the heel pad to be peeled back to expose the reddened and extremely sensitive deeper tissue. The heel pad shriveled as healing of the deep layers progressed and eventually an area of grey-white scar tissue covered the bulb of the heel.

Temperature telemetry was a decided asset to these studies. Although the test deer had been raised in captivity and were semidomesticated, their natural excitability made the taking of rectal temperatures a difficult procedure. Even with the use of xylazine, the temperature readings were extremely erratic. Deer No. 1 had abnormally high readings, and others had subnormal readings after the administration of the drug. Telemetry allowed the acquisition of temperatures from completely unrestrained animals. Continuous recording of body temperatures demonstrated a diphasic response in one of the intranasally inoculated deer and in the steer infected by contact exposure. Earlier studies in partly restrained cattle had shown a similar pattern in a steer infected with FMD virus by tongue inoculation but not in one intranasally inoculated.\textsuperscript{13}

All of the deer tested 4 weeks after exposure had virus in the OPF and therefore could be classified as carriers. Generalization is not possible with such a small experimental group but the presence of virus in the OPF of one animal 11 weeks after exposure makes the existence of relatively long term carriers a distinct possibility. The results confirm that whitetailed deer are a factor to be taken into account should an outbreak of FMD occur in an area where they might come in contact with cattle.
FMD IN WHITE-TAILED DEER

(HPC)- the steer’s temperature rose from baseline to a peak of 107.3 and then declined to 105.2. A second rise started almost immediately, and the temperature stayed between 106.0 and 107.0 until a technical problem interrupted the transmission at 44 HPC. When radio contact was reestablished at 62 HPC, the temperature was 105.3 and, with the exception of a single hour’s reading, remained above 103.0 until 108 HPC when the equipment was removed. The animal had remained clinically normal through 2 DPC but at 3 DPC was showing obvious signs of severe generalized FMD. Epithelium, harvested from an oral lesion at 4 DPC was tested by complement fixation and found to be FMD virus subtype 01.

At 12 DPC, Deer No. 4 was found dead. Although the carcass was in a rather advanced state of decomposition, we could see typical lesions of FMD in the heart muscle. The oral and foot lesions were all in various stages of healing.

The purpose of the final experiment was to test the transmission of FMD from cattle to deer. A steer was confined against one wall of the larger section of the isolation unit, and 2 adult deer (Deer No. 5 and No. 6) and 2 fawns (Fawns C and D) were allowed access to both sections through the open door in the partition. As before, the deer were apprehensive of the steer. The four deer were instrumented and the steer was inoculated intranasally with $10^6$ pfu of virus. Three days later the steer had a rectal temperature of 106.2 F and obvious signs of FMD. Telemetry indicated a temperature rise in Deer No. 6 and in both fawns, which started between 4 and 5 DPI. On 6 DPI, these 3 were showing signs of depression, anorexia, drooling of saliva, and slight nasal discharge. Deer No. 5 remained afebrile and showed none of these signs but at 7 DPI was viremic and had a small lip lesion and vesicles on 2 feet. The other 3 animals were also viremic, but these had more extensive lesions and developed clinical courses similar to those seen in the deer described previously.

A summary of assayed blood samples is presented in Table 1. The data are fragmentary but apparently a viremia of 3 to 4 days was the rule.

Table 2 contains a summary of virus isolations from the OPF. Samples taken from all of the 7 animals 5 weeks after exposure contained virus but isolations were less frequent after that. Interestingly, one of the 4 animals tested at 11 weeks still had infectious virus in the OPF.

Results of plaque reduction neutralization tests on serums from the deer showed the titers to be comparable to those seen in cattle, sheep, and goats after infection with FMD virus.

Discussion

Recent reports from Great Britain$^{23}$ indicate that the clinical syndrome of FMD in deer can differ between species. Some differences in the clinical course have also been seen in sheep and goats exposed to different FMD virus strains.$^{12}$ Results of this limited series of experiments must be interpreted with those facts in mind. However, white-tailed deer were clearly susceptible to infection with this strain of FMD virus both by intranasal inoculation and by contact exposure.

The clinical syndrome in deer was somewhat variable but in general the severity of the disease was intermediate between that seen in cattle and in
10^6 pfu of virus. This animal and the single animal (Deer No. 4) had been instrumented but unforeseen technical difficulties prevented the continuous accumulation of temperature data. At 3 DPI, the inoculated deer and the one in close contact (Deer No. 3) were visibly depressed, and all 3 animals were examined closely and sampled. The rectal temperature of the inoculated deer was 105.6 F whereas that of the contact animal was 105.0; both animals had oral lesions and virus in the blood and OPF. Deer No. 4 on the other side of the partition was clinically normal, and virus was not detected in either blood or OPF. This animal was then moved into the smaller section with Deer Nos. 2 and 3 and 2 fawns about 8 months of age were placed in the larger section of the unit. Fawn A was instrumented and its temperature taken by telemetry over the next several days. The next day (4 DPI), the inoculated deer was recumbant and moribund and was euthanized and necropsied. Lesions were present on the gums and tongue, in the nostrils, and on the rumen pillars; but the feet were normal. The cardiac musculature contained numerous 1- to 4 mm pale areas typical of the degenerative lesions seen with FMD in other species. A small section of heart muscle containing one of these lesions was removed and yielded 10^{3.1} pfu of virus.

On this same day, which was 4 days after the start of contact (DPC), Deer No. 3 was extremely depressed and had extensive oral lesions and beginning foot lesions. Deer No. 4 had no lesions although telemetry indicated a slight (0.5 to 1.0 F) rise in temperature. On this day (1 DPC for fawns) both fawns were normal.

The next day (5 DPC) Deer No. 3 was still very ill, and the foot lesions had become more severe. Deer No. 4 had developed a fever during the night and had a few small oral lesions. Both of these animals had high titers of virus in the blood (10^{4.6} - 10^{5.8}) and OPF (10^{6.0} - 10^{6.2}). Both fawns (2 DPC) remained normal.

At 6 DPC, Deer No. 3 was recovering whereas Deer No. 4 was still quite ill. The instrumented fawn (Fawn A, 3 DPC) was febrile. The temperature had started to rise 64 hours after the start of exposure (Fig. 2). Both fawns were visibly depressed and reluctant to move. A pool of lesion material from the animals was examined by complement fixation* and proved to be FMD virus subtype 01.

At this time (Deer 6 DPC, Fawns 3 DPC), to test transmission of the infection from deer to cattle, we put a steer into the unit. The steer was confined against the wall farthest from the partition, and the door in the partition was left open. Although the deer and fawns had free access to both sections of the unit, they were never observed to leave the smaller section and seemed to be extremely apprehensive of the steer.

The next day the deer (7 DPC) were both slowing clinical improvement. The fawns (4 DPC) were quite ill although the telemetry showed that Fawn A's temperature had returned to the baseline (Fig. 2). The steer (1 DPC) remained normal and was instrumented; the baseline temperature was approximately 102.2 F (Fig. 3). Between 27 and 34 hours after contact

*Kindly performed by Dr. R. J. Yedloutschnig.
isolated from these 2 unexposed animals nor from others in the reserved group. Lesions to be described in the present report as resulting from FMD were verified by isolations of FMD virus. The possibility exists however, that the syndrome which we will describe could have been complicated by the presence of the as yet unknown condition.

To observe the reaction of the deer to the particular strain of virus chosen, a single adult (Deer No. 1) was placed in an isolation unit and instrumented for telemetry. Administration of xylazine required physical restraint and caused a very brief period of excitement. It was observed over the next 2 days that a very erratic pattern of high temperature readings developed. Temperatures as high as 107.2 F were recorded usually within a few hours after the administration of xylazine. The temperature invariably dropped below 103.0 within 24 hours but rose again each time the animal was handled and xylazine was given. On the third day, the animal was given xylazine, and 1 ml of virus suspension containing $10^3$ pfu was slowly instilled intranasally. Within 8 hours the temperature had reached 106.2 but 2 hours later dropped to 102.2 (Fig. 1). A baseline temperature of approximately 101.8 was soon established and maintained until 44 hours after inoculation (HPI) when an abrupt increase was noted. Over the next 11 hours, the temperature reached a peak of 103.6 and then declines to near baseline level. A second increase followed, and a plateau of approximately 103.6 was reached at 65 HPI and maintained for the next 34 hours. A rather steady decline followed, and baseline temperatures were reached toward the end of the 5th day (120 hours). The animal had not been handled during this period but was observed at rather close range because the isolation unit was entered daily for feeding. The deer behaved in an entirely normal manner during the first 2 days after inoculation (DPI) but on the 3rd and 4th days became progressively less active and ate very little. At 5 DPI, blood and OPF samples were taken. At this time, there was a slight mucopurulent nasal discharge and small epithelial defects in the right nostril and on the upper and lower lip. Virus was present in substantial amounts in the blood ($10^{4.7}$ pfu/ml), OPF ($10^{4.6}$ pfu), and lesion harvest ($10^{3.8} - 10^{4.3}$ pfu). The telemetry equipment was removed at this time.

The next day (6 DPI), Deer No. 1 was quite depressed and was limping. At 7 DPI, both nostrils were partly occluded with a mucopurulent discharge, and lesions had developed on 3 feet, but the oral lesions had not spread or increased in size. Virus was not detected in a blood sample taken on this day but was present in the OPF ($10^{3.4}$ pfu) and in material harvested from a foot lesion ($10^{5.0}$ pfu). At 10 DPI, all lesions were healing; by 17 DPI the animal had returned to normal activity, and scars on the feet were the only remaining sign of illness.

The next experiments were done to test the transmissibility of FMD between deer and cattle. For this purpose, a partition was built across an animal isolation unit in order to divide it into 2 sections with approximate dimensions of 6 x 12 and 12 x 12 feet. The partition was 8 feet high, the bottom half was solid and the top was of coarse wire mesh. A 3-foot door in the partition controlled access between the 2 sections. Initially 3 adult deer were placed in the unit, 2 in the smaller section and one in the larger one. One of the pair (Deer No. 2) was inoculated intranasally with
REFERENCES


CLINICAL AND SEROLOGICAL RESPONSE OF THE NINE-BANDED ARMADILLO (DASYPUS NOVEMCINCTUS) TO VIRUSES OF AFRICAN SWINE FEVER, HOG CHOLERA, RINDERPEST, VESICULAR EXANTHEMA OF SWINE, VESICULAR STOMATITIS AND FOOT-AND-MOUTH DISEASE

F. W. Wilder, A. H. Dardiri and R. J. Yedloutschnig

United States Department of Agriculture, Agricultural Research Service, Plum Island Animal Disease Center, Greenport, New York 11944.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Summary
The armadillo (Dasypus novemcinctus) is gradually expanding its range in south-central and southeast areas of the United States. Its susceptibility to six economically important exotic diseases was investigated. The armadillos showed classic vesicles with foot-and-mouth Type A5 after inoculation with the virus. Tissue harvested from 1 armadillo and inoculated into pigs reproduced the disease. There was no clinical evidence of susceptibility to African swine fever, hog cholera, rinderpest, vesicular exanthema of swine or vesicular stomatitis. Some serologic conversion occurred with rinderpest.

Introduction
The nine-banded armadillo ranges east of the Andes from Uruguay to and including parts of the southwest and southeast areas of the United States (Fig. 1). Since the mid-19th century, it has spread from south Texas into northeast Texas, Oklahoma and parts of Arkansas, Louisiana and Mississippi. Armadillos that escaped from a circus train wreck near Cocoa and Titusville have spread over much of Florida.1 A second focus has migrated from Mobile county, Ala. through southern Alabama and into northwestern Florida.2

Because this animal is establishing itself in much of southern United States, we need to investigate its possible importance in the dissemination of certain exotic diseases of economical importance.

Materials and Methods
Test Animals. Ten nine-banded armadillos (3 males and 7 females were obtained through Dr. H. L. Rubin* from Dr. O. Frye, Jr.** in Florida.

The armadillos were housed on a concrete floor in a 3 x 4 meter isolation unit. The air temperature was kept at 24°C because armadillos are not able to control their body temperature, and sufficient hay or straw was kept in the room to easily cover all the animals. They were provided with a continual

---

*Animal Disease Diagnostic Laboratory, Live Oak, Florida 32060.
**Director, Florida Game and Fresh Water Fish Commission, Tallahassee, Florida, 32304.
water supply, and once a day the animals were fed canned Alpo*** beef chunks and meat by-products containing vitamins and minerals. The animals averaged 7 to 11 pounds on arrival. Each gained an average of 2 pounds under these conditions.

The armadillos were observed for 14 days post inoculation (DPI) for clinical signs. Blood samples were taken by cardiac puncture at 0 and 14 DPI. Often only 1 milliliter (ml) of serum was obtained from a 5 ml sample. Two men were required to restrain an adult armadillo on its back and a third to obtain the blood with a syringe and 20 gauge, 1½ inch needle.

African Swine Fever (ASF)

Animals and Viruses. Nine armadillos were used. Five were inoculated intraperitoneally (IP) with 1 ml of the Lisbon '60' strain of African swine fever virus (ASFV) in suspensions of 1 part spleen plus 4 parts blood which were further diluted 1:20 with phosphate buffered saline (PBS). Four other armadillos housed in the same room served as contact controls. Two weeks before the trial, the inoculum was assayed in leukocyte cultures prepared by the method of Hess and DeTray³ and was found to have a hemadsorption (HAD) titer of 10⁸HAD50/ml. In another room, a control pig inoculated intramuscularly (IM) with 1 ml of the same virus expired in 7 days with classical signs of ASF.

Agar Gel Diffusion Precipitin Test (AGDP). Serums were tested by AGDP as described by Coggins and Heuschele.⁴ However, the test antigen consisted of sonicated debris from African green monkey kidney (Vero) cell cultures infected with Lisbon '60' ASF virus and prepared by the method of Pan et al.⁵

Immunoelectroosmophoresis Test (IEOP). The same serums were also tested by the IEOP test; the same antigen was used.

Results. No abnormal signs were observed in armadillos inoculated with ASFV. One 14 DPI serums from an inoculated armadillo produced a precipitin line in both the AGDP and the IEOP tests. The nine 0 DPI serums and the eight other 14 DPI serums produced no lines in the same test.

Hog Cholera (HC).

Animals and Viruses. Eight armadillos that were used in the ASF trial were also used in this trial. Four served as contact control, and the other four were inoculated with the Ames strain of hog cholera virus (HCV) in blood from an infected pig. Each of the four armadillos was given IP 1 ml of a suspension of one part blood containing HCV plus nine parts PBS. Two control pigs were inoculated with one-half the above dosage and became moribund within 1 week.

Results. Elevation of body temperature was noted in armadillos inoculated with HCV and in armadillos serving as contact controls. Normally, armadillos had 33 or 34°C temperatures. Inoculated and un inoculated animals had 35°C temperatures sporadically over a 2 week period. One inoculated animal and one control were rather listless and slept apart from the other animals.

***Allen Products Co., Allentown, Pennsylvania 18001.
One-ml heparinized blood samples were collected from five armadillos that showed signs deviating from normal, such as lethargy. These samples were pooled and injected into a pig. No abnormal signs were seen in the pig for 14 days, and white blood cell counts were normal on 3 and 8 DPI. At 14 DPI, upon challenge by IM injection of 0.5 ml of 1 part spleen and 4 parts blood suspension of virulent HCV, the pig came down with persistent temperatures of 40-41°C and severe depression. After 10 days, the pig was considered moribund and euthanized. Necropsy revealed severely congested lymph nodes and "turkey egg" kidneys.

**Vesicular Exanthema of Swine (VES).**

**Animals and Viruses.** The same eight animals that were used in the previous infectivity trials were inoculated with VES virus (VESV). Half the animals were inoculated intradermally between each toe, in the foot-pad and in the upper lip on each side. The other half were uninoculated contact controls in the same room.

Tissue culture fluid of VESV type B-51, passage 12 in the primary swine kidney (PSK) culture, was used as inoculum. The virus titer was $10^{6.5}$ TCID$_{50}$/ml (50% tissue culture infective doses) assayed in PSK cells. When 1 ml of undiluted virus was injected intradermally into the feet of four pigs, it produced high temperatures and vesicles within 24 hours after inoculation.

**Virus Neutralization Test.** Seven 14 DPI serums were examined for VESV antibodies by the virus neutralization test. Constant amounts of virus mixed with varying dilutions of test serums were added to PSK cultures. The cultures were examined daily for cytopathic effect (CPE).

**Results.** No clinical signs were noted after inoculation with VESV. The virus neutralization test revealed a slight retardation of CPE, the lowest dilution was of one 14 DPI serum from an inoculated armadillo.

**Rinderpest (RP).**

**Animals and Viruses.** Five or eight armadillos that had previously been exposed to ASFV, HCV and VESV were inoculated IP with 0.5 ml of undiluted RP virus (RPV) suspension. The Pendik strain of RPV was used as inoculum. The virus was a suspension of one part mesenteric lymph nodes plus nine parts of blood suspension. The same dose killed two steers in 6 days with typical clinical signs and lesions of RP. At 200 DPI, the armadillos were hyperimmunized in the same manner.

**Complement-Fixation Test.** Serum samples obtained pre-inoculation and post-inoculation were tested against RP antigen and normal tissue antigen in the complement-fixation (CF) test. The antigen consisted of homogenized mesenteric lymph nodes from a steer infected with RPV. One ml of slurry was diluted with 63 ml of PBS. The CF test was performed as the laboratory branch CF test method but the complement was titrated in the presence of the antigen dilution, and the appropriate quantity of it was added to the test to overcome the antigen's anticomplementary effect.

**Results.** The armadillos showed no abnormal clinical signs after inoculation with RPV. Armadillo serums taken at 21 days after they were hyperimmunized at 200 days post-inoculation fixed complement to dilutions of 1:45. Although some fixation was nonspecific, definite complement fixing antibodies were present (Table 1).
Vesicular Stomatitis (VS).

Animals and Viruses. Seven armadillos that were used in the previous trials were also used in these trials. Four armadillos were inoculated intradermally with 0.5 ml suspension of vesicular stomatitis virus (VSV) Indiana Type I containing one part chicken embryo membrane in four parts amnionic allantoic fluid. Four armadillos were left in the room as contact controls. Five pigs showed classical clinical signs of VS after they were inoculated intradermally in the snout with 1 ml of the above suspension. The pigs had large vesicles and wide extensions on the snout surface on 2 DPI.

Virus Neutralization Test. Seven 0 and seven 14 DPI serums were diluted with four parts Hanks' basic salt solution containing 0.5% lactalbumin hydrolysate (HLH) plus antibiotics and combined with varying decimal dilutions of VSV and inoculated into the chorioallantoic sac (CAS) of 10 day-old embryonated chicken eggs. The eggs were examined daily for deaths.

VSV Isolation. Seven heparinized blood samples drawn on 2 DPI were diluted with 1, 4, 9 and 99 parts of HLH, and an aliquot of each dilution was inoculated into the CAS of four embryonated eggs.

Results. No clinical signs were noted in the armadillos up to 14 DPI. No VSV was isolated from the heparinized blood samples drawn on 2 DPI. Tissue from six dead embryos of the first egg inoculation was harvested and used as inoculum for a second passage. No embryos were killed on the second passage. The virus neutralization test for VSV in 10 day-old embryonated chicken eggs was negative.

Foot-and-Mouth Disease (FMD)

Animals and Viruses. Seven armadillos that were used in the previous trials were also used in this trial. Four were inoculated intradermally in the feet and snout; the other three were designated as contact controls. Tissue culture fluid from the third passage of FMD virus (FMDV) Type A5, Westerwald in primary bovine kidney cultures diluted with 19 parts of Thioglycolate medium was used as inoculum. Two control pigs in a different room were inoculated intradermally and developed clinical signs and lesions typical of FMDV.

Agar Gel Diffusion Precipitin Test for FMD. Seven 0 DPI and seven 21 DPI serums were tested for antibodies to the virus-infection-associated (VIA) antigen common to all types of FMDV by the method described by Cowan. Two positive guinea pig serums were used as controls.

Results. Two days after inoculation with FMDV, the 4 inoculated armadillos showed vesicles on the footpads, sides and edges of the toes. The vesicles, though small, were discrete and contained fluid. The vesicles were not at the site of inoculation. The three contact control armadillos showed ruptured vesicles on all footpads between 7 to 14 DPI. No deviation of body temperature was noted during the course of the infection. The footpads appeared healed 3 to 4 weeks post-infection. The incubation period varied from 3 days in the inoculated armadillos to approximately 10 days in the uninoculated armadillos. Some armadillos exhibited mucopurulent ocular discharge, anorexia and serous nasal discharge for 4 days after vesicles appeared. One armadillo dies 1 month later with lesions of hemorrhagic enteritis.
When armadillo serums were tested for VIA antigen by agar diffusion, a single precipitin line was obtained with two of seven 14 DPI serums, but not with their corresponding 0 DPI serums.

Two pigs were inoculated with one part vesicular tissue and fluid harvested from one armadillo and two parts PBS. The pigs showed lesions typical of FMD, including blanched necrotic tissue around the coronary bands with severe extensions and high temperatures.

Complement-Fixation Test. Vesicular tissue from the two aforementioned pigs was washed in saline, ground, diluted with nine parts PBS and centrifuged at 800xg for 10 minutes. The supernatant was used as the antigen in a complement-fixation (CF) test against 1:6 dilutions of hyperimmune guinea pig serums for 7 types of FMDV, 4 types of VS and swine vesicular disease. The CF test was performed as the laboratory branch method, but the complement was titrated in the presence of the antigen and added accordingly to overcome the presence of anticomplementary activity of the antigen. Vesicular tissue harvested from the pigs exhibited complete fixation with Type A FMDV guinea pig hyperimmune serum in the CF test.

Discussion

During the HC trial, elevations of armadillo temperatures was noted. Varying room temperatures caused by malfunctioning of the air conditioning system may have had an effect on their temperatures. One pig injected with pooled blood samples of five infected armadillos did not show any immunological protection upon subsequent challenge with HCV.

The armadillos not only proved susceptible to FMDV, but also transmitted the disease to each other, which suggests the possibility that they might infect other susceptible hosts.

Campion found that the hairy armadillo (Chaetophractus villosus) was susceptible to FMDV. This animal is a different genus than the nine-banded armadillo but illustrates the possible susceptibility of other members of the order Edentata such as anteaters, pangolins or sloths to FMD. The nine-banded armadillo should be added to the host list of FMD.

Acknowledgements

The excellent technical assistance of Mrs. Marjory Llewellyn, Mrs. Elizabeth King, Mr. David Perkins, Mr. Peter Mikiciuk and Mr. Thomas Franke and the assistance rendered by Dr. H. L. Rubin, Animal Disease Diagnostic Laboratory, Live Oak, Florida and Dr. O. E. Frye, Jr., Director Florida Game and Fresh Water Fish Commission, Tallahassee, Florida in supplying the armadillos are greatly appreciated.
Table 1. Challenge Results

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical</th>
<th>Serology</th>
<th>Viremia</th>
<th>Infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASF</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VES</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RP</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FMD</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
REFERENCES

SUSCEPTIBILITY OF ONE-TOED PIGS TO CERTAIN DISEASES EXOTIC TO THE UNITED STATES


Abstract

Mexican One-toed pigs were challenged with seven economically important exotic diseases. One-toed pigs were found clinically susceptible to foot-and-mouth disease, hog cholera, African swine fever, vesicular stomatitis, vesicular exanthema swine, and swine vesicular disease, but not to rinderpest. Clinical signs nor sero-conversion did not occur following exposure to rinderpest virus.

Introduction

The One-toed pig is small, relatively hairless, slow maturing, and has one digit enclosed in a hoof like that of a horse. Syndactylism results from a dominant gene, however 2 toes are occasionally seen in certain individuals, especially on the hind limbs.1

The completion of the Pan American Highway increases the threat that foot-and-mouth (FMD) will spread from South American into Central and North America. Few, if any One-toed pigs or mule foot hogs exist in the United States, but this animal is domesticated and feral in the Caribbean coastal areas of Mexico south of Tampico.

A need existed to investigate this animal, since little information is available concerning its susceptibility to certain diseases exotic to the United States or Central America.

It was thought early in the 1900's that these pigs were immune to hog cholera, but this idea was disproved at the Indiana State Experiment Station in 19102.

Materials and Methods

Twelve adult One-toed pigs were obtained through the USDA, Animal Plant Health Inspection Service group in Mexico City. These twelve pigs were divided into groups of 3 and then one domestic Tamworth pig was added to each group. Each group was confined in a 3 x 4 meter concrete enclosed indoor isolation unit, and established laboratory husbandry procedures were followed3.

All pigs were observed for clinical signs for 14 days post inoculation (DPI). Blood or epithelial tissue was taken “ad lib” for virus isolations, and serums were collected for antibody assay at 14 DPI. When the animals recovered from one disease they were inoculated with a different virus.

*USDA, APHIS, VS, Tres Picos No. 79, Mexico, D. F.
**C.M.A.P.F.A. Vesicular Disease Laboratory - I.N.I.P. Km. 15.5 Highway, Mexico, Toluca, Mexico, D. F.
Samples of epithelium from vesicular lesions were washed in PBS, ground with a mortar and pestle, diluted 1 part tissue with 9 parts PBS containing 200 U Penicillin and 100 μg Streptomycin (P/S) per milliliter (ml) and centrifuged at 2000 rpm for 10 minutes. Infectivity of the supernatent fluids was assayed in primary swine kidney tissue culture (PSK) or chicken embryos. Inoculated cultures were examined daily for cytopathic effect (CPE).

**Experiments and Results**

**Foot and Mouth Disease.** One One-toed pig and 1 Tamworth pig were inoculated intradermally in the snout and the lateral coronary bands with a total of 1 ml of virus suspension containing $10^{6.5}$ 50% tissue culture infective doses (TCID$_{50}$)/ml of A$_5$ type foot-and-mouth (FMD) virus. Two One-toed pigs were left as uninoculated contact controls.

Both contact control pigs displayed a temperature rise, lameness and vesiculation of the feet and snout by the 4th day after exposure. One One-toed pig was euthanized at 9 DPI because it was unable to rise to eat or drink.

Vesicular epithelium was collected from the 2 uninoculated contact control One-toed pigs and treated as described in Materials and Methods except that P/S was excluded. This suspension was used as antigen in the complement fixation (CF) test. However, complement was titrated in the presence of the antigen, to determine the presence of the anticomplementary activity. Then complement was added in the test to overcome this activity. Pre-exposure and 14 DPI sera from the 4 pigs was examined for FMD neutralizing antibodies using a 1:10 constant serum-10 fold dilution virus neutralization (VN) test performed with primary bovine kidney cultures. The CF test and the VN test both indicated infection with A type FMD virus had occurred.

**Hog Cholera.** Recovered survivors of the group infected with FMD were subsequently used to test for susceptibility to hog cholera (HC) virus. A single One-toed pig served as a contact control while another and the Tamworth were each inoculated intramuscularly (IM) with 1 ml of HC virus in infected $10^5$TCID$_{50}$/ml swine blood.

Inoculated pigs displayed elevated temperatures, depression and cynosis by 6 DPI. At 9 DPI the trial was terminated after the Tamworth pig died and the other 2 pigs were euthanized. Necropsy of the inoculated One-toed pig showed congested visceral capillary beds, peripheral hemorrhages of the lymph nodes, petechial hemorrhages on the serous surfaces of the small intestine and diffuse hemorrhages of the skin. The contact control One-toed pig had an elevated temperature beginning on 5 DPI, but no definite lesions were found on necropsy. Leukocyte counts were made at 2, 3, 6, 8, 9, DPI. Table 1 indicates that all pigs displayed leukopenia by 6 DPI.

**Vesicular Stomatitis.** In another group the snouts of a One-toed pig and a Tamworth pig were scarified and each inoculated with 1 ml of amnionic-allantoic fluid (AAF) from chicken embryos infected with Indiana type 2 vesicular stomatitis (VS) virus. The inoculum contained $10^{5.5}$ 50% chicken embryo infective doses.

Temperatures of all pigs were elevated at 4 DPI and large vesicles developed on the snout of both inoculated pigs. Vesicular epithelium from
infected pigs was harvested and prepared as described under Material and Methods. This material was inoculated onto the chorio-allantoic sac of 10 day old chicken embryos. All chicken embryos inoculated with suspensions of 1:100 or less died in 2 to 3 days. Infective AAF was harvested from inoculated eggs the same day that the embryos died. This material was passed 3 times in chicken embryos. A VN test was performed with chicken embryos using ten-fold dilutions of the AAF mixed with constant 1:10 dilution final Indiana type 2 VS swine antiserum. Results showed the infective agent had been Indiana type 2 virus.

Another aloquet of the suspension of vesicular epithelium was used in a CF test, and also indicated that the infectious agent was Indiana type 2 virus.

*African Swine Fever.* Recovered pigs from the group infected with VS were inoculated IM with a 1 ml of a suspension of tissue culture fluid containing the Tengani (stain ) of African swine fever (ASF).

All 4 pigs developed high temperatures, became recumbant, and by 7 DPI were dead. Necropsy lesions included an enlarged spleen, and severe congestion of the lymph nodes, kidneys, heart and liver. Spleens collected at necropsy were treated as described in Materials and Methods and inoculated into swine buffy coat cultures. All spleen samples produced hemadsorption in the buffy coat cultures.

*Vesicular Exanthema of Swine.* In another group, 2 One-toed pigs were inoculated intradermally in the snout and lateral coronary bands with 10⁸ TCID₅₀ of A₄₈ type vesicular exanthema of swine (VES) virus in 1 ml of cell cultured virus. The remaining 2 pigs served as contact controls. Both the inoculated pigs and the 2 contact control pigs developed fevers and became lame by 5 DPI. Necrotic epithelial lesions spread from the coronary bands of all animals to the soles of the feet.

Epithelial tissue was harvested from vesicular lesions, prepared for virus isolation as described in Materials and Methods, and inoculated into primary swine kidney tissue culture (PSK). Tissue cultures showing CPE were passaged once. In a VN test ten fold dilutions of virus were mixed with a constant 1:10 final dilution of convalescent swine A₄₈ antiserum and added to PSK monolayers. Virus was isolated from vesicular epithelium collected from each animal and shown in the VN test to be A₄₈ type virus.

*Rinderpest.* Two One-toed pigs of the group previously infected with VES virus were inoculated IM with 1 ml of a 1:9 slurry of bovine spleen and mesenteric lymph nodes in blood containing 10⁴ 50% animal infective doses from a steer infected with Pendik (strain) of rinderpest (RP) virus.

Signs of clinical disease did not develop in the One-toed or Tamworth pigs.

A VN test was performed in green monkey kidney cell cultures (VERO) using 14 DPI serums at 1:10 final dilution and 10 fold dilutions of RP virus. The 14 DPI serums of inoculated pigs did not possess neutralizing activity to RP virus.

*Swine Vesicular Disease.* Two One-toed pigs used in the VES and RP trials were inoculated intradermally in the coronary bands with 1 ml of swine vesicular disease (SVD) virus suspension obtained from infected swine vesicular epithelium. This virus was originally isolated during the United Kingdom outbreak of 1972 and passed once in swine at PIADC.
All pigs developed vesicular lesions on the snout and feet by 4 DPI. Heat-inactivated vesicular fluid, obtained from one of the contact control pigs produced a CF titer of 1:10. Vesicular epithelial tissue harvested during this trial was prepared as described in Materials and Methods and inoculated in PSK cells. The agent was passed 3 times in PSK cells after CPE appeared. This cell-cultured SVD virus when diluted 1:4, produced a CF titer of 1:40.

Discussion

*One year previous to the current study, the original 12 One-toed pigs were inoculated with either New Jersey or Indiana 1 types VS virus. All swine became infected. This, with the present results, demonstrated that One-toed pigs were susceptible to 3 types of VS virus.

In all except the RP trial, contact control pigs became infected. The other diseases infected One-toed pigs in the same manner as domestic varieties of swine. (Table II). RP challenge caused no clinical signs, nor did any sero-conversion occur with 14 DPI serums from inoculated or uninoculated pigs. This finding is in contrast to data showing the javelina to be highly susceptible to RP virus. During the vesicular disease trials, One-toes pigs suffered more lameness than usually found in domestic breeds of swine. This may be due to the difference in the anatomy of the foot.

Acknowledgements

The competent technical assistance of Mr. David Perkins and Mr. Peter Mikiciuk is greatly appreciated.

TABLE 1

<table>
<thead>
<tr>
<th>HOG CHOLERA TRIAL LEUKOCYTE COUNTS</th>
<th>2 DPI</th>
<th>3 DPI</th>
<th>6 DPI</th>
<th>8 DPI</th>
<th>10 DPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-toed pig*</td>
<td>ND</td>
<td>17,000**</td>
<td>6,400</td>
<td>10,300</td>
<td>10,100</td>
</tr>
<tr>
<td>One-toed pig</td>
<td>8,600</td>
<td>5,700</td>
<td>6,300</td>
<td>11,100</td>
<td>5,000</td>
</tr>
<tr>
<td>Tamworth</td>
<td>9,600</td>
<td>5,300</td>
<td>6,600</td>
<td>5,400</td>
<td>dead</td>
</tr>
</tbody>
</table>

* Uninoculated

** Number of WBC per cmm; average normal swine value is 16,000 WBC per cmm.

(*Work done at Vesicular Disease Laboratory of C.M.A.P.F.A. and in process of being published.*)
TABLE II.

**ONE-TOED PIG INOCULATION RESULTS**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical Signs</th>
<th>Sero-Conversion</th>
<th>Infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot and Mouth</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hog Cholera</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>African Swine Fever</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vesicular Stomatitis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vesicular Exanthema of Swine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Swine Vesicular</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rinderpest</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

REFERENCES

THERMAL INACTIVATION DATA FOR SWINE VESICULAR DISEASE VIRUS

H. R. Cunliffe

From the Plum Island Animal Disease Center, Northeastern Region, Agricultural Research Service, U.S. Department of Agriculture, Greenport, New York 11944.

“Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.”

Introduction

Swine vesicular disease (SVD) is a contagious virus disease of swine clinically indistinguishable from foot-and-mouth disease (FMD), vesicular stomatitis, vesicular exanthema of swine and San Miguel sea lion viruses. A serological relationship between SVD virus (SVDV) and Coxsackie B-5 virus\(^1\) and the fact that serological evidence indicated that illnesses in laboratory workers handling SVDV were probably due to this virus suggest a possible interspecific transmission of these two agents.\(^2\)

Importation of various fresh and processed pork products, therefore, is of great epidemiological importance because data regarding the stability of SVDV indicate rather substantial resistance to acid pH and to heat.\(^3\)

This report presents data derived from studies designed to define the thermal inactivation kinetics and death points of SVDV. Although these objectives were not achieved, a variety of interesting data were obtained regarding the effect of sodium ion concentration on the thermal inactivation of SVDV. This effect is of special interest because the processing of many pork products involves mild heating and high NaCl concentrations.

Materials and Methods

**Virus:** SVDV, United Kingdom 27/72 (SVDV-UKG) was obtained as infectious swine tissue and passaged twice in roller cultures of a pig kidney cell line (MVPK); serum-free F-15 medium (Grand Island Biological Co., Grand Island, New York 14072) was used. Virus harvested in fluids from the second passage was clarified by high-speed centrifugation and stored as 2-ml samples at -20 C.

**Inactivation Procedures:** SVDV-UKG was heat treated in a precision water bath in temperatures maintained within 0.1 C. Before heating, a 0.1-ml sample of the thawed virus was titrated for infectivity of the untreated virus. For heat treatments, 1 ml of virus was thoroughly mixed with 9 ml of preheated buffer; samples were taken after desired time intervals and added to ice-cold diluent in the first tube of each dilution set. All such tubes were held in an ice water bath during the preparation of dilution series and plaque assay procedures.

**Plaque Assays:** Virus samples were diluted 5-fold in serum-free F-15, and 0.1 ml of each dilution was pipetted onto each of 2 MVPK cultures in 4-ounce prescription bottles. At dilutions where minimal plaque counts
were expected, 6 to 10 replicates were used. Virus absorption was allowed for 20 minutes at 37 °C, after which 5 ml of F-15 containing 4% normal bovine serum, 0.6% gum tragacanth, 0.01 M Tris base and antibiotics were added to each culture. Tris base was found to be a critical additive necessary for discrete plaques with SVDV-UKG. Cultures thus prepared were incubated at 37 °C for 48 hours, after which plaques were counted on the stained cultures. SVDV-UKG infectivity titers were computed from plaque counts and are expressed as common logarithms of the PFU/ml.

Results

Although other buffer systems were tested, data from only Tris-buffered 0.15 M saline, pH 7.6 (TBS) and serum-free F-15 medium are presented because significant differences were not apparent between other buffer fluids containing 0.15 M NaCl or 0.15 M NaCl alone.

Data in Table I indicate that at 45 °C, SVDV-UKG was stable for at least 3 hours in TBS whereas the virus was moderately labile in serum-free F-15. At 50 °C, the data show that SVDV-UKG was still relatively stable in TBS. However, at 55 °C and 60 °C, SVDV-UKG shows an increasing lability to heat in both buffers. Less than 3 logs of virus infectivity survived after 1 minute at 60 °C in TBS whereas substantial infectivity was measurable in F-15 for at least 16 minutes. This difference in sparing effect is even more obvious at 65 °C and 70 °C (Table II) where detectable infectivity is apparent in F-15 for several minutes but not in TBS after only 0.5 minutes. Apparently, some component(s) of F-15 other than sodium ion has a protective effect on SVDV-UKG at 65 °C and 70 °C, but this effect is not apparent at the lower temperatures when compared with effects of buffers containing 0.15 M NaCl.

The effect of 0.62 M and 3.37 M NaCl solutions on the thermolability of SVDV at 37, 50 and 70 °C was tested because these salt concentrations are comparable with those used in processed pork sausages and partly cooked, salted, canned hams. Results of this test (Table III) indicate that the sparing effect of 3.37 M NaCl was marked on the virus at 70 °C compared with that of F-15 and TBS (Table II) whereas the sparing effect of 0.62 M NaCl was slight. At 37 °C (Table III), no sparing effect was detected in any of the salt concentrations whereas at 50 °C some sparing effect was noted in 0.62 M and 3.37 M NaCl.

Discussion

The ability of 1-2 M cationic solutions to stabilize enteroviruses against thermolability at 50 °C was reported in 1961. Earlier, Cottral et al. cited literature and presented data indicating a sparing effect of sodium salts on FMDV in animal tissues against adverse conditions of pH and temperature. Although divalent cations stabilize enteroviruses at all temperatures between 4 and 50 °C, monovalent cations (i.e., sodium) appear more selective in their effective temperature range. The same may be true for SVDV-UKG; at a relatively high temperature (70 °C), the sparing effect of 3.37 M saline against a demonstrated thermolability of the virus in 0.15 M and 0.62 M saline was marked. However, at 45 and 50 °C (Table I), the infectivity of SVDV-UKG appeared to be stable in 0.15 M saline compared with the infectivity of the virus in F-15 medium.
Reasons for the sparing effect of F-15 on SVDV-UKG at 60 to 70 °C remain obscure because of the complexity of this medium.

The inability of McKercher et al.6 to detect infective SVDV-UKG in partly cooked, salted (ca. 3.37 M NaCl) hams may be explained in terms of total caloric input. Although data of the present study show that the effect of 3.37 M NaCl against thermolability at 70 °C for at least 4 minutes was sparing, the processed hams were heated for 4 to 5 hours before reaching approximately 70 °C. For at least 1 hour of this total heating period, the temperature was above 60 °C. Thus, although such hams are cooled rapidly after reaching 69 °C (156 °F), the total caloric input far exceeds that applied to SVDV-UKG at 70 °C in the present study. In addition, virus titers of infective meat used to prepare canned hams6 were much lower (3.5-4.5 TCID50/gm) than titers of SVDV-UKG (> 10^7 PFU/ml) used in this study.

Although relatively high NaCl concentration are commonly used to cure many pork products, the effective sodium ion concentration surrounding infective SVDV-UKG particles that may be resident in the meat is unknown. However, because most of the salt probably dissolves in the products' free water, its actual concentration may be much higher than the reported molarities calculated from percent-weight values. Further, much of the free water is evaporated during the curing phase of many pork products. Thus, survival of SVDV-UKG infectivity in the pork products reported by McKercher et al.6 may be related to very high sodium ion concentration in the environment of resident virus.

Acknowledgements

The author wishes to express his gratitude to Mr. Ronald Trower for competent technical assistance throughout this study and also to Dr. William Mengeling of the National Animal Disease Center, Ames, Iowa, who kindly provided the pig kidney cell line.

TABLE I. Stability of SVDV-UKG in Two Buffer Systems at 45, 50, 55 and 60 °C.

<table>
<thead>
<tr>
<th>Min.</th>
<th>45 °C</th>
<th>50 °C</th>
<th>55 °C</th>
<th>60 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-15**</td>
<td>TBS**</td>
<td>F-15**</td>
<td>TBS**</td>
</tr>
<tr>
<td>0</td>
<td>7.39</td>
<td>7.52</td>
<td>0</td>
<td>7.60</td>
</tr>
<tr>
<td>10</td>
<td>7.46</td>
<td>7.42</td>
<td>10</td>
<td>6.27</td>
</tr>
<tr>
<td>20</td>
<td>7.15</td>
<td>7.32</td>
<td>20</td>
<td>4.81</td>
</tr>
<tr>
<td>40</td>
<td>6.91</td>
<td>7.30</td>
<td>40</td>
<td>3.51</td>
</tr>
<tr>
<td>80</td>
<td>5.43</td>
<td>7.36</td>
<td>80</td>
<td>2.43</td>
</tr>
<tr>
<td>160</td>
<td>4.08</td>
<td>N.D.</td>
<td>160</td>
<td>2.61</td>
</tr>
<tr>
<td></td>
<td>1.98</td>
<td>N.D.</td>
<td>1.08</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>1.18</td>
<td>N.D.</td>
<td>0.00</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

*SVDV infectivity titers expressed as the common logarithm of the PFU/ml computed from plaque counts.

**F-15 = a serum-free cell culture medium; TBS = 0.01 M Tris buffer, pH 7.6, containing 0.15 M NaCl.

Min. = minutes; N.D. = not done.
**TABLE II. Stability of SVDV-UKG in Two Buffer Systems at 65 and 70 C**

<table>
<thead>
<tr>
<th>Minutes</th>
<th>65 C</th>
<th>70 C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-15**</td>
<td>TBS**</td>
</tr>
<tr>
<td>0</td>
<td>8.43</td>
<td>7.56</td>
</tr>
<tr>
<td>0.5</td>
<td>2.70</td>
<td>&lt;3.0</td>
</tr>
<tr>
<td>1</td>
<td>2.96</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>2</td>
<td>1.98</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>3</td>
<td>2.86</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>4</td>
<td>2.05</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>5</td>
<td>1.22</td>
<td>0.0</td>
</tr>
<tr>
<td>8</td>
<td>N.D.</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*SVDV infectivity titers expressed as the common logarithm of the PFU/ml computed from plaque counts.

**F-15** = a serum-free cell culture medium; **TBS** = 0.01 M Tris buffer, pH 7.6, containing 0.15 M NaCl.

N.D. = not done.

**TABLE III. Stability of SVDV-UKG in 0.15 M, 0.62 M and 3.37 M NaCl at 37, 50 and 70 C**

<table>
<thead>
<tr>
<th>37 C</th>
<th>50 C</th>
<th>70 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.</td>
<td>0.15 M</td>
<td>0.62 M</td>
</tr>
<tr>
<td>0</td>
<td>7.00</td>
<td>7.00</td>
</tr>
<tr>
<td>15</td>
<td>6.91</td>
<td>6.81</td>
</tr>
<tr>
<td>30</td>
<td>6.96</td>
<td>6.88</td>
</tr>
<tr>
<td>60</td>
<td>6.60</td>
<td>6.37</td>
</tr>
<tr>
<td>120</td>
<td>6.90</td>
<td>6.66</td>
</tr>
<tr>
<td>180</td>
<td>6.88</td>
<td>6.80</td>
</tr>
</tbody>
</table>

*SVDV infectivity titers expressed as common logarithm of PFU/ml computed from plaque counts.

0.15, 0.62 and 3.37 M = saline solutions in 0.02 M sodium phosphate buffer, pH 7.6.

Min. = minutes; N.D. = not done.
REFERENCES

ANTIGENIC VARIATION OF VENEZUELAN STRAINS OF FOOT AND MOUTH DISEASE VIRUSES

G. Gomez*
J. Castaneda**
M. Espinoza*
A. Maldonado*

Summary

The latest isolations of Foot And Mouth Disease virus in Venezuela are described. Relationship of the new strains compared to strains common in the country is characterized by analysis of complement fixation assays.

Subtype relatedness is expressed by the formula \( R=100 \sqrt{1+\frac{x}{r^2}} \). Strains of type “O” virus exhibited a wide range of serologic differences. Characteristics of strain “O” Manamito suggest the emergence of a new strain of type “O” virus. Another strain, “O”1 Bolivar, is characterized by high infectivity titers in suckling mice and short incubation periods in cattle. Strains of type “A” virus corresponded to subtype “A”32 which is predominant in Venezuela, although the presence of subtype “A”27 has been confirmed in some Venezuelan states bordering the Republic of Colombia.

Introduction

Foot-And-Mouth Disease (FMD) first appeared in Venezuela in 1950 when type “O” virus spread across the central and mid-occidental regions (5). Type “O” virus was most prominent during 1950, 1951, 1956, 1960, 1962 and 1966 through 1968. Until 1966, subtype “O”3 (Lara strain) was common in Venezuela but thereafter a new subtype known as subtype “O”1 (Strain “O”1 Cura 66) became the predominant strain of type “O” virus (6).

In 1971 a strain known in our laboratory as “O”1 Cura 71 appeared and in 1972 the presence of strain “O”1 Carora 71 (4) was detected. These strains have distinct antigenic differences as defined by serologic methods. During 1973 two additional strains of type “O” virus were isolated and each had distinct epizootiologic and serologic characteristics.

Type “A” virus first appeared in 1951 and was identified as “A” Alpargaton. A new strain of type “A” virus, identified as “A” Táchira, appeared in 1954 and spread throughout Venezuela by 1957. In 1962 a strain identified in Venezuela as “A” Zulia was isolated and later classified in the FMD World Reference Laboratory as type “A” subtype “A”18. This virus had epizootiologic and serologic characteristics totally different from the “Táchira” strain.

Late in 1969 FMD appeared in the state of Bolívar, which previously had been free of the disease. This virus was identified in our Laboratory as “A” Venezuela

---

*Sección de Enfermedades Vesiculares, Instituto de Investigaciones Veterinarias, Centro Nacional de Investigaciones Agropecuarias, Ministerio de Agricultura y Cría, Apartado 70, Maracay, Estado Aragua; Venezuela.

**Director Instituto Investigaciones Veterinarias, Centro Nacional de Investigaciones Agropecuarias, Ministerio de Agricultura y Cría, Apartado 70, Maracay, Venezuela.

Profesor of Epizootiology, Facultad de Ciencias Veterinarias, Universidad Central de Venezuela, Apartado 4563, Maracay, Estado Aragua; Venezuela.
70 (7) and was designated subtype "A"\textsubscript{32} by the FMD World Reference Laboratory.

This report presents data from studies of the most recent isolations of FMD strains in Venezuela.

**Materials and Methods**

**Virus:** The following strains of FMD virus were used in these studies:

a) "O"\textsubscript{1} Bolívar strain was isolated on a cattle ranch in the state of Bolívar where vaccine had not been used during the previous two years. This virus was passed three times in BHK-21 cells before testing.

b) "O" Manamito strain which was isolated from cattle in the Delta Amacuro Territory with no history of FMD vaccination, was passaged several times in suckling mice and finally 3 times in BHK-21 cells.

c) Control strains of type "O" commonly used in our laboratory are identified as: "O"\textsubscript{3} Lara (BHK-3); "O"\textsubscript{1} Cura 66 (BHK-9) "O"\textsubscript{1} Cura 71 (BHK-5); "O"\textsubscript{1} Campos (BHK-3); and "O"\textsubscript{1} Carora 72 (BHK-7).

d) Strains of type "A" virus used originated from isolates obtained in different regions of Venezuela and are identified by the following numbers: 10998 (Monagas), 11004 (Lara), 11044 (Guárico), 11088 (Zulia), 11104, 11113 and 11114 (Bolívar).

**Antiserums:** The antiserums used in these studies were prepared in our laboratory from guinea pigs immunized with "O" Manamito or "A"\textsubscript{32} antigen. Control antiserums for "O"\textsubscript{1} and "O"\textsubscript{3} antigen were supplied by the Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brasil.

The "O"\textsubscript{1} and "A"\textsubscript{32} control antiserums were produced in our laboratory using previously described procedures (4).

Type "A" antiserums, other than "A"\textsubscript{32}, used in this study, were also supplied by the PanAmerican Foot-and-Mouth Disease Center.

The specificity of each antiserum was confirmed by complement fixation tests using two 50% hemolytic units of complement. Optimal concentration of antigen was determined using two-and a-half 50% hemolytic units of complement. For differentiation of the viruses, complement fixation procedures described by Camargo et al (2) and by Brooksby (1) were used.

**Experimental Animals:** Six-day-old suckling mice were used for passage and titration of virus as well as for serum protection tests.

Fully susceptible, sero-negative cattle from the state of Bolívar were used in pathogenicity tests.

**Results**

Complement fixation data shown in Table I and Figure I indicate that "O" Manamito is serologically distinct from "O"\textsubscript{3} Lara and the "O"\textsubscript{1} strains tested.

Cross fixation ratios (Figure II) obtained from reactions of "O" Manamito antiserum with the same heterologous strains of type "O" viruses shown in Table I also indicates that "O" Manamito is a distinctly different strain of type "O" virus.

Results presented in Figure III indicate relationships between the strain "O"\textsubscript{1} Bolívar and "O"\textsubscript{1} strains already known in Venezuela ("O"\textsubscript{1} Cura 66, "O"\textsubscript{1} Cura 71 and "O"\textsubscript{1} Carora 72).

Table II presents results of pathogenicity and infectivity studies of strain "O"\textsubscript{1} Bolívar in cattle and suckling mice. Infectivity titers in suckling mice ranged from
10^7.3 to 10^9.1 LD\textsubscript{50}/ml. Cattle inoculated in the epithelium of tongue (IDL) with 10^4 mouse LD\textsubscript{50} of original virus showed local (Tongue) and generalized lesions (feet) in 24 and 48 hours respectively.

Table III presents data from cross immunity studies between virus "O"\textsubscript{1} Cura 71 and "O" Manamito using the procedure described by Cunha (3).

Results obtained with type "A" strains of FMDV (Table IV) show the Monagas and Lara isolates to have a broad serologic spectrum relative to other type "A" viruses including subtype "A"\textsubscript{27} which has been detected in some states of Venezuela in 1974.

On the other hand the Guarico and Zulia isolates and those from the state of Bolivar tended to be less broad and identified more closely with subtype "A"\textsubscript{32}. Homologous antiseraums against these new type "A" isolates are being prepared in order to complete these studies.

Discussion

Use of the complement fixation test, serum-protection test, cross immunity assays and cross fixation ratio studies (1), facilitated the identification and characterization of FMD virus field strains. This information is critical in the selection of the most effective FMD virus strains for vaccine production. Previous work by Jimenez et al (4) demonstrated broad antigenic variation among the "O"\textsubscript{1} strains of FMD virus. Our results indicate that the "O" Manamito strain has all the traits of a new subtype of type "O" FMD virus and thus has been submitted to the World Reference Laboratory at Pirbright, England for final verification.

Recent isolates of type "A" virus in Venezuela indicate that subtype "A"\textsubscript{32} is the only one present in Bolivar state but it is also present in other states throughout the country. Other strains of type "A" virus are also present in Venezuela but not in Bolivar state e.g. Monagas and Lara isolates which appear to be more closely related to "A"\textsubscript{27}. This fact complicates the sanitary control of FMD virus in the field and has stimulated an intense effort in our laboratory to further characterize the antigenic variants isolated in Venezuela.

Acknowledgements

The authors gratefully acknowledge the generosity of Dr. Harry Cunliffe and Dr. John W. Mc. Vicar for their comments and revision of this paper.
### TABLE I

**RESULTS OF COMPLEMENT FIXATION TESTS ON VARIOUS TYPES OF FOOT AND MOUTH DISEASE VIRUS ISOLATED IN VENEZUELA**

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>O. LARA</th>
<th>O. CURA 66</th>
<th>O. CURA 71</th>
<th>O. CAMPOS</th>
<th>O. CAR. 72</th>
<th>O. MANAMITO</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. LARA</td>
<td>100 +</td>
<td>47</td>
<td>43</td>
<td>94</td>
<td>49</td>
<td>7</td>
</tr>
<tr>
<td>O. Cur. 66</td>
<td>35</td>
<td>93</td>
<td>71</td>
<td>92</td>
<td>66</td>
<td>2</td>
</tr>
<tr>
<td>O. Cur. 71</td>
<td>69</td>
<td>97</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td>O. Campos</td>
<td>32</td>
<td>92</td>
<td>100</td>
<td>100</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>O. Car. 72</td>
<td>48</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>O. Manamito</td>
<td>77</td>
<td>89</td>
<td>94</td>
<td>100</td>
<td>90</td>
<td>100</td>
</tr>
</tbody>
</table>

+ Percent complement fixation

### TABLE II

**INFECTIVITY AND PATHOGENICITY OF O. BOLIVAR STRAIN OF FOOT AND MOUTH DISEASE VIRUS ISOLATED IN VENEZUELA**

<table>
<thead>
<tr>
<th>VIRUS ISOLATED</th>
<th>Suckling Mouse Titers</th>
<th>Pathogenicity 24 hour</th>
<th>For cattle 48 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. P.S 10865 (Bolivar)</td>
<td>$10^{7.9+}$</td>
<td>T</td>
<td>4 F</td>
</tr>
<tr>
<td>O. Exp-P.S. 10865 l bov.</td>
<td>$10^{9.1}$</td>
<td>T</td>
<td>4 F</td>
</tr>
<tr>
<td>O. Exp-P.S. 10865 l bov.</td>
<td>$10^{8.6}$</td>
<td>T</td>
<td>4 F</td>
</tr>
<tr>
<td>O. Exp-P.S. 10865 l bov.</td>
<td>$10^{8.8}$</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>O. Bolivar BHK 1</td>
<td>$10^{7.9}$</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>O. Bolivar BHK 2</td>
<td>$10^{8.0}$</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>O. Bolivar BHK 3</td>
<td>$10^{7.3}$</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

+ 50% suckling mouse LD/50
T Local lesion in tongue
4F Lesions in 4 feet
ND Not done
### TABLE III

RESULTS OF CROSS IMMUNITY TESTS OF VIRUS "O" MANAMITO IN SUCKLING MICE

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>ANTISERUM O₁ Cura 71</th>
<th>&quot;O&quot; Manamito</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₁ Cura 71</td>
<td>&gt; 3.16*</td>
<td>2.40</td>
</tr>
<tr>
<td>&quot;O&quot; Manamito</td>
<td>4.17*</td>
<td>4.50</td>
</tr>
</tbody>
</table>

* Serum protection index in suckling mice expressed as Log (base 10)/0.1 ml. of serum

### TABLE IV

Results of complement fixation tests on several Type A Foot and Mouth Disease virus isolated in Venezuela

<table>
<thead>
<tr>
<th>Virus A isolates</th>
<th>ANTISERA A10</th>
<th>A11</th>
<th>A12</th>
<th>A24</th>
<th>A25</th>
<th>A26</th>
<th>A27</th>
<th>A29</th>
<th>A30</th>
<th>A31</th>
<th>A32</th>
</tr>
</thead>
<tbody>
<tr>
<td>10998 – Monagas</td>
<td>77</td>
<td>95</td>
<td>91</td>
<td>93</td>
<td>95</td>
<td>82</td>
<td>99</td>
<td>83</td>
<td>89</td>
<td>95</td>
<td>69</td>
</tr>
<tr>
<td>11004 – Lara</td>
<td>68</td>
<td>97</td>
<td>72</td>
<td>97</td>
<td>93</td>
<td>78</td>
<td>99</td>
<td>65</td>
<td>80</td>
<td>92</td>
<td>72</td>
</tr>
<tr>
<td>11044 – Galrico</td>
<td>49</td>
<td>90</td>
<td>82</td>
<td>57</td>
<td>90</td>
<td>21</td>
<td>69</td>
<td>62</td>
<td>51</td>
<td>69</td>
<td>96</td>
</tr>
<tr>
<td>11088 – Zulia</td>
<td>14</td>
<td>59</td>
<td>22</td>
<td>15</td>
<td>30</td>
<td>30</td>
<td>44</td>
<td>10</td>
<td>25</td>
<td>55</td>
<td>65</td>
</tr>
<tr>
<td>11104 – Bolivar</td>
<td>3,4</td>
<td>46</td>
<td>27</td>
<td>21</td>
<td>22</td>
<td>7</td>
<td>28</td>
<td>25</td>
<td>3</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>11113 – Bolivar</td>
<td>64</td>
<td>65</td>
<td>41</td>
<td>45</td>
<td>52</td>
<td>2.1</td>
<td>68</td>
<td>97</td>
<td>79</td>
<td>38</td>
<td>83</td>
</tr>
<tr>
<td>11114 – Bolivar</td>
<td>2</td>
<td>41</td>
<td>21</td>
<td>4</td>
<td>30</td>
<td>1</td>
<td>37</td>
<td>65</td>
<td>1</td>
<td>2</td>
<td>87</td>
</tr>
</tbody>
</table>

* Percent of complement fixation
COMPLEMENT FIXATION RESULTS OBTAINED BY REACTIONS BETWEEN O MANAMITO ANTISERUM AND VARIOUS TYPE O VIRUSES

![Bar chart showing complement fixation results for various type O viruses with different percentages of CF. O3 LARA, O1 CU66, O1 CU71, O1 CAMPOS, O1 CAR 72, O MANAMIT.](https://example.com/bar-chart.png)
FIGURE II

CROSS FIXATION RATIO OF SERUM O MANAMITO

\[ R = 100 \sqrt{\frac{Y}{X}} \]

\begin{center}
\begin{tabular}{ccccccc}

\textbf{VIRUS} & \textbf{O3 LARA} & \textbf{O1 CU66} & \textbf{O1 CU71} & \textbf{O1 CAMPOS} & \textbf{O1 CAR72} & \textbf{O MANAM} \\

\hline

\textbf{R VALUES} & 20 & 10 & 40 & 30 & 20 & 100 \\

\end{tabular}
\end{center}
FIGURE III

COMPLEMENT FIXATION RESULTS OBTAINED BY REACTION BETWEEN O1 BOLIVAR VIRUS AND TYPE O ANTISERUMS

1 = O1 CURA 1.966
2 = O1 CURA 1.971
3 = O1 CARORA 1.972
ANTIGENIC VARIATION

REFERENCES


SWINE VESICULAR DISEASE:
VIRUS SURVIVAL IN PORK PRODUCTS

P. D. McKercher, J. H. Graves, J. J. Callis, and F. Carmichael*

INTRODUCTION

Swine vesicular disease (SVD) is a contagious viral disease of swine clinically indistinguishable from foot-and-mouth disease, vesicular stomatitis, vesicular exanthema of swine, and infection by San Miguel sea lion virus. SVD was described by Nardelli et al.1 in 1968 and has since been identified in England, Italy, Austria, Poland, France, Japan, and Hong Kong. Investigations of outbreaks incriminate the feeding of garbage contaminated with SVD virus (SVDV)-infected meat scraps as a major source of infection.

The serological relationship between SVDV and Coxsackie B-5 virus suggests the possibility of transmission of the virus between man and swine2 although Brown et al3 have shown that Coxsackie B-5 virus can be clearly distinguished from SVDV by immunodiffusion tests. Three workers at the Animal Virus Research Institute, Pirbright, Surrey, England, engaged in experiments with SVDV became ill, and tests of their serums taken during and after the illness suggested that the causative agent could be SVDV.

The possibility of human infection with SVDV and the susceptibility of the United States swine population to infection with this virus have led to careful consideration of importation of pork products from countries where SVD exists. Products imported into the United States that might be potential hazards for the spread of SVD include pasteurized cooked canned pork products such as hams which are labeled “Perishable Keep Under Refrigeration”, dry salami sausage, dry pepperoni sausage, and natural hog casings.

The stability of the virus is such that it is not inactivated by the acid changes that occur in the musculature after death; thus, the virus can be expected to withstand the various processes used in the production of dry sausages such as salami. Infectivity decreases little or not at all in cold storage, and thus uncooked pork and pork products could remain a hazard indefinitely.4

MATERIALS AND METHODS

Source of Meat: Cross-bred Tamworth swine varying in weight from 125 to 300 pounds were infected by intravenous inoculation of SVDV UKG 24/72 strain and slaughtered at either 48 or 72 hours after inoculation when all had severe clinical signs of SVD. Previous studies indicated this period best for recovering virus from swine muscle that would be in products such as hams,

From the Plum Island Animal Disease Center, Northeastern Region, Agricultural Research Service, U.S. Department of Agriculture, Greenport, New York11944.

“Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.”

belly, and flank parts used for sausages. Because space limitations permitted the production of only 1 or 2 products at a time, 4 or 5 swine were infected at different intervals when products were to be prepared. Carcasses were kept at 39 F for 48 hours after slaughter.

In the following experiments the meat used for salami and pepperoni preparations was treated for possible live Trichinae according to method #1 as prescribed in section 318.10 of the Meat Inspection Regulations.

A. Preparation of hams:
After deboning and removal of excess fat, hams weighing approximately 2 pounds were injected with a 16% brine solution by weight and submerged in brine at 39 F for 24 hours. The hams were then tightly packed in cans, and the cans were sealed. Some of the canned hams were submerged in a water bath and an equal number were retained as unheated controls. The temperature of the water in the bath was 129 F, and the internal temperature of the ham as determined by a thermocouple in the center was 57 F. The bath temperature was gradually increased so that in approximately 5 hours the internal temperature of the hams reached 156 F. The hams were then placed in a cooling bath for 2½ hours, at which time the internal temperature had reached 44 F (Fig. 1). The processed canned hams were then stored at 39 F.

Hams, shoulders, flanks, and belly parts were ground for preparation of salami. The meat, in 100- to 140-pound lots, was ground mixed to a ratio of 80% lean and 20% fat and stored at 39 F. It was then thoroughly mixed with recommended amounts of salt, sugar, dextrose, white pepper, garlic, sodium nitrite, and sodium nitrate. After a 72-hour curing period at 39 F, a lactacel (lactic acid) starter culture (Merck and Co., Inc., Rahway, New Jersey) was thoroughly mixed with the meat. The mixture was stuffed into synthetic casings previously soaked in water. Salami sausages were stuffed in 3-inch casings to a weight of 2 to 3½ pounds and in .75-inch casings to a weight of ½ pound. After stuffing, they were held in a "green room" at 68 F dry bulb, 64 F wet bulb for 24 hours and then exposed to smoke produced from hickory chips as follows:

12 hours — 90 F dry bulb
12 hours — 85 F wet bulb
4 hours — 100 F dry bulb
4 hours — 95 F wet bulb

The sausages were then removed from the smokehouse, washed with warm water to remove surface juices and fat, and then placed in the drying room. The drying room was maintained at 53 to 55 F and a relative humidity of 72 to 74%. The products were held in the drying room for a minimal time of 25 days. The pH of the meat was determined at the time of grinding, after mixing, and in the finished product.

B. Preparation of dry salami sausage:
Hams, shoulders, flanks, and belly parts were ground for preparation of salami. The meat, in 100- to 140-pound lots, was ground mixed to a ratio of 80% lean and 20% fat and stored at 39 F. It was then thoroughly mixed with recommended amounts of salt, sugar, dextrose, white pepper, garlic, sodium nitrite, and sodium nitrate. After a 72-hour curing period at 39 F, a lactacel (lactic acid) starter culture (Merck and Co., Inc., Rahway, New Jersey) was thoroughly mixed with the meat. The mixture was stuffed into synthetic casings previously soaked in water. Salami sausages were stuffed in 3-inch casings to a weight of 2 to 3½ pounds and in .75-inch casings to a weight of ½ pound. After stuffing, they were held in a "green room" at 68 F dry bulb, 64 F wet bulb for 24 hours and then exposed to smoke produced from hickory chips as follows:

12 hours — 90 F dry bulb
12 hours — 85 F wet bulb
4 hours — 100 F dry bulb
4 hours — 95 F wet bulb

The sausages were then removed from the smokehouse, washed with warm water to remove surface juices and fat, and then placed in the drying room. The drying room was maintained at 53 to 55 F and a relative humidity of 72 to 74%. The products were held in the drying room for a minimal time of 25 days. The pH of the meat was determined at the time of grinding, after mixing, and in the finished product.

C. Preparation of dry pepperoni sausage:
Meat was prepared as for the salami sausage and thoroughly mixed with salt, sugar, dextrose, sodium nitrate, sodium nitrite, cayenne pepper, pimento, aniseed, garlic powder, and pepperoni pepper and held for 48 hours at 39 F. The temperature was then lowered to 32 F and the lactacel
starter culture was added and thoroughly mixed with the meat. This mixture was stuffed into 1- to 1½-inch casings as twin links of 10 to 12 inches in length and weighing about ½ pound each. The sausages were held in a “green room” at 68 °F dry bulb, 64 °F wet bulb for 48 hours and then placed in the smokehouse for 8 hours at a dry heat of 90 °F and relative humidity of 85%. They were then placed in the drying room at 53 to 55 °F and a relative humidity of 72 to 74% for at least 16 days.

D. Preparation of intestinal casings:

Intestines were removed from SVD-infected swine similar to those from which the meat was used for the various product preparations. The intestines were hand stripped and placed in water at 108 °F for 30 minutes, flushed with cold water (70 °F), and scraped with a plastic scraper until white inside. The casings were then placed in saturated brine solution, packed in ice, and placed in a 39 °F holding room; they were then flushed with water and used or packed in salt and stored at 39 °F until required.

Pasteurized cooked hams, dry salami, and pepperoni sausage were also prepared from noninfected swine in order to determine the characteristics of the products in respect to color, texture, consistency, and taste. Three batches of each product were prepared, once from noninfected swine and twice from infected swine.

Virus: SVDV was obtained from the Animal Virus Research Institute at Pirbright, as epithelium from swine involved in the first outbreak in Staffordshire and designated UKG 24/72. It was passaged once in swine and twice in pig kidney cell line IBRS-2. Swine from which products were prepared were inoculated intravenously with 2 ml of tissue culture fluid at a titer of 10⁷⁻⁰/ml.

Antiserum: Antiserum was prepared by hyperimmunizing guinea pigs with the SVDV produced in the brains of newborn mice. Complement-fixation tests showed this to be SVD specific. The neutralizing index of the swine serum was determined in cultures of IBRS-2 cells in Falcon flasks (Falcon Plastics, Division of BioQuest, 1950 Williams Drive, Oxnard, California) or in microtiter plates; a constant serum dilution mixed with varying dilutions of virus was used.

Virus Assay: The virus content of the meat samples was determined by inoculating small flasks (25 cm) of IBRS-2 monolayer cultures with 0.2 ml of the required dilution and absorbing for 30 minutes. Final examination of the cultures was at 72 hours, at which time the virus content per gram of meat was calculated and the virus type was confirmed by serum neutralization.

Feeding Tests: Swine used for feeding the test products varied in size and weight in different tests but within each test were of similar size and weight. Their weights varied from 125 to 300 pounds. Generally 9 swine were used per feeding test — 3 swine kept in separate rooms for each dose level fed. Doses fed were either 1, 4, and 16 ounces, or 2, 8, and 32 ounces. Food was withheld for 24 hours before the test to assure that the swine were sufficiently hungry to eat the test samples. Blood samples were collected at 0 and 14 days after feeding, their temperatures were taken daily, and the swine were examined periodically for clinical signs of SVD. Each test was concluded after 14 days of observation. In later tests, contact swine were added to each room to detect virus that might be shed by the animal that ate the sample because in some of the earlier tests, swine
developed specific antibody without having had apparent signs of disease.

Feeding tests were conducted with the hams that had been processed through 156 F and with those kept as controls. Samples from the hams were also tested in cultures of IBRS-2 cells for virus isolation.

The virus content of dry salami and pepperoni sausage was determined before mixing, after mixing, and before stuffing of the casings.

RESULTS

Virus content of the meat from infected swine varied from a titer of $10^3$ to $10^{4.5}$ per gram.

Results of feeding tests varied considerably. In some tests, the swine became infected when fed 2 ounces of infected meat and in other instances when fed 32 ounces. In one feeding test, the animals did not show any clinical manifestations of SVD; however, 5 of 18 developed neutralizing antibody; 3 of these 5 were contact swine. Six feeding tests involving 39 swine were used to indicate that swine may become infected when fed products from meat from infected animals. However, in these tests, only 12 of the 39 swine showed clinical evidence of SVD.

Virus isolations in tissue culture are indicated in Fig. 2. Virus was not recovered from hams after their internal temperature had reached 156 F — the temperature to which these products are heated commercially. However, virus was recovered from dry salami sausage, dry pepperoni sausage, and intestinal casings for at least 200 days throughout the processing period.

The pH of the meat at the time of processing was 5.6 to 5.7, but dropped to 4.5 and remained relatively unchanged through the 200-day test period.

Control products made from meat from noninfected swine proved comparable to those produced commercially. The moisture protein ratio of the processed dry pepperoni was 1.6/1 and 1.9/1 for the dry salami. The water activity for both was approximately .85.

DISCUSSION

The equipment used to produce the test products was modified at this Center from existing equipment and was of limited capacity. However, all equipment and the products produced were inspected and found to be satisfactory for experimental use by a representative of the Meat and Poultry Inspection Program, USDA Technical Services. The objective of the experiment was to determine whether SVDV would survive in products of infected pigs prepared similarly to those exported to the United States from other countries. The products were prepared from the carcasses of swine infected with SVD to determine which, if any, of the treatments is detrimental to the virus. From our experiments, it would appear that the time of slaughter is extremely critical; virus titers are highest from 2 to 3 days after virus inoculation. Burrows et al. reported peak virus concentrations in samples collected 2 to 5 days after inoculation. This discrepancy might be accounted for by the different method of inoculation; i.e., foot versus intravenous. Approximately 700,000 TCID$^{50}$ of SVDV are required to produce clinical manifestations of the disease in swine. The highest titer obtained per gram of meat used for producing the experimental pork products was $10^3$ to $10^{4.5}$. 
In general, the assumption could be made that the amount of SVDV found in pork products would be very small unless they were prepared from meat from a herd undergoing active infection or possibly from one having inapparent infection. The virus does not appear to be appreciably affected by any of the commercial processing procedures except heating above 156 F, and thus could remain viable for long periods of time in contaminated meat scraps. The opinion that SVDV could be expected to withstand the various processes used in the production of sausages and salami is also shared by British workers. Thus, imported pork products produced in countries where SVD is present are potential sources of infection to a susceptible swine population. Cured and dried products originating from countries where SVD is present are not permitted entry into the U.S. except for further processing by heating to an internal temperature of 166 F.

Acknowledgements: The authors wish to thank Messrs. William Doroski and Nicholas Shout for their excellent technical assistance, Dr. G. R. Snyder, Meat Group, Inspection Standards and Regulations Staff, Technical Services, for his advice and cooperation in making consultant personnel available, and Mr. Truman Cook and his staff of Engineering and Plant Management for technical support in modifying and adjusting available processing equipment.
Fig. 1. Curve indicates temperature range for treatment of pasteurized cooked, canned hams.

Fig. 2. Infectivity (measured by cytopathogenic effect) curves indicating isolation of virus from dried pepperoni and salami sausages and intestinal casings.
REFERENCES

REPORT OF THE COMMITTEE ON FOREIGN ANIMAL DISEASES

Chairman: T. G. Murnane, Bethesda, Md.
Co-Chairman: H. Q. Sibley, Austin, Texas


The annual meeting of the Committee on Foreign Animal Diseases was held in open sessions on 15 and 16 October 1974. Twenty-three of the 35 man committee membership participated in these sessions. The committee also met in July 1974 in conjunction with the American Veterinary Medical Association.

The committee addressed itself primarily to the status of exotic diseases which pose a threat to the health of livestock on the North American Continent particularly the United States and to the research pertinent to these diseases.

Last year this committee reiterated the necessity for further action to secure the participation of the United States, as an official member, in the Office of International Epizootics. The committee wishes to commend Mr. Olin Timm, President of the USAHA, and the many livestock organizations, i.e., the American National Cattlemen's Association, American Horse Council, National Wildlife Federations, National Wool Growers, and the Poultry and Egg Institute who responded to his request to communicate with the US Department of State on this vital matter. The committee believes these efforts have advanced significantly our cause.

The continued concern of the committee for improved safeguards to preclude entry of exotic agents into the United States was expressed and resolutions were proposed to intensify US/Mexico border surveillance and to amplify regulations regarding inspection for ectoparasites of all mammals and subhuman primates imported into the United States. The committee in conjunction with the committee on Infectious Diseases of Horses proposed a resolution urging continued surveillance of VEE.

The committee further expressed its support for continued research of the potential of certain indigenous wild animal species of North America to serve as hosts of exotic animal diseases.

The revision of the handbook Foreign Animal Diseases is over 80%
completed. The committee looks forward to submission of the completely revised handbook to the publishers in 1975.

During the past year there have been significant changes in status of exotic diseases of animals and important new developments in the research of these diseases.

THE WORLD EPIZOOTIOLOGY OF VESICULAR DISEASE

Foot-and-Mouth Disease (FMD) - Russia continued to have outbreaks caused by FMD virus type A22 during 1973. In Turkey, outbreaks due to the Asia-I virus have not been reported since September 1973, but type A22 and type 0 persist. Vaccination of all cattle, sheep and goats over 4 months of age is required in Thrace and Istanbul provinces. Czechoslovakia reported outbreaks of types 0 and C during the first half of 1973 while A22 and 0 were present in Greece during the latter half of the same year. Bulgaria reported FMD for the first time in 7 years and the disease occurred sporadically in Romania and Yugoslavia.

The outlook has, in general, been favorable in Western Europe. Spain had outbreaks involving types 0, A, and C but a marked decrease in incidence followed the institution of the slaughter policy early in 1973. During the spring and summer of 1973 Austria experienced its first outbreaks since 1966. France had a limited outbreak near the Spanish border in January of 1973 and another in the spring of 1974 in Normandy. The department of Finistere escaped the latter outbreak which was rather quickly brought under control by a combination of vaccination, slaughter, and restricted movement of animals and people. The last case occurred on May 22. An outbreak in Belgium in April and May was traced to vaccination. FMD due to virus Type C was diagnosed on the island of Jersey in February 1974. The 10 cattle involved were destroyed and no spread occurred. This was the first outbreak in the Channel Islands since 1957. Isolated outbreaks were reported in Switzerland after 4 years of freedom and the disease occurred sporadically in the Federal Republic of Germany, Italy, and Israel.

FMD occurred throughout much of Africa and, in several countries, virus types were identified which had not been reported there before. Virus type SAT-1 was isolated from cattle in Zambia where this type had not been recorded since 1956. The same type was also isolated from wild buffaloes in the area. In November of 1973 virus type SAT-1 was isolated from impala in an outbreak in South Africa's Kruger National Park. No cattle were involved but 4 months later a limited outbreak occurred in cattle near the park, the first such outbreak since early 1971.

Early in 1973 outbreaks of FMD caused by type A virus were reported from areas in Thailand and Western Malaysia previously free of the disease.

FMD continued to be enzootic in South America, although the number of outbreaks was considerably reduced from 1973. Epidemics did occur in Chile, Western Paraguay and in Northern Brazil. Subtype A22, FMDV was identified. An extensive outbreak of type C FMDV occurred in Paraguay (Chaco Region). It is believed that the disease was introduced from Brazil. From Paraguay the disease spread into Argentina probably by cattle movement.
In August 1973 FMD occurred in Guyana close to the border with Brazil. Apparently the outbreak was related to illegal movement of cattle from Brazil. The outbreak was controlled by vaccination and strict isolation procedures. The last case in the area occurred in March 1974.

Pan American Highway

In May 1972 the governments of Panama and the United States signed an agreement to cooperate in the prevention of FMD in Panama and eradication if the disease should occur. The Panamanian Director and U. S. Co-director of the program have been named and a field program has been initiated. Control posts are being established, patrol boats have been purchased, inspection personnel have been hired and the program is getting underway.

An agreement has also been signed by Colombia and the United States. The purpose of this agreement is to control and eradicate FMD in certain portions of Northwest Colombia and to prevent dissemination of the disease from Colombia as a result of the construction of the Pan-American Highway through the so called "Darien Gap". Completion of this highway will for the first time provide an overland connection between Panaman and Colombia thus completing the highway link between North and South America. The program got underway in February 1973 but has progressed slowly and has not achieved the goals agreed upon by the two countries. Administrative difficulties in Colombia have slowed purchases of equipment, building of quarantine facilities, movement of cattle herds located near the border and other similar activities.

The danger associated with building the highway is not only movement of products and possibly livestock on the highway, but also the development of the livestock industry in Colombia immediately adjacent to the border. That development has already started. Plans call for limiting this development and moving those herds that are close to the Panama border and near the proposed highway right-of-way.

There are approximately 20,000 head of livestock in Colombia within 25 miles of the Panama-Colombia border. Within this area the following actions are planned:

1. Regular inspection of all livestock.
2. Accurate census and identification of all livestock.
3. Eradication by slaughter of any outbreaks which may occur.
4. Laboratory tests to determine if livestock in the area are exposed.
5. Establishment of quarantine posts to prevent illegal entry of livestock into the area.
6. Study of wildlife populations to determine their susceptibility to FMD and to find out if they have been exposed to the disease.
7. Suspension of FMD vaccination in the area.
8. Control of the importation of breeding animals including a requirement that they come from FMD free areas or farms.

During the last two years two outbreaks of FMD have occurred near the border. Although the origin of these outbreaks was not officially announced it is believed the virus was introduced with breeding animals introduced from
infected areas of Colombia. The infected herds were slaughtered and buried and the premises were cleaned and disinfected.

Construction of those portions of the highway already in progress will continue, however, no new sections will be started until an adequate FMD prevention program has been instituted.

*Vesicular Stomatitis (VS)* - The appearance of VS outside of the Western Hemisphere is certainly rare and perhaps difficult to verify but sporadic occurrences were reported during 1973 in China, Nepal, Saudi Arabia, and Mozambique. Central American and the northern countries of South America again reported the highest incidence of the disease.

*Swine Vesicular Disease (SVD)* - The virus of SVD was identified in samples from Great Britain, France, Switzerland, Austria, Italy, Poland, Japan and Hong Kong. The disease was also reported in the Federal Republic of Germany. SVD continues to spread in Great Britain with new outbreaks in the summer of 1974 in the southwestern part of the country where it had previously been unreported. The disease has never been recorded in Northern Ireland. A full "stamping out" policy has been applied in Great Britain, The Federal Republic of Germany, Switzerland, and Japan. Limited slaughter was practiced in Italy, Austria, and France.

*Vesicular Exanthema of Swine (VES)* - This disease remains extinct, however, there are continued reports of isolations of viruses from marine mammals which closely resemble the VES virus. One such agent has been shown to produce vesicles much like those of VES when inoculated into domestic swine.

### RINDERPEST

Once the most devastating of cattle diseases, Rinderpest killed over 2 million head of cattle in Africa alone each year prior to 1950. Presently, the disease is indigenous to many parts of Asia, the Far East and Africa. Long a serious plague of tropical Africa, Rinderpest is now under control as a result of extensive vaccination campaigns using attenuated tissue culture vaccine. It is a tribute to Plowright's tissue culture-attenuated vaccine and all of those who contributed to and took part in the African vaccination campaign that the disease there is essentially under control. Members of this committee played a major role in the African immunization campaign.

The severe drought persisting in Sahelian, Africa, has so disrupted the cattle industry, that the vaccination of young animals has not been uniformly maintained with the result that small outbreaks were reported in 9 West African countries in 1973. Those countries were Chad, Upper Volta, Ghana, Niger, Mauritania, Mali, Ivory Coast, Dahomey & Nigeria. So far in 1974, one additional outbreak has been reported in Niger, and 1 in Ghana.

The highest incidence continues in India where 135 new outbreaks were reported between April and December 1973, and 10 more in January to February 1974.
There have also been 3 outbreaks in Lebanon and 2 reported in Viet Nam.

In view of the proven effectiveness of the attenuated tissue culture vaccine being used in Africa, there is an opportunity and need for a persistent world-wide eradication campaign. And, in view of the present limited distribution of the disease, a United Nations world-wide eradication campaign is urgently recommended. To let these few foci of infection persist is to leave the world's livestock, hence a major part of the world's food resources, remain exposed to the constant threat of destruction.

PESTE DES PETIT RUMINANTS (PPR)  
(PLAGUE OF SMALL RUMINANTS)

In view of the progress toward control of rinderpest, the committee wishes to invite attention to PPR, a disease of small ruminants which remains as a threat to the animal protein resources of West Africa. PPR is an acute or sub-acute virus disease of sheep and goats. It was first reported in 1942 in some of the previous French territories of West Africa where it persists today. Signs and lesions associated with frank clinical form of the disease are similar to those observed in cattle rinderpest. Mortality in goats may range from 10-90%. Sheep are less susceptible than goats, and the rate of recovery is significantly higher. In spite of the close resemblance of PPR disease signs, lesions and the viral properties of RV, cattle exposed to PPR do not exhibit any clinical reaction but do acquire a solid immunity to RV. An attenuated PPR virus vaccine obtained from repeated passage of PPR virus in embryo kidney cell culture is efficient in the protection of goats and sheep. Methods which are applied to RV eradication are useful in the eradication and control of PPR.

CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP)

Australis was officially declared free of CBPP on August 6, 1973. This marked the successful conclusion of the intensive national eradication campaign carried out over many years. The last case was recorded in 1967 and all vaccination has been prohibited since January 1972.

AFRICAN SWINE FEVER (ASF)

During 1973 African Swine Fever (ASF) was reported in Spain, Portugal, Malawi, Angola, South Africa and Mozambique. The number of outbreaks reported in the O.I.E. Monthly reports totaled: Spain 652; Portugal 482; Malawi 2; South Africa 12; Angola 10; and Mozambique 2.

The incidence in Spain and Portugal increased from September to December. From January through August 1973 the incidence decreased in both countries. The persistence of the disease in domestic swine in Spain and Portugal has markedly reduced the mortality commonly associated with the classical disease. France reported 2 outbreaks in January 74, 6 outbreaks in February and 2 outbreaks in March. Each outbreak was quickly eradicated. After freedom from the disease for 6 years, outbreaks of ASF were reported
from the Island of Madeira during February 1974. Total outbreaks reported
in the O.I.E. Monthly reports from January through August 1973 were:
Apain 197; France 10; Portugal 79; South Africa 2; Angola 4; Malawi 1
and Mozambique 1.

VENEZUELAN EQUINE ENCEPHALITIS

Minimal occurrence of disease in equidae was reported in Colombia and
Venezuela; Mexico continued the extensive vaccination campaign with over
3.5 million doses of vaccine administered; and for the third consecutive year,
results of surveillance activities in the United States were negative. Re-
portedly such surveillance is to be greatly curtailed next year; this curtail-
ment is of utmost concern to members of this committee, who feel that it is
premature to assume that the negative findings to date are sufficient indica-
tors that the virus has been eliminated from the ecology of Mexico and Texas.
Such a conclusion is not in keeping with the history of this disease.

RESEARCH ON VESICULAR DISEASES

Swine Vesicular Disease (SVD)

A bibliography of SVD has been compiled by R. J. Uskavitch.1
At 5°C. at a pH of 7.54 the virus survived without loss of titer for 164
days, but at pH values of 2.88 and 10.14 more than 6 log units of virus were
lost by 164 days. The virus was inactivated in 30 minutes at 56°C and in
2 minutes at 60°C. In feces the virus was viable at 138 days at 12 to 17°C., but not
viable at 200 days when temperature rose to 25°C.3

A serological relationship by virus neutralization tests has been establish-
ed between swine vesicular disease virus (SVDV) and the human pathogen
Coxsackie B5 virus.4 Antigenic differences can be shown between the
Italian, UK, French and Hong Kong strains of SVDV and Coxsackie B5
virus is immunodiffusion tests in agar. Immunodiffusion was used to verify
that 2 human laboratory infections were due to SVDV infection and not
Coxsackie B5 virus. Antigenic differences are even being noted between
isolates of the 1972-1973 UK outbreaks collected 6 months apart.5

Pigs in contact with experimentally inoculated animals were killed at
intervals before the appearance of clinical disease. The distribution and
amounts of virus in various tissues indicated entry of virus through the skin
and the mucosa of the digestive tract.6 Attempts to recover virus from
feces of convalescent pigs for to 9 weeks after infection by Italy/66 strain
were unsuccessful.7

Airborne excretion of SVDV appears to be 160-fold less than foot-and-
mouth disease virus (FMDV) infected pigs. Excretion is more likely from
lesions of the skin than from the respiratory tract.8 The incubation period
varies between 3 and 7 days and the emission of virus can be as early as
the second day after infection and is usually completed within 8 days of the
appearance of lesions, but may persist up to the fourteenth day. No virus
carrier state has been found. Virus is shed in high titer from vesicular
fluid, but high concentrations of virus have also been obtained from skin
surfaces and excretion of the virus through the skin may occur. Feces
contain an appreciable quantity of virus. Morbidity is high (up to 100%) in affected pens but spread to other pens depends mainly on mechanical transmission of infected material.9

Pigs inoculated intravenously with SVDV (Hong Kong strain) developed diffuse encephalomyelitis. Lesions (perivascular cuffing with lymphocytes and formation of neuroglial cell foci) were most severe in the diencephalon, mesencephalon, metencephalon, and myelencephalon. Encephalomyelitis was of mild to moderate intensity and was most severe on the sixth day after inoculation.10

The fluorescent antibody (FA) technique was used to detect virus in tissue cultures inoculated with suspensions of vesicular epithelium from lesions. Positive results were obtained after 3 h from vesicles less than 5 days old and after 3 to 5 h from lesions 6 to 10 days old. The infectiousness of vesicular material declined from approximately $10^{10}$ plaque forming units/gram (pfug) in vesicles 1 day old to $10^6$ pfug in lesions 10 days old.11

French researchers feel that vaccination against SVDV is possible and may be a valuable tool.12

Vesicular Stomatitis (VS)

A bibliography of Vesicular / Stomatitis Virus (VSV) October 1972 to September 1973 was compiled by R. Uskavitch.13

At least 98% agreement was obtained on typing (VS) isolates from bovine epithelium by FA technique when compared to the complement fixation (CF) and/or the virus isolation (VI) and neutralization techniques.14

Epidemiological inquiry is underway in Brazil to establish the presence of antibodies of 5 viruses of VSV group (N.J., Indiana 1, 2, 3, and Cocal).15 Indiana and N.J. subtypes neutralizing antibodies have been found in Colombian rodent serum survey.16 Attempts continue to characterize factors influencing the growth of Cocal virus, closely related serologically, to the Indiana serotype, in vitro in continuous mosquito cell lines.17

A non-antibody inhibitor of VSV was found in low levels in most lots of fetal calf serum.18

Vesicular Exanthema of Swine (VES)

Several virus isolates from Pinnipeds appear to have a host range, morphology and physio-chemical properties identical to vesicular exanthema of swine virus (VESV).19,20 Serological evidence exists of transmission of virus between marine animals and feral swine on Santa Cruz Island off California.21 Viral agents recovered from oral and fecal swabs of sea lions in California had electron micrographic profiles identical to those of VES. Intradermal inoculations into the snouts, lips, and feet of pigs resulted in development of fever, pustules, vesicles, erosions and skin necrosis. Lesions appeared in the mouth and feet of suckling piglets from a sow with lesions on the mammary and teat skin. However, contact exposure mostly resulted in a transient fever and inapparent disease. Neutralizing antibodies were detected 7 DPI reaching high levels about 21 DPI. Precipitin antibodies were common to different sea lion isolates but the neutralizing antibodies were serotype specific. The range of reciprocal virus neutralizing indices
between the sea lion viruses and the 11 known VES virus types was 0.0-1.5 log units. It is known that serologic relations or similar range exists between the known types of VES viruses. Therefore, sea lion viruses may be considered new VESV types.22

Foot-And-Mouth Disease (FMD)

The species particularly implicated as a carrier of foot-and-mouth disease virus in Africa is the cape buffalo which curiously is rarely seen to exhibit lesions of the disease; however, older animals transmit the infection to young buffalo soon after maternal immunity has waned. The system would seem to be self-perpetuating in that some herds of buffalo have been found to be infected at approximately the same level over many years.23 Significant antibody titers to all 3 SAT types were demonstrated in serum samples; however, there is no evidence of transmission from buffalo to cattle.24,25

An African elephant (Loxodenta Africana) showed clinical disease upon inoculation, but no transmission of the disease to other elephants.26 Mechanical transmission of FMDV was reported by starling to cattle.27 Of 3 types of deer - red, fallow and roe - only roe were severely infected with FMDV in studies done in England. Aerosol virus quantity during clinical disease was comparable to that recorded for cattle and sheep. The longest carrier state was reported in the species that showed the least clinical involvement, the fallow deer.28,29

No foot lesions were noted in sheep; and disease signs are such, that the disease might go unnoticed by farm workers. Seasonal movements of sheep bands may be of importance in certain parts of the world in spreading the disease.30 In one study of the carrier state in the Brazilian state of Sao Paulo where FMD had not been reported for 6 months, oesophageal pharyngeal samples (OP) from 150 normal bovine destined for the abattoir were surveyed for FMDV. Seventy-nine were positive to VI, 49.4% for Type C, 38% for Type 0 and 12.6% for Type A.31

Of 5 bovine in 1 experimental trial, 3 developed high serum and OP fluid neutralizing antibody titers. Wehn challenged 30 weeks later with homologous virus there was no appreciable virus growth in their throats. Five other steers who had low neutralization titers in serum and OP fluid showed virus growth in their throats when challenged 30 weeks later.32

Evidence exists that infection with FMD and bovine enterovirus or bovine rhino-tracheitis may cause an alteration of typical clinical signs of FMD and/or a much delayed incubation period of the disease. Evidence of virus transencapsidation could explain these observations.33

Wind can convey infectious FMDV on dust particles 3 to 6 mm in size long distances. These particles would normally settle out in still air. Rain even may play a vital part in the spatial distribution of outbreaks. Some evidence points to wind distribution of protein molecules without natural support of dust particles or water droplets.35,36,37 Statistical analyses of the spread of FMD points to relative humidity and rainfall as being very important.38 Infectivity of aerosols from milk and fecal slurries containing virus at 55% relative humidity was 0.15 to 5% as compared to aerosols from saliva which was 0.0005 to 0.005%.39

Apparently the instability of virus in aerosols of saliva at high relative
Himidity is caused by an undefined dialyzable organic molecule which is sensitive to heating at 70°C.\textsuperscript{40}

Milk from cows showing no clinical signs of disease was a source of disease among pigs.\textsuperscript{41}

In cattle killed at 24 to 120 hours post inoculation, virus was recovered from the lymph nodes in most cases, from the lungs in 80% of cases and from the thyroid gland in one case. In no case was virus isolated from the skeletal muscle, heart muscle or pancreas. In cattle slaughtered at 5 to 8 days post-inoculation (DPI) virus was recovered from the lymph nodes, lungs, bone marrow and thyroid. Virus could not be detected in any tissue or organ samples taken from cattle killed at 12 to 33 DPI. The blood of young cattle contained virus for 7 DPI.\textsuperscript{42}

In Russia foot-and-mouth disease virus was found in the ejaculate of two artificially infected breeding bulls in the incubation and clinical periods of the disease. The virus was not found in the ejaculate on or after the 21st DPI.\textsuperscript{43}

Infected premises are routinely cleansed and disinfected using a 1% solution of NaOH and are again treated with 1% solution of NaOH before restocking 8 weeks later.\textsuperscript{44} In another study, trucks contaminated with FMDV were sprayed with jet aerosols of 38 to 40% formaldehyde solution generated at the rate of 60 ml per cubic meter air temperatures-not below 10°C relative humidity 60 to 95% and exposed for 20 minutes. Formaldehyde neutralization after exposure was performed with a 12.5% liquid ammonia solution at the rate of 40 to 50 ml per cubic meter. This method was found reliable for disinfection.\textsuperscript{45}

\textit{Vaccine and Prophylaxis}

Vaccines against FMD virus are almost exclusively prepared with inactivated virus as antigen in combination with an adjuvant. The antigen production is generally done in tissue explant cultures or in cell cultures. Formaldehyde or ethyleneimine derivatives are agents employed for inactivation. The most commonly used adjuvant is aluminum hydroxide, sometimes in combination with saponin. Recent research on oil emulsions as adjuvants indicate the induction of a longer lasting immunity with this type of vaccine.\textsuperscript{46}

The major limiting factor in South America in the use of the Frenkel method is the lack of bovine tongue epithelium. The use of baby rabbit antigen in Brazilian plants is promising.\textsuperscript{47}

A procedure has been described for the large-scale culture of vaccine virus in a suspension of cattle tongue epithelial cells in combination with cells from the ruminant stomach.\textsuperscript{48}

A French foot-and-mouth disease institute has patented a vaccine process which consists of obtaining 2 successive cultivations using the same bovine tongue cells but with different batches of culture media from which production then proceeds in the conventional Frenkel manner.\textsuperscript{49}

Baby hamster kidney cell line BHK\textsubscript{21} grown in suspension produced virus with the titer 1.0 log higher than virus grown in primary calf kidney cells.\textsuperscript{50} This method is very suitable for virus culture in large quantities because the size of the containers is theoretically unlimited; however, some-
times problems arise in adapting new strains of virus to this type of culture. 

BHK\textsubscript{21} cells being heterologous protein have the greater potential of sensitizing the recipient than the homologous material used in the Frenkel method.\textsuperscript{51}

Ethylenimine (DI) inactivated vaccine is as potent an immunogen as N-acetylthelyleneimine (AEI) inactivated vaccine, but inactivates virus at a faster rate with the linear loss of infectivity.\textsuperscript{52}

Other aziridine derivatives, N-carbethoxyethylenimine (CEI), two ethyl ethylenimine (EEI) and N-acetyl propylenimine (API) were found to induce neutralizing antibody in guinea pigs comparable to AEI.\textsuperscript{53} Prior treatment with 0.05% formaldehyde followed by a 0.05% acetyl ethylenimine stabilized more of the I40S component for inactivation than AEI alone.\textsuperscript{54}

A bivalent vaccine O/C using 1/4 the normal dose of antigen, EI inactivating agent and DEAE dextran as an adjuvant protected 84% of 92 pigs against challenge with heterologous strains. No correlation between neutralization antibody titer and immunity was observed. Mild fever and dullness occur for 2 days post injection if it was given intramuscularly into the neck.\textsuperscript{55}

The Pan American Foot-and-Mouth Disease Center at Rio de Janeiro carried out experiments at a field level with an oil-adjuvanted vaccine in a cooperate program with the Plum Island Animal Disease Center, (USDA). Tests in 3,000 cattle have shown that good protection can be achieved with 2 vaccinations a year instead of 3 or 4.\textsuperscript{48}

Serum neutralization titers of bovine vaccinated with a vaccine emulsified with another oil-adjuvant, and inactivated with AEI were shown to remain at the same level during the last 3 months of a 6 months' trial as opposed to an aluminum hydroxide-saponin vaccine inactivated with formalin whose titers declines.\textsuperscript{56} In Hungary, it was shown that oil-adjuvant vaccines consistently render pigs immune longer than natural infection.\textsuperscript{57}

In Germany, frequency of vaccination and use of non-purified tissue culture vaccine are causing more post-vaccinal allergic reactions, including abortion. This is 0.27% more adverse reactions than found with the Frenkel type vaccine.\textsuperscript{58} A polyethylene glycol (PEG) purified virus vaccine tested in guinea pigs was antigenically potent and showed no signs of allergies correlated with other vaccine components.\textsuperscript{59}

Pigs and calves were treated with a double-stranded RNA of fungal origin before, during and after being exposed to pigs infected with FMD. In pigs given 0.1 mg. per kg. bodyweight and calves 5 mg. per kg. subcutaneously for 10 days failed to develop lesions or they developed later and to a lesser extend that in untreated animals. Interferon was demonstrated in the blood of calves or pigs given one large dose intraperitoneally but not in animals given the preparation subcutaneously in a smaller dose over a number of days.\textsuperscript{60} Polyriboinosinic-polyriboctydyl acid, itaconic-acrylic acid copolymer acid, divinyl ethamaleicanhydride copolymer, three synthetic interferon induers failed to demonstrate any differences in either course or severity of disease upon challenge with virulent virus. Two attenuated FMD virus used as interferon inducers protected adult mice against death but did not protect suckling mice, guinea pigs or calves. Generalized diseases were delayed by a matter of days.\textsuperscript{62}
Passive immunity conveyed by the administration of concentrated interferon produced in tissue culture and animal blood serum do not have as long a period of action as direct stimulation of interferon.\textsuperscript{63}

Exposure to inactivated live virus does not produce nasal or salivary antibodies but will produce serum antibodies. Nasal inoculation with live virus will produce nasal and salivary antibodies.

Passively acquired antibodies were detected as long as 7 months after birth.\textsuperscript{64}

When vaccinating young animals consideration must be given to whether the respective animals were from vaccinated dams.\textsuperscript{65} Vaccination of cattle 1 year old or less requires more than 1 vaccination.\textsuperscript{66}

Rapid reduction in infectivity of bovine kidney cells and cattle occurred by various combinations of temperature and dilution methods using a Type C mutant of FMDV grown in chicken embryos.\textsuperscript{67}

Immunological study of C virus from Yugoslavia adapted to cattle and serological study of the Type A virus isolated in Spain during 1972 concluded that present vaccines protect well when challenged with strains of the 1972/73 epizootics.\textsuperscript{68}

Wellcome Foundation Limited now owns all or partially 8 FMD vaccine production units in Europe, Africa and South America.\textsuperscript{69}

Diagnosis and Serology

Three immuno-peroxidase techniques (direct, indirect and peroxidase-anti-peroxidase) were compared for their potential in FMD research. Each technique was shown to offer a simple and efficient means for the detection of the virus of FMD and of virus-infection-associated antigen in infected cells.\textsuperscript{70}

Guinea pig antisera against the various sub types in each type of FMD have been pooled and used in the rapid CF test used for diagnosis of FMDV.\textsuperscript{71}

Specific antibody to FMD virus was produced in ascitic fluid of an established line of white rats sensitive to ovarian tumor formation.\textsuperscript{72}

Results from an indirect fluorescent antibody test agreed well with those in a virus neutralization test using 96 serums from acutely infected, convalescent and immunized cattle.\textsuperscript{73} The CF properties of Type O virus remains unchanged after inactivation by gama radiation.\textsuperscript{74} Lapinized virus concentrated with polyethylene glycol retained its antigenic properties and may be useful in complement fixation and diffusion-precipitation tests for differentiation of virus types.\textsuperscript{75}

REFERENCES

226 REPORT OF THE COMMITTEE


29. FORMAN, A.J., GIBBS, E.P.J., BABER, D.J., HERNIMAN, K.A.J., and BARNETT, I.D.


55. BAUER, J., WITTMANN, G., GEILHAUSEN, H., and IRION, E. [Vaccination of pigs with a DEAE dextran containing bivalent foot and mouth disease vaccine.] Berl. Munch. Tierarztl. Wochenschr. 87(9):170-173, 1974 (Ger., engl.).


69. THE WELLCOME FOUNDATION LIMITED. Wellcome foot-and-mouth disease vaccines.


RESEARCH ON VESICULAR DISEASES

SWINE VESICULAR DISEASE (SVD)

A bibliography of SVD has been compiled by R. J. Uskavitch.1

At 5°C. at a pH of 7.54 the virus survived without loss of titer for 164 days, but at pH values of 2.88 and 10.14 more than 6 log units of virus were lost by 164 days. The virus was inactivated in 30 minutes at 56°C and in 2 minutes at 60°C.2

In feces the virus was viable at 138 days at 12 to 17°C., but not viable at 200 days when temperature rose to 25°C.3

A serological relationship by virus neutralization tests has been established between swine vesicular disease virus (SVDV) and the human pathogen Coxsackie B5 virus.4 Antigenic differences can be shown between the Italian, UK, French and Hong Kong strains of SVDV and Coxsackie B5 virus in immunodiffusion tests in agar. Immunodiffusion was used to verify that 2 human laboratory infections were due to SVDV infection and not Coxsackie B5 virus. Antigenic differences are even being noted between isolates of the 1972-73 UK outbreaks collected 6 months apart.5

Pigs in contact with experimentally inoculated animals were killed at intervals before the appearance of clinical disease. The distribution and amounts of virus in various tissues indicated entry of virus through the skin and the mucosa of the digestive tract.6 Attempts to recover virus from feces of convalescent pigs 5 to 9 weeks after infection by Italy/66 strain were unsuccessful.6

Airborne excretion of SVDV appears to be 160-fold less than foot-and-mouth disease virus (FMDV) infected pigs. Excretion is more likely from lesions of the skin than from the respiratory tract.8 The incubation period varies between 3 and 7 days and the emission of virus can be as early as the second day after infection and is usually completed within 8 days of the appearance of lesions, but may persist up to the fourteenth day. No virus carrier state has been found. Virus is shed in high titer from vesicular fluid, but high concentrations of virus have also been obtained from skin surfaces and excretion of the virus through the skin may occur. Feces contain an appreciable quantity of virus. Morbidity is high (up to 100%) in affected pens but spread to other pens depends mainly on mechanical transmission of infected material.9

Pigs inoculated intravenously with SVDV (Hong Kong strain) developed diffuse encephalomyelitis. Lesions (perivascular cuffing with lymphocytes and formation of neuroglia cell foci) were most severe in the diencephalon, mesencephalon, metencephalon, and myelencephalon. Encephalomyelitis was of mild to moderate intensity and was most severe on the sixth day after inoculation.10

The fluorescent antibody (FA) technique was used to detect virus in tissue cultures inoculated with suspensions of vesicular epithelium from lesions. Positive results were obtained after 3 h from vesicles less than 5 days old and after 3 to 5 h from lesions 6 to 10 days old. The infectiousness of vesicular material declined from approximately $10^{10}$ plaque forming units/gram (pfug) in vesicles 1 day old to $10^6$ pfug in lesions 10 days old.11

French researchers feel that vaccination against SVDV is possible and may be a valuable tool.12
VESICULAR STOMATITIS (VS)

A bibliography of Vesicular Stomatitis Virus (VSV) October 1972 to September 1973 was compiled by R. Uskavitch.\textsuperscript{13} At least 98% agreement was obtained on typing (VS) isolates from bovine epithelium by FA technique when compared to the complement fixation (CF) and/or the virus isolation (VI) and neutralization techniques.\textsuperscript{14}

Epidemiological inquiry is underway in Brazil to establish the presence of antibodies of 5 viruses of VSV group (N. J., Indiana 1,2,3, and Cocal).\textsuperscript{15} Indiana and N. J. subtypes neutralizing antibodies have been found in Colombian rodent serum survey.\textsuperscript{16} Attempts continue to characterize factors influencing the growth of Cocal virus, closely related serologically, to the Indiana serotype, \textit{in vitro} in continuous mosquito cell lines\textsuperscript{17}

A non-antibody inhibitor to VSV was found in low levels in most lots of fetal calf serum.\textsuperscript{18}

VESICULAR EXANTHEMA OF SWINE (VES)

Several virus isolates from Pinnipeds appear to have a host range, morphology and physio-chemical properties identical to vesicular exanthema of swine virus (VESV).\textsuperscript{19 20} Serological evidence exists of transmission of virus between marine animals and feral swine on Santa Cruz Island off California.\textsuperscript{21} Viral agents recovered from oral and fecal swabs of sea lions in California had electron micrographic profiles identical to those of VES. Intradermal inoculation into the snouts, lips, and feet of pigs resulted in development of fever, pustules, vesicles, erosions and skin necrosis. Lesions appeared in the mouth and feet of suckling piglets from a sow with lesions on the mammary and teat skin. However, contact exposure mostly resulted in a transient fever and inapparent disease. Neutralizing antibodies were detected 7 DPI reaching high levels about 21 DPI. Precipitin antibodies were common to different sea lion isolates but the neutralizing antibodies were serotype specific. The range of reciprocal virus neutralizing indices between the sea lion viruses and the 11 known VES virus types was 0.0-1.5 log units. It is known that serologic relation or similar range exists between the known types of VES viruses. Therefore, sea lion viruses may considered new VESV types.\textsuperscript{22}

FOOT-AND-MOUTH DISEASE (FMD)

\textit{Epidemiology and Pathogenesis}

The species particularly implicated as a carrier of foot-and-mouth disease virus in Africa is the cape buffalo which curiously is rarely seen to exhibit lesions of the disease; however, older animals transmit the infection to young buffalo soon after maternal immunity has waned. The system would seem to be self-perpetuating in that some herds of buffalo have been found to be infected at approximately the same level over many years.\textsuperscript{23} Significant antibody titers to all 3 SAT types were demonstrated in serum samples; however, there is no evidence of transmission from buffalo to cattle.\textsuperscript{24 25}

An African elephant (Loxodonta africana) showed clinical disease upon inoculation, but no transmission of the diseases to other elephants.\textsuperscript{26} Mechanical transmission of FMDV was reported by starlings to cattle.\textsuperscript{27} Of 3 types of deer — red, fallow and roe — only roe were severely infected with FMDV in studies done in England. Aerosol virus quantity during clinical disease was comparable to
that recorded for cattle and sheep. The longest carrier state was reported in the species that showed the least clinical involvement, the fallow deer.28,29

No foot lesions were noted in sheep; and disease signs are such, that the disease might go unnoticed by farm workers. Seasonal movements of sheep bands may be of importance in certain parts of the world in spreading the disease.30 In one study of the carrier state in the Brazilian state of Sao Paulo where FMD had not been reported for 6 months, oesophageal pharyngeal samples (OP) from 150 normal bovine destined for the abattoir were surveyed for FMDV. Seventy-nine were positive to VI, 49.4% for Type C, 38% for Type O and 12.6% for Type A.31

Of 5 bovine in 1 experimental trial, 3 developed high serum and OP fluid neutralizing antibody titers. When challenged 30 weeks later with homologous virus there was no appreciable virus growth in their throats. Five other steers who had low neutralization titers in serum and OP fluid showed virus growth in their throats when challenged 30 weeks later.32

Evidence exists that infection with FMD and bovine enterovirus or bovine rhino-tracheitis may cause an alteration of typical clinical signs of FMD and/or a much delayed incubation period of the disease. Evidence of virus transencapsulation could explain these observations.33

Results show that the conjunctival route gives a response similar to intranasal inoculation; however, aerosolized virus probably has more chance of being trapped in the respiratory tract than in the conjunctiva.34

Wind can convey infectious FMDV on dust particles 3 to 6 mm in size long distances. These particles would normally settle out in still air. Rain even may play a vital part in the spatial distribution of outbreaks. Some evidence points to wind distribution of protein molecules without natural support of dust particles or water droplets.35,36,37 Statistical Analyses of the spread of FMD points to relative humidity and rainfall as being very important.35 Infectivity of aerosols from milk and fecal slurries containing virus at 55% relative humidity was 0.15 to 5% as compared to aerosols from saliva which was 0.0005 to 0.005%.39

Apparently the instability of virus in aerosols of saliva at high relative humidity is caused by an undefined dialyzable organic molecule which is sensitive to heating at 70°C.40

Milk from cows showing no clinical signs of disease was a source of disease among pigs.41

In cattle killed at 24 to 120 hours post inoculation, virus was recovered from the lymph nodes in most cases, from the lungs 80% of cases and from the thyroid gland in one case. In no case was virus isolated from the skeletal muscle, heart muscle or pancreas. In cattle slaughtered at 5 to 8 days post-inoculation (DPI) virus was recovered from the lymph nodes, lungs, bone marrow and thyroid. Virus could not be detected in any tissue or organ samples taken from cattle killed at 12 to 33 DPI. The blood of young cattle contained virus for 7 DPI.42

In Russia foot-and-mouth disease virus was found in the ejaculate of two artificially infected breeding bulls in the incubation and clinical periods of the disease. The virus was not found in the ejaculate on or after the 21st DPI.43

Infected premises are routinely cleansed and disinfected using a 1% solution of NaOH and are again treated with a 1% solution of NaOH before restocking weeks later.44 In another study, trucks contaminated with FMDV were sprayed with jet aerosols of 38 to 40% formaldehyde solution generated at the rate of 60
ml per cubic meter air temperatures-not below 10°C, relative humidity 60 to 95% and exposed for 20 minutes. Formaldehyde neutralization after exposure was performed with a 12.5% liquid ammonia solution at the rate of 40 to 50 ml per cubic meter. This method was found reliable for disinfection.45

VACCINE AND PROPHYLAXIS

Vaccines against FMD virus are almost exclusively prepared with inactivated virus as antigen in combination with an adjuvant. The antigen production is generally done in tissue explant cultures or in cell cultures. Formaldehyde or ethylenimine derivatives are agents employed for inactivation. The most commonly used adjuvant is aluminum hydroxide, sometimes in combination with saponin. Recent research on oil emulsions as adjuvants indicate the induction of a longer lasting immunity with this type of vaccine.46

The major limiting factor in South America in the use of the Frenkel method is the lack of bovine tongue epithelium. The use of baby rabbit antigen in Brazilian plants is promising.47

A procedure has been described for the large-scale culture of vaccine virus in a suspension of cattle tongue epithelial cells in combination with cells from the ruminant stomach.48

A French foot-and-mouth disease institute has patented a vaccine process which consists of obtaining 2 successive cultivations using the same bovine tongue cells but with different batches of culture media from which production then proceeds in the conventional Frenkel manner.49

Baby hamster kidney cell line BHK21 grown in suspension produced virus with a titer 1.0 log higher than virus grown in primary calf kidney cells.50 This method is very suitable for virus culture in large quantities because the size of the containers is theoretically unlimited; however, sometimes problems arise in adapting new strains of virus to this type of culture. BHK21 cells being heterologous protein have the greater potential of sensitizing the recipient than the homologous material used in the Frenkel method.51

Other aziridine derivatives, N-carbethoxyethylenimine (CEI), two ethyl ethylenimine (EEI) and N-acetyl propylenimine (API) were found to induce neutralizing antibody in guinea pigs comparable to AEI.53 Prior treatment with 0.05% formaldehyde followed by a 0.05% acetyl ethylenimine stabilized more of the 140S component for inactivation than AEI alone.54

A bivalent vaccine O/C using 1/4 the normal dose of antigen, E1 activating agent and DEAE dextran as an adjuvant protected 84% of 92 pigs against challenge with heterologous strains. No correlation between neutralization antibody titer and immunity was observed. Mild fever and dullness occur for 2 days post injection if it was given intramuscularly into the neck.55

The Pan American Foot-and-Mouth Disease Center at Rio de Janeiro carried out experiments at a field level with an oil-adjuvanted vaccine in a cooperative program with the Plum Island Animal Disease Center, (USDA). Tests in 3,000 cattle have shown that good protection can be achieved with 2 vaccinations a year instead of 3 or 4.48

Serum neutralization titers of bovine vaccinated with a vaccine emulsified with another oil-adjuvant, and inactivated with AEI were shown to remain at the same level during the last 3 months of a 6 months' trial as opposed to an
aluminum hydroxide-saponin vaccine inactivated with formalin whose titers declined.\textsuperscript{56} In Hungary, it was shown that oil-adjuvant vaccines consistently render pigs immune longer than natural infection.\textsuperscript{57}

In Germany, frequency of vaccination and use of non-purified tissue culture vaccine are causing more post-vaccinal allergic reactions, including abortion. This is 0.27\% more adverse reactions than found with the Frenkel type vaccine.\textsuperscript{58} A polyethylene glycol (PEG) purified virus vaccine tested in guinea pigs was antigenically potent and showed no signs of allergies correlated with other vaccine components.\textsuperscript{59}

Pigs and calves were treated with a double-stranded RNA of fungal origin before, during and after being exposed to pigs infected with FMD. In pigs given 0.1 mg. per kg. bodyweight and calves 5 mg. per kg. subcutaneously for 10 days failed to develop lesions or they developed later and to a lesser extent than in untreated animals. Interferon was demonstrated in the blood of calves or pigs given one large dose intraperitoneally but not in animals given the preparation subcutaneously in a smaller dose over a number of days.\textsuperscript{60} Polyriboinosinic-polyribocytidylc acids, itaconic-acrylic acid copolymer acid, divinylethermalei-canhydride copolymer, three synthetic interferon inducers failed to demonstrate any differences in either course or severity of disease upon challenge with virulent virus.\textsuperscript{61} Two attenuated FMD virus used as interferon inducers protected adult mice against death but did not protect suckling mice, guinea pigs, or calves. Generalized diseases were delayed by a matter of days.\textsuperscript{62}

Passive immunity conveyed by the administration of concentrated interferon produced in tissue culture and animal blood serum do not have as long a period of action as direct stimulation of interferon.\textsuperscript{63}

Exposure to inactivated live virus does not produce nasal or salivary antibodies but will produce serum antibodies. Nasal inoculation with live virus will produce nasal and salivary antibodies.

Passively acquired antibodies were detected as long as 7 months after birth.\textsuperscript{64} When vaccinating young animals consideration must be given to whether the respective animals were from vaccinated dams.\textsuperscript{65} Vaccination of cattle 1 year old or less requires more than 1 vaccination.\textsuperscript{66}

Rapid reduction in infectivity of bovine kidney cells and cattle occurred by various combinations of temperature and dilution methods using a Type C mutant of FMDV grown in chicken embryos.\textsuperscript{67} Immunological study of C virus from Yugoslavia adapted to cattle and serological study of the Type A virus isolated in Spain during 1973 concluded that present vaccines protect well when challenged with strains of the 1972/73 epizootics.\textsuperscript{67}

Wellcome Foundation Limited now owns all or partially 8 FMD vaccine production units in Europe, Africa and South America.\textsuperscript{69}

**DIAGNOSIS AND SEROLOGY**

Three immuno-peroxidase techniques (direct, indirect and peroxidase-antiperoxidase) were compared for their potential in FMD research. Each technique was shown to offer a simple and efficient means for the detection of the virus of FMD and of virus-infection-associated antigen in infected cells.\textsuperscript{70}

Guinea pig antiserums against the various sub types in each type of FMD
have been pooled and used in the rapid CF test used for diagnosis of FMDV.\textsuperscript{71}

Specific antibody to FMD virus was produced in ascitic fluid of an established line of white rats sensitive to ovarian tumor formation.\textsuperscript{72}

Results from an indirect fluorescent antibody test agreed well with those in a virus neutralization test using 96 serums from acutely infected, convalescent and immunized cattle.\textsuperscript{73} The CF properties of Type O virus remains unchanged after inactivation by gama radiation.\textsuperscript{74} Lapinized virus concentrated with polyethylene glycol retained its antigenic properties and may be useful in complement fixation and diffusion-precipitation tests for differentiation of virus types.\textsuperscript{75}
FOREIGN ANIMAL DISEASES

BIBLIOGRAPHY


REPORT OF THE COMMITTEE


55. BAUER, J., WITTMANN, G., GEILHAUSEN, H., and IRION, E. [Vaccination of pigs with a DEAE dextran containing bivalent foot and mouth disease vaccine.] Berl. Munch. Tierarztl. Wochenschr. 87(9): 170-173, 1974 (Ger., engl.).


FLORIDA'S PROGRAM AGAINST E.I.A.

C. L. Campbell, D.V.M.
State Veterinarian, Director, Division of Animal Industry
Florida Department of Agriculture and Consumer Services
Tallahassee, Florida

This is the second time in less than six months that I've been asked to talk on E.I.A. This past May at the Southern Animal Health Association Meeting I was assigned a subject designated on the program simply as “E.I.A.” The way we do things in the Southern group is that the president or program chairman decides how much time needs to be killed in particular spots, assigns some innocuous characters to speak on selected subjects for such periods, has the program printed, and about a week before the association convenes, mails copies to the participants, who only then realize that they are supposed to give a talk on the particular subject shown. Well, as I said, I was drafted to discuss “E.I.A.” for about thirty minutes. Actually, I was half way through my paper when, as I glanced out at the audience and noted the puzzled facial expressions of some of my compatriots, I realized that I must have misinterpreted the meaning of the letters “E.I.A.” and was talking on the wrong subject. So, in case you are wondering about my topic, I'll tell you right now that it has noting to do with, nor do I intend to talk about, “Elephants in Africa.”

In putting together this presentation, it occurred to me that nearly nine years ago this organization started to really get involved in equine infectious anemia at the time I was President of the group. I recalled convening the Executive Committee of what was then the United States Livestock Sanitary Association in Atlanta early in 1966 for the purpose of ratifying the first draft of the “Prospectus on Equine Infectious Anemia with Guidelines.” As a result of that, and other meetings with practitioners, allied veterinary associations and state racing commissions, we were able, at the time, to quell what could have been a chaotic situation in connection with the disease at racetracks throughout the nation.

In the intervening years steady progress has been made toward, and this association has been kept abreast of the developments directed to, the eventual elimination of this insidious disease. Of course, we have a long way yet to go to rid the country's equine population of infectious anemia, but after a year's personal experience of being on the firing line in a statewide test and control program, I am convinced that we are on our way.

Recently, I had occasion to review some remarks which I made at the 1966 Convention of the American Association of Equine Practitioners' meeting in Los Angeles:

“There will probably be delays and pitfalls in developing an effective vaccine or of even finding remedial agents against infectious anemia. This, I believe, we can afford for awhile. We can ill afford, however, the luxury of additional delays in the perfection of a practical test for EIA. Without it, further progress toward eradicating the disease cannot be made.”

I have found over the years that quite often some of the statements
which I had earlier made have come back to haunt me; however, in this case with official recognition of that recently developed milestone, namely, the AGID test for locating foci of infection, we are now able to make progress in the initial eradication phases as we buy that time necessary for the receipt of results of crash measures now being directed toward therapeutic relief. I do want to caution those of you who are conducting work in vaccine and therapeutic studies, though, to not yield to the often highly emotional pressures, to which you will be subjected to shortcut sound scientific challenge procedures that will be demanded by your professional peers in proving your experimental efforts. It has been my experience during this past year in this new ball game involving proper disposal of diseased animals, the majority of which have greater emotional than monetary attachment, that one's professional judgment is too frequently placed in jeopardy of compromise in making a proper decision when faced by weeping adolescents. Certainly, I do not want to leave the impression that I am without compassion in dealing with these, for the most part, younger reactor horse owners and their parents; however, what the veterinarian, be he regulatory, research, or practice oriented, must keep foremost in his mind is that ethically he has a primary obligation to those owners of healthy horses in precluding exposure to disease.

Now let's look at a few of the details of Florida's program, so that you who are contemplating the initiation of similar measures in your state might benefit from some of our pioneering efforts and errors.

Although we did not make this mistake, it would have been sheer folly to attempt to launch even a semi-compulsory testing plan throughout the state without strong industry support, for there always exists that element of owners who are opposed to being inconvenienced in spite of what benefits might accrue to the industry. Our equine infectious anemia control program was put into effect on October 15, 1973, following a series of well advertised meetings with representatives of the State's equine industry. Thoroughbred, standardbred, quarter horse, and all pleasure horse breeds were invited and participated in discussions of the equine infectious anemia problem, and the regulations now in effect were recommended by the equine industry representatives to the Commissioner of Agriculture.

The program is designed to control the spread of equine infectious anemia through restricting the movement of known infected animals to concentration points where large numbers of equines may be exposed to infection, and by preventing known infected animals from entering the State from other states and foreign countries. As such it serves to protect susceptible horses from exposure to infected animals.

It should be understood that this is not an eradication program, but a "crawl-before-you-walk" concept, as an initial step toward eventually eliminating the disease should industry elect to do so. The main thrust of the program at this time is to control the disease by prohibiting the movement of infected animals to concentration points such as racetracks, rodeos, shows, fairs, public boarding stables and similar public assembly points. All equine animals moving to such points or changing ownership must have had a negative test within the preceding 12 months. Horses entering Florida
from other states must be negative to test within six months prior to entry.

I might state at this point that it is quite important to know that you have sufficient resources to implement your planned program, once it has been determined the direction which is to be taken, whether it be that of control or eradication. As an example, you might be interested to know that for the Fiscal Year 1973-74 we spent $73,500 for EIA antigen test kits only. That's for 1,255 kits, or enough to make 110,250 tests. Of course, there are additional costs such as other laboratory supplies and labor, clerical help and supplies, postage (not a minor expenditure by far), salary and expenses of involved field personnel, tattooing and branding equipment, and a myriad of other costs. It was because of this tremendous outlay of funds that we realized right after the first of this year that we could not continue at this level of expenditure and, with the cooperation of industry, amended our regulations to extend our testing requirements for intrastate purposes from six to the present twelve month requisite. As expected, this has decreased the number of tests being conducted at our four state laboratories. the only ones approved for running official tests within Florida. While there is a fee charged by accredited veterinarians for taking blood samples and preparing them for submission, there is no charge made by our department for conducting the tests. Thus we are unable to recoup from horse owners, other than from taxes which they might pay, any fees to defray our costs.

As to the mechanics of the program, equine animals over nine months of age which give a positive reaction to the AGID test for EIA are required to be permanently identified and are subject to quarantine and isolation or destruction. Animals under nine months of age giving a positive reaction to the test are subject to quarantine and retest when reaching nine months, and if positive at that time are handled in the same manner as adult reactors.

Upon disclosure of a horse as a reactor to the test, our involved laboratory notifies my office, the practicing veterinarian submitting the sample, and the owner who also receives concurrently a notice of quarantine which, thus, legally restricts its movement. As soon as possible thereafter, the owner is contacted by one of our employees for the purpose of identifying the horse, either by lip tattoo or body brand, as a reactor. At that time side and head drawings are made of the animal for purposes of future positive identification, should the owner elect to again have the horse tested at his expense rather than submit to reactor tattooing at this time. In such event this drawing, as well as the other attendant papers involved in the case, are turned over by our inspector to the selected veterinarian who then takes a second blood sample and forwards it to our laboratory for test. If the retest is again positive, the animal is identified as a reactor. If the retest is negative, a third sample is drawn, but this time by one of our state-employed veterinarians, and submitted for test to a reference or referee laboratory such as the Veterinary Services Diagnostic Laboratory at Ames, Iowa. Additionally, because of the probability of error or mix-up in animal identification, all other horses on the premises are tested at that time. The final status of the horse in question is determined upon the basis of results received from the reference laboratory.

Since there is no indemnity paid on EIA reactors, an owner may elect not to destroy them; however, reactors which are to be retained must be placed in approved isolation facilities. Approval of isolation will depend upon
the physical facilities of the infected premise and its location in proximity to other areas where horses are kept. It may require individually screened stalls if other horses are stabled in the same facility, or an isolated pasture located 200 or more yards from other horses, depending upon the terrain, density of the insect population, and other factors. Isolation facilities are approved by the regulatory veterinarian following inspection of the premises at the time quarantine is placed.

Reactors may be moved only upon written permit from a representative of the Department. Such movements are restricted to approved isolation facilities, approved research facilities, approved slaughtering establishments, or to approved EIA quarantine lots for assembling and movement to approved slaughtering establishments.

Horses not known to be infected which are located on quarantined premises may move without permit provided reactor animals are properly isolated in approved facilities.

The enforcement of program requirements is, of course, the responsibility of regulatory personnel of the Department's Division of Animal Industry. The limitation on personnel of the Division will not permit regulatory veterinarians to routinely test animals for EIA. Likewise, Division representatives cannot be present at all assembly points, and these must necessarily be covered on a spot-check basis. It is, therefore, necessary to seek the cooperation of management at race tracks, rodeos, shows, fairs and similar organizations as well as individual horse owners in seeing that test requirements are met on animals entering these assembly points.

Statistically, for the four year, lacking two months, period between December 1, 1970, and September 30, 1974, our laboratories have tested 118,617 horses, of which 8,553 were disclosed as reactors. This represents an infection rate of 7.2%.

Of this 118,000 plus figure, 25,012, or somewhat less than one-fourth of them were tested in the first two and a half years of the period. The 2,311 reactors disclosed represented an incidence of 9.2%. In the main, these horses were clinically ill from one cause or another, and we would expect a higher rate of infection than that which we are now finding on tests in order to meet our existing regulations.

The remainder, or 93,605 horses have been tested since July 1, 1973. The 6,242 reactors gives us an incidence of 6.7%. However, when one considers that the great majority of these reactors have been retested at least once, this incidence should probably be extrapolated to a figure more closely approximating some 3.5%.

As you might suspect, the greatest concentration of reactors have been detected in those Florida counties where we seem to have the greater vector problem, extending from the mid and south central east coastal counties south-westward to the lower west coast counties.

Based upon this past year's experience, we can unequivocally state that without the cooperation of all facets of Florida's horse industry, the reduction of the incidence of equine infectious anemia to the point where eradication is feasible will be most difficult. The realization of this ultimate goal, however, will be of inestimable value to the future of the equine industry of our state.
THE HORSE AS A MODEL FOR
NATIONAL INDIVIDUAL IDENTIFICATION OF ANIMALS

Dr. R. Keith Farrell* and Mr. Lee S. Garner**

As a nation, we consider ourselves the most advanced in adapting scientific findings to agricultural pursuits, but in animal identification we are woefully deficient. Proper identification is the key that could solve most control problems under discussion by the USAHA.

It is our belief that one major stumbling block to a better approach to animal identification for disease control has been the willingness of the industry to accept existing ownership marks as the epitome of identification. This attitude is about 4,000 years old and past due for an evaluation. Modern society has data retrieval systems that allow us to identify each animal as an individual instead of only as to an owner. One major fallacy accepted by owners is that ownership marks are unique. The present system is nonfunctional as a method of identifying even ownership from one state to another and to make it more confusing, a county registry prevails in Texas, allowing identical marks to occur in two or more counties.

Despite the known deficiencies, there are states that are now drafting brand laws for the first time that are being guided by existing brand laws. They are thereby perpetuating an outdated symbol system without considering the improvement of the program with symbols functional across state lines.¹

The need for re-evaluation and revamping of our identification procedures is not unknown to the United States Animal Health Association. In three major stockyards in the southwest, cattle were backtagged and followed through primary, secondary, and tertiary movement. We were astounded to learn that cattle from these three major stockyards hit 170 counties in 16 states in three days.² We know what these statistics indicate in time of national emergency, such as an outbreak of foot and mouth disease in any one of these major stockyards. Only one-third of our tuberculosis reactors are being traced back to point of origin from the slaughterhouse.³ Further, there is waste in veterinary manpower for traceback procedures.

Fraud is a continuing factor in horse identification. Six horses raced under 12 different identities in at least 41 different races at 12 different tracks in nine different states.⁴

There is no animal in association with man that would not benefit by individual identification on a national basis. There is strong interest in individual identification of horses and they might serve as the pilot animal on which to work out the principles of individual identification.

Individualizing of all livestock is too broad a subject to cover here, thus, we will confine our discussion to individual identification of horses.

It is important to identify the horse as an individual to aid in:

a. Equine health programs; vaccination, EIA testing, health certifi-

---

**New Mexico Livestock Board, Albuquerque, New Mexico 87103.
cates, quarantine regulations, etc.
b. Tracing blood lines.
c. Effective change of ownership.
d. Preventing fraud at shows, races, etc.
e. Obtaining loans and certifying insurance claims.
f. Tracing stolen horses across state lines and international boundaries and to act as a deterrent to theft.

Identification by signalment (appearance)

Present known ways to individualize a horse are be tattoo, freeze mark, or signalment. Signalment, the natural marks that distinguish one animal from another is the oldest means of identification, and can now be adapted to data retrieval. In the absence of computer-adapted applied marks, signalment should be more widely utilized.

This technique finds wide usage in breed registries throughout the world. In its simplest form, identification by signalment consists simply of drawing in all obvious marks on an outline of the animal. The technique leaves a lot to be desired, as presently practiced, but in the hands of skilled identifiers, it is a valuable tool.

Establishing a number to represent a given mark of signalment is of assistance when attempting to trace an animal back to records of a group of animals known to be identified.\(^5\)\(^6\) The term “signalment key” simply implies a numerical designation of the outward appearance of the horse. This key is functional, especially in the hands of the individual that assigned the numbers in the first place. Some discrepancies in number assignment will exist between one identifier and another, even when skilled. We believe we can, with additional studies, establish a confidence interval on combinations of marks of signalment that will give the signalment key great value. It should be emphasized that, without some numerical designation of the appearance of the horse, an orderly record search for a horse by appearance alone would be impossible.

Figure 1 depicts an experimental form to generate a signalment key. Keys are necessarily imperfect because of difference in judgment and they should be used only to assist in an orderly search for an individual horse. The errors in the program are due to human differences in observation.

The columns marked A, B, C, D, E, etc., are used to generate a numerical description of the horse. Column A (sex) is the easiest category, and very little error creeps into this key number. The white marks of description for the legs are categorized in B, C, D, and E with “0” designating no white hair at all, and “1” designating white coronets, heels, etc., providing that no white occurs above the fetlock. No 2 is used to describe horses that have white up to the knee and these horses may have black between the fetlock and coronet.

No. 3 category has white hair above the knee or hock, and includes horses that have a white stocking that reached above the knee or hock and horses that do not have white below the knee or hock.

The chestnut key is filled out at the time attention is focused on the legs. Following is a way of evaluating the chestnut as to basic shape.

No. 1 covers chestnuts pointed at top and broader based.
No. 2 is broad at the top, tapering to a narrower bottom.
No. 3 is pointed on both ends.
No. 4 is oval or round.
No. 5 is notched at the top with both points being equal in height.
No. 6 is bulged to the left.
No. 7 is bulged to the right.
No. 8 has three peaks.
No. 9 is for peculiar chestnuts that are not covered by the 1-8 classification. If no chestnut appears, a zero indicates its absence.

The chestnut key is designated J, K, L, M, in an attempt to establish the least important portion of the signalment key to the far right. The chestnut key was given this low position because individual evaluation of chestnuts will vary greatly; the chestnut looks pointed to one identifier while it still looks oval to another. Also, surgical alteration of chestnuts is easily accomplished and difficult to impossible to detect.

The white marks of signalment on the face are in the F position, a lower rating than the white marks of signalment on the legs, because it is easier to cryogenically alter the face of a horse than the legs. For those interested in the detection of fraudulent alteration by freezing, polarization stress analysis is the present technique of choice for detecting cryogenic fraud in the horse.

The position and number of hair whorls is a very interesting identification technique. They will eventually be more important than the present position in the key indicates. They are still under study to determine how much confidence we can generate in utilizing the hair whorl. At the present time, for experimental purposes only, we've included the number of hair whorls on the head and on the neck. The hair whorls are required as a means of identification for sale and registration of cattle and horses in the Philippine Islands.

Coat color, "1" on the key, is a very misunderstood category and is very difficult to assess. Our present thinking is to lump as many similar colors into one classification as possible. Color is probably one of the most changeable of all classifications. For example, a black horse looks brown in the spring when it is shedding, and color can be changed simply by clipping a horse during the hair growth cycle.

The numerical signalment key is entered under the picture of the horse. While teeth are observed, it has been found that tooth development and wear vary greatly in horses of the same age. Teeth are, therefore, not considered a reliable indicator of age and are not given a position in the signalment key. Of great value in depicting signalment is the picture of the horse with all marks carefully drawn on. Note that the anatomical lines used to categorize the white marks of the legs are drawn in and the body of the horse is broken down into its parts. The head is split into 6 regions with the top 2 regions being an area above a line drawn from the medical canthus of one eye to the medial canthus of the other. The middle sections fall between this line and the line drawn at the bottom of the facial crest. The bottom portion of the face is that area below the line drawn through the facial crest. The head is vertically divided in half by drawing a line through the middle of the head from top to bottom.

Sectioning of the face is of great value, particularly for the unartistic, in correctly depicting location of white marks and hair whorls. If double hair whorls occur in the middle of the forehead, this is depicted, etc. Most horses
are not exactly symmetrical in the manner in which the white marks and
whorls occur on the face.

The state of New Mexico started recognizing horses as individuals in
1969. Arizona, Wyoming, and Colorado have now instituted similar pro-
grams. New Mexico identifiers draw all natural markings, scars, and applied
marks on a horse outline. They also draw the chestnuts and note coat color.
An owner is issued a card with the drawings and description of the horse.
Use of the signalment key is being studied.

The state of Washington is preparing legislation solely concerned with
individual identification of horses. The signalment key will be used for
computerized record keeping. For each registered horse, the numerical key
and drawing of markings will be reduced to a credit card size passport which
is carried by the owner.

A signalment key, adaptable to computer, under which horses can be
individualized by use of natural signalment has been described. Use of a
signalment key will also be useful to reinforce identification programs which
use hot brands, freeze marks, or tattoos.

**Blood Typing**

Blood typing is the most valuable adjunct to applied and natural marks
for horse identification and will play an increasingly important role in the
future. The technique has proven its worth in cases of questionable parentage
and will play a bigger role in genetic abnormalities and weaknesses as pre-
sent research develops. Its use in a planned parentage program in such
problems as genetic immunodeficiency is immediately evident.

Dr. Clyde Stormont reports that it is now theoretically possible to define
1,032,192 blood types in horses and at least 70% of all paternity cases in-
volving 2 stallions can be solved.\(^\text{10}\)

**Individual Blood Sample of Record**

We have proposed another use of genetic blood determinates for those
horses in areas where deliberate fraud is especially prevalent, such as race
horses and show horses. This proposal suggests that we simply take a blood
sample on the foal at the time it is being identified by signalment key,
and freeze marking or tattooing. This sample would be kept in the frozen or
lyophilized state and used only if a question arises. Then, a second blood sample
is drawn and compared to the blood sample on record.

The advantages of this approach is a reduction in the cost because we
won't have to bloodtype each horse. It is obviously much easier to identify
the animal as itself than to establish the possibility of parentage, and the
blood sample on record could act as a deterrent to deliberate fraud such as
counterfeiting foal certificates, cryogenic alteration of coat color, tattoo
removal by laser and alteration of chestnuts.

**Tattooing**

The lip tattoo has been used to individually identify horses for many
years. It is most upsetting to find people taking blood samples for EIA,
certifying insurance claims, etc., without recording the tattoo number. The
unalterable tattoos used by the TRPB with their multi-needle dies\(^\text{11}\) are serv-
ing a purpose at the track and these existing marks could be used more efficiently by people in the health related sciences. Use of the TRPB tattoo system on all horses is not feasible because the system would require more symbols than the lip will accommodate.

Several registries which have recently popped into existence are using alterable Arabic numerals for tattoo identification. We view this development with alarm because of the alterability of the symbols and the fact that the tattoo does not designate a given registry where records on the animal may be found.

Poor sanitation is of considerable concern on some tattoo identification programs. It should be born in mind that EIA and other equine diseases can be spread by poor tattoo techniques. The TRPB spends approximately $2,000.00 per technician\textsuperscript{12} to have them equipped to do a hygenic job but few other tattoo programs are careful enough about this point.

The angle numerical symbols and alpha-angle alphabet\textsuperscript{13} have been successfully adopted for use as lip tattoos. This approach has the advantage of a one step application of the symbols, patented symbols for protection of the registry, and enough symbols for all horses in the world (the symbols can be three to five times smaller than Arabic symbols). A registry designation is included in the symbols. Angle symbols cannot be easily changed from one to another without laser removal of the tattoo.

Unfortunately, this angle tattoo system shares the same disadvantages as previous tattoo systems. The most troublesome problem with all lip tattoos is that they are not adaptable to the very young foal. When tattoos are put in young foals they fade, and we feel an identification system should function on a very young animal. Identifying horses as two year olds is akin to putting a motor number on a car after it leaves the showroom floor.

Also, tattoos can be removed by "zapping" with a burst of coherent light in the proper wave length and power (laser). Laser alteration is not considered a major threat at present but could constitute a future problem to be considered.

Freeze mark identification

A system to individually identify all horses with a permanent, unalterable mark has been devised. This system combines the freeze marking technique with unalterable, angle numerical and alphabetical symbols to apply a registration number to the animal.

Freeze marking is a painless technique of altering the brand site by destroying the melanocyte in the hair follicle or by destroying the hair follicle itself.\textsuperscript{14}

Angle numerical symbols have been developed which are unalterable and three to five times as visible as Arabic numbers. The numbers 2 through 9 are each represented by a right angle. The system is easily remembered if you visualize a basic square which represents the even numbers (Fig. 2). Turning the square 45° will give you the placement of the odd numbers. The number "1" is represented by two vertical lines, and the "0" is represented by two horizontal lines. Double lines are used for the "1" and "0" to avoid fraudulent alteration of angles. A horizon line is always used in conjunction with the angle numbers in order to interpret possible growth distortion.
An alphabetical symbol is used as part of an official identification. The alphabet has been designed so that there are eight positions of each letter (Fig. 3). Each letter in any of its eight positions is distinctive from any other letter in any of its eight positions. One of these 208 symbols will represent a single state or breed registry. It is this alphabetical symbol which is the key to finding where registration details of a particular animal will be located.

The marking device

The appropriate angle alphabet symbol is combined with a series of angle numbers into a single marking device. Each number plug is reversible with an angle at one end and parallel lines at the other end. Each plug rotates so one angle makes all numbers 2 through 9, or with the plug reversed, 1 or 0. Thus the device is self-contained/ no extra digits are required.

Figure 4 demonstrates the total symbol structure. The A which is in the first position (A1) designates the Arabian Horse Registry. The stacked angles represent the year of birth. The last six digits represent the horse's individual number assigned by the registry. Indents on the top of each plug give you the Arabic equivalent of the angle in position.

On a dark horse it is recommended to make a mark with white edges and bald centers. On a white animal, or a grey which will lighten, you can easily produce a bald mark with a darker appearance of the bald skin. The mark is placed on the neck, midway between poll and withers and about one inch below the eruption of the mane. It is usually placed under the mane where it is not obvious. There has been objection voiced to making an obvious mark on the animal—particularly on show stock. However, as the freeze marking method becomes more widespread, and people realize that the mark is protecting their horse, the objections fade away.

In a registry program, breed or state, the actual freeze mark symbols are put on registry papers by use of an imprinter (Fig. 5). The freeze marking device is held in position in a special box and, by means of carbon paper and roller, the impression of the iron is transferred to the official papers. In Washington, if the owner wishes to have the horse freeze marked as part of its identification program, the freeze mark impression will be made on the identification papers and will thus be included on the horse's passport (Fig. 6).

Freeze marking is now recommended by the Arabian Horse Registry for its membership, is in use by Anglo Arabs and will soon be used by the National Trotting Pony Association. All Arabians owned by the Egyptian Agricultural Organization and most privately owned Arabians in Egypt are freeze marked. Sweden has a state sponsored program for freeze marking horses and a Standardbred cannot be raced in New Zealand unless it has positive identification by freeze marking. New Zealand, however, uses a symbol system which is alterable.

The freeze mark has several advantages over tattoos as an applied mark. The most obvious is the ability to mark the animal at a young age. Another advantage is the safety from accidental inoculation of pathogens with the tattoo pins.

The unalterable angle system used as a freeze mark allows enough num-
bers to have a distinct mark for every horse in the world so that states, registries, and other official programs can mark each horse in a unique manner.

Figure 1. State of Washington equine passport form with signalment key attached.
Figure 2. The Angle System of numbers.

Figure 3. Eight positions of letter A.
Figure 4. A complete symbol system for equine identification. The alphabet symbol represents the registry where further information can be found on the animal.
Figure 5. Imprinter for making an impression of the marking irons on official papers.

Figure 6. After removal of signalment key, the Washington state passport form is reduced and folded into a laminated pocket card.
THE HORSE AS A MODEL

REFERENCES

12. Drayton, Jr., Spencer J.: Personal communication, Thoroughbred Racing Protective Bureau, Lake Success, N.Y.
1974 REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

Chairman: Dr. R. C. Knowles, Silver Spring, Md.
Co-Chairman: Dr. W. O. Kester, Golden, Colo.


Equine Infectious Anemia (EIA)

"The Canadian program on equine infectious anemia was outlined:* In Canada the eradication and control of animal diseases is a federal responsibility. The testing program for EIA started in February, 1971. EIA was made a "named disease" in the Canadian Animal Disease Regulations; and because it is "named," veterinary practitioners or owners of horses are required to report suspected cases to the Health of Animals Branch of the Canadian Department of Agriculture. Animals suspected of EIA are tested by the Agar-gel immunodiffusion (AGID) method. Once it has been established that an animal is a reactor, a quarantine is placed on the premises and all other horses on the property are tested. The Canadian branch veterinarian orders the reactor destroyed and awards compensation up to $200, depending on the value of the horse. The animal is branded on the shoulder with the letter "R" using a cold brand. The owner will also receive any payment for the carcass if the animal is slaughtered in an abattoir, rendering plant or similar establishment instead of being put down. Of course, whatever method of disposal is chosen, the animal is destroyed under the supervision of the Branch veterinarian. In a few cases they have permitted the owner to remove the animal to an approved premises where it is permanently quarantined in fly-proof quarters.

If the horse is a clinical reactor, it is removed and the quarantine on the premises is kept in force and other horses on the premises are retested after 40 days. The quarantine is withdrawn when it has been established that the horses retested are negative to the Coggins test. If, however, the reactor is a non-clinical reactor, it is removed; and the quarantine is withdrawn provided the other horses on the property have been found to be negative to a Coggins test.

An epidemiological investigation is carried out by our veterinarian and any horses which are considered to be a risk due to contact with the reactor are traced and tested.

Now for some statistics. Up to the end of June, 1974, 171,642 samples

*By Dr. D. J. Skinner, Health of Animals Branch, Canada Department of Agriculture.
were tested and out of these, 5,234 were positive. This gives a percentage of 3.04%. On a yearly basis, figures for the fiscal year, i.e., April 1 to March 31 are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Number Tested</th>
<th>Positive</th>
<th>Percentage Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 1971-March 1971</td>
<td>602</td>
<td>70</td>
<td>11.2%</td>
</tr>
<tr>
<td>April 1971-March 1972</td>
<td>49,402</td>
<td>2,009</td>
<td>4.0%</td>
</tr>
<tr>
<td>April 1972-March 1973</td>
<td>50,771</td>
<td>1,623</td>
<td>3.19%</td>
</tr>
<tr>
<td>April 1973-March 1974</td>
<td>49,004</td>
<td>1,126</td>
<td>2.29%</td>
</tr>
<tr>
<td>April 1974-June 1974</td>
<td>149,819</td>
<td>4,828</td>
<td>3.23%</td>
</tr>
<tr>
<td>Total February 1971-June 74</td>
<td>171,642</td>
<td>5,234</td>
<td>3.04%</td>
</tr>
</tbody>
</table>

It can be seen that the percentage of positive animals has decreased each year.

I must point out that these figures will include race horses, some of which may be being tested for the second or third time so that they can be issued a certificate stating that they were tested within the last six months. The reason for this will be mentioned later.

We have found that some foals have given a positive result due to maternal antibodies. Our experience has been that these have become negative when retested at six months of age.

Our program at present is a control program and rather than farm to farm testing, we test only on premises where we suspect there is infection or when requested to do so. This method was used with success in the eradication of glanders and dourine.

In the United States since the first of July, 1973, seventeen states have initiated requirements regarding horses and other equidae for a negative AGID test prior to entry. Among these states, seven require a negative test at certain assembly points such as auction sales, horse shows and race tracks. These states are: Florida, Kentucky, North Carolina, New York, Virginia, Vermont, and West Virginia. An outline for a state control program for equine infectious anemia was reviewed and refined. This outline is intended to give guidance to state animal health officials in combating equine infectious anemia. (Refer to outline included with this report)

The matter of treating EIA reactors in the field with the drug thiouracil was discussed. It is recognized that chemotherapy investigations are being conducted in several states by workers at the University of Florida under a veterinary investigational new drug application which has been in effect for approximately six months; and any data collected concerning efficacy and safety of thiouracil will necessarily have to be submitted to the Bureau of Veterinary Medicine, Food and Drug Administration, Department of Health, Education and Welfare (FDA, HEW). It is the committee's recommendation that State and Federal animal health officials, in addition to USAHA, provide information to the horse industry as to the experimental nature of this use of thiouracil and the limits related thereto. The safety and efficacy of this drug has not been established, and it has not been approved by
FDA for other than experimental use; and until such is done, FDA advises there is no legal way animal health officials can sanction its use.

**Importation of Horses**

It is evident that horses offered for importation must be evaluated as to their potential for introducing EIA and VEE, as well as any other infectious disease. The present scrutiny in the case of EIA is inadequate in that the AGID test is not applied. (Copy Resolution)

It should be recognized that zebras are susceptible to and do harbor the parasites *Babesia caballi* and *Babesia equi*, and when offered for entry into the United States should be tested by the complement fixation test to determine their status. (Refer to resolution)

**Venezuelan Equine Encephalomyelitis**

Surveillance has been continued for VEE, which includes investigation of horse cases and deaths suspicious of VEE. No evidence of viral activity has been observed since November, 1971. The committee proposes the following resolutions (Refer to Resolutions).

**Horse Identification**

The sub-committee on horse identification reported the following. It is imperative that a uniform system of identification of horses be established for the following reasons:

1. To assist in the control of intrastate and interstate movement of horses relative to infectious diseases.
2. To help minimize theft of horses.
3. To help minimize the promiscuous substitution of similarly marked horses in exhibitions, sales and at horse race tracks.

It is established fact that accurate health certification and efficient disease prevention programs involving testing, vaccination or isolation of horses are not practical without a system for permanent identification of individual horses.

The present system of health certificates, certification of vaccination and certification of certain tests does not include adequate identification of the horses involved. It is recommended that animal health officials review their own systems and take positive steps to improve this matter relative to horse identification. This improvement should include liaison with equine practitioners to remind them of their responsibility in this matter.

This Committee recommends that the US Animal Health Association recommend to all states that in order to protect the horse industry against spread of disease, theft and promiscuous substitutions, each state adopt rules and regulations that would provide for a system to adequately identify horses. Such a system could be similar to that used in Arizona, New Mexico, and Colorado (Passport System) or comparable to the system used by England, Ireland and France. Such a system should operate in a manner similar to that presently used for automobiles in the United States.

The Passport description of an individual horse should include owner's name and address, horse's name or registration number, age, sex, height,
weight, body color, color markings, size and location of chestnuts and location of whorls. Also, if marked by brands or scars, these should be included. This passport should be kept with the horse. In the case of sale or transfer of ownership, a new passport should be issued.

It is recommended that such passports have reciprocity among states comparable to automobile registrations.

EQUINE INFECTIONS ANEMIA
An Outline for a State Control Program

1. Introduction
In an effort to help provide national uniformity to the control and eventual eradication of equine infectious anemia (EIA) the Infectious Diseases of Horses Committee, United States Animal Health Association (USAHA) offers this outline as a guide to animal health officials and others concerned with the fight against EIA.

The practical application of the agar-gel immunodiffusion (AGID) test for EIA, otherwise known as the Coggins test, involves the identification and control of reactor animals (those known to be affected). Successful control of EIA should be preceded by an educational program directed toward horse owners in all facets of the horse industry. The presence of the inapparent EIA carrier animals among the horse population makes it important that owners understand how EIA is perpetuated and spread in nature.

The AGID test, or other official diagnostic tests recognized by USDA, should be considered as official tests for EIA. Definitions for the purpose of this outline should be the same as, or similar to, those contained in 9 CFR. Amendment to part 75 concerning EIA.

II. Testing Requirements
(Phase in each item B through D below at 6 to 12 month increments)

It is advisable to initiate requirements for testing of exhibition or competition horses at least 6 months before the active season begins for these events.

A. Only USDA approved laboratories will conduct the AGID test and all tests will be official. Samples can be accepted only from accredited veterinarians or fulltime State or Federal regulatory veterinarians.

B. All horses and other equidae entering the State must have been AGID tested negatively within 6 months prior to entry and must be accompanied by a certificate describing the horse and signed by an accredited veterinarian.
C. All horses and other equidae sold, traded, or given away within the state must have been AGID tested negatively within 12 months prior to this transaction and be accompanied by a certificate describing the horse, signed by an accredited veterinarian.

D. All horses and other equidae from within the state entered in exhibitions or competitive events must have been tested negatively within 12 months prior and be accompanied by a certificate describing the horse and signed by an accredited veterinarian.

E. An importing state may wish to consider the adoption of regulations to provide for the importation of non-tested animals that are to be consigned directly to slaughter.

III. Control of Reactors
A. Reactors must be officially permanently identified using the National Uniform Tag code number assigned by USDA to the State in which the reactor was tested followed by the letter "A", such markings shall be permanently applied to the animal by a Veterinary Services inspector or State inspector, or accredited veterinarian who shall employ for the purpose a hot iron or chemical brand, freezebranding, or a lip tattoo. If hot iron or chemical branding or freezebranding is employed, the markings shall not be less than two inches high and shall be applied to the left shoulder or left side of the neck of the reactor. If a lip tattoo is employed, the tattoo shall be not less than 3/4 in. high and 1/2 of an inch wide and shall be applied to the inside surface of the lip of the reactor.

B. Reactors must be properly isolated.

C. A reactor may be moved when:

1. It is identified as in Paragraph A above and is moved intrastate or interstate, for immediate slaughter, to a federally inspected slaughtering establishment operated under the provisions of the Federal Meat Inspection Act (21 U.S.C. 602 et seq.) or to a State inspected slaughtering establishment which has inspection by a State inspector at the time of slaughter, or to a diagnostic or research facility designated by the Deputy Administrator, Veterinary Services, APHIS, USDA, or a state approved rendering establishment.

2. The reactor is disclosed on an official test conducted in a State other than the State in which the home farm or ranch of the reactor is located, and such reactor is moved interstate to its home farm or ranch under a certificate issued by a State or Veterinary Services inspector upon his determination, after consultation with and approval by the State officials concerned, that the reactor so moved will be maintained segregated from other equine animals and quarantined under State authority on the premises of its home farm until natural death, slaughter, or disposition by euthanasia.
IV. Suggested Procedures for Eliminating EIA from Infected Herds

A. Herd is defined as:

1. On a ranch, farm, or stable: all equidae on one premises or where the owner has several units with interchange of equine animals all of these units.

2. At a horse racetrack: all animals handled by the same trainer who is in charge of the reactor or, if animal health officials so deem it necessary (based on vector pressures), all animals in the same shed row or the whole stable area.

B. AGID Testing Schedule and Vector Control

1. Official test on all horse stock in herd. Identify and remove all reactors.

2. Repeat "1" above (does not apply to horse racetracks) until all horses are negative on three consecutive tests, the first two to be conducted at 30-40 day intervals with the final test to be conducted 60-90 days following the preceding test.

3. All introduction of horses into herd shall have a negative test conducted within 90 days prior to entry.

4. Vector control practices be followed in herds to reduce exposure in these herds. These practices should include insect and human vector control such as periodic application of insecticides to horses and facilities occupied by these animals, and cleaning and disinfection of equipment used among animals in the herd.
PROPER DRUG USAGE AND THE PUBLIC HEALTH

R. E. McKinley, V.M.D.*

For Presentation at the
U.S. ANIMAL HEALTH ASSOCIATION ANNUAL MEETING
Roanoke, Virginia

Mr. President, members of the Association and guests: I wish to thank your program committee and your chairman of the Committee on Pharmaceuticals, Dr. Roland Gessert, for this opportunity to present this discussion of Proper Drug Usage to this Association. Because this is a regulatory oriented organization and because of your long history of leadership in infectious disease control procedures, biologics development and regulation of the biologics half of the animal drug industry, this audience is acutely aware of the many facets of Proper Drug Usage. While it is my intention to provide you with some facts and to make some suggestions that some of you may find useful in your contacts with the public and colleagues, my views and comments should not be considered to represent the views or policies of either the Animal Health Institute or Hoffmann-La Roche.

I wish also to express my appreciation of your published Proceedings and for this opportunity to have my presentation become a part of those proceedings. These annual publications are a valuable reference source and record of the contributions made to the protection of the public health by the livestock industry, the animal biologics and pharmaceutical industry and the regulating state and federal agencies.

I have chosen to limit this discussion to:
1. Drug residue considerations,
2. A statement of currently reported experience relative to the public health and animal drug residues,
3. A brief listing of major benefits from animal drug usage,
4. Some reasons why we must concern ourselves with the subject of Proper Drug Usage,
5. Mention of new legislation and policies that may affect our concepts of proper drug usage, and
6. A listing of some safeguards that may assist in informing the public of some of the reasons why they should continue to have faith in the purity and wholesomeness of their food supply.

One key question that may be asked concerning this topic, Proper Drug Usage and the Public Health, is "What information is available regarding the effects, good or bad, of animal drug usage on the public health?" With the passage of the 1962 amendments to the Food and Drug Act and the requirements for records and reports with emphasis on the collection of adverse reaction information, one would expect a wealth of information to have been gained relative to any particular effects experienced during the

*Chairman, Animal Drug Section, Animal Health Institute, Washington, D.C.; Assistant Director, Dept. Drug Regulatory Affairs, Hoffmann-La Roche, Inc., Notley, N.J.
past 12 years.

Inquiry of Bureau of Veterinary Medicine/Food and Drug Administration officials has disclosed that, while there have been many speculative attacks on animal drugs, there are, in fact, no reports of the experience of adverse effects on humans as a result of pharmacological or biological effects from animal drug residues. To my knowledge, prior to 1962 there was one report of an allergic reaction in man to penicillin residues in milk. With today's media concentration on newsworthy alarming items, extensive monitoring of FDA/industry activities by Congress, and the wide-ranging investigations of the consumer protection groups, this absence of ill effects suggests that the animal health industry's proposals and performance and the FDA/USDA decisions as to the safety of animal drugs relative to the public health have been correct.

Let me hasten to add that this kind of experience should not be permitted to give us an undue sense of security or satisfaction with a job well done by both the responsible Federal agencies and members of the animal drug and livestock industries. The United States Department of Agriculture reports of drug residue monitoring, while showing major improvements in the reduction of incidents of positive drug residue findings, continue to show some limited number of positives, especially in certain classes of livestock. The significance of these positive findings relative to public health is difficult to determine, despite the long history of the absence of reports of adverse effects in humans. Furthermore, the previously mentioned speculative attacks on animal drugs are matters of concern to all of us, not only because science has been and will be unable to prove absolute safety despite all of our technological advances and abilities, but also because, while we believe these USDA findings represent drugs and drug amounts that are insignificant relative to the public health, the animal drug approval system is designed to avoid completely such incidents. Thus, where failures of the system occur, it is in our best interest and the public interest to ascertain the cause of the incidents and to take corrective measures.

On the other side of the coin, what are the good effects of proper drug usage on the public health? Here the list can be very long indeed, so I will list only a few.

1. We have, through the proper use of animal drugs, been able to control effectively most of the historically and potentially devastating infectious diseases of our vast livestock and pet populations. Here I include in the term "Animal drugs" all of the vaccines and immunizing agents, as well as therapeutics, diagnostics, preventives, and other usual drug categories. This has been no small accomplishment, even considering only the large number of animal diseases that are transmissible to man and the dramatic effects on the cost and availability of food for man and animals.

2. We have alleviated suffering and the painful effects of disease in man and animals and therefore made major contributions to the comfort of animals and the physical well-being and mental health of the human population.

3. Through various management tools made possible by drug usage, we have enabled the livestock industry to make major breakthroughs in efficient animal food production - thereby making possible, in this country,
the highest nutritional standard of living in the world and vastly improving
the standards of other countries.

With this introduction, one might conclude that proper drug usage should
not be a matter for concern. I hold the opposite view, and I base this
opinion on the following:

(1) We are enjoying a phase of the application of technological advances
in the fields of toxicology, chemistry, and analytical instrumentation, which
application raised more questions than we can answer readily—thus we are
going through a period of uncertainty. Some recent experiences with the
withdrawal of approvals for certain animal drugs vividly illustrate this
point.

(2) The facts stated above relative to the good and bad effects of animal
drug use on the public health suggest that benefit/risk decisions made thus
far by both industry and the regulating agencies have been very sound.
However, we are now attempting to develop more precise benefit/risk
decisions. Hence, our procedures to define, describe and communicate
benefit/risk decisions for animal drugs effectively are being reformed.
We have not yet had sufficient experience with the National Environmental
Protection Act to assess its full impact on the use of drugs in animals.
We are now entering a new era in our attempts to live with the Delaney
Clause of the Food and Drug Act. The zero tolerance concepts expressed
by this clause and in acts such as the Clean Air Act and the Federal
Water Pollution Control Act are the subject of an extensive report by the
Council for Agricultural Science and Technology, as well as a presenta-
tion by Dr. Albert Kolbye, Associate Director for Sciences, Bureau of Foods,
Food and Drug Administration, at the 1974 Animal Science Meeting in
Maryland. It is uncertain how these related Federal laws and policies will
affect our concepts of proper drug usage.

(3) The previously mentioned USDA reports of continuing positive findings
of drug residues in some classes of livestock suggest that either some drugs are
not being used properly or that appropriate withdrawal intervals are not being
followed. Unfortunately, these findings have not always been determined by the
use of the official residue methodologies. For whatever reasons that unofficial
methods have been used, these positive findings have not always been believed
reliable enough to permit prosecution. As a result, both FDA and USDA have
created or are creating new standards for acceptable residue methodologies.
Again, the effects of the method developments on proper drug usage are difficult
to predict at this time.

Additionally, all parties (the regulating agencies, the regulated industries,
and the animal drug users) must be concerned that the public does not
lose faith in the purity and wholesomeness of our food supply. In this
regard I suggest that it is important for all parties involved to give more
prominence to the fact that animal drugs go through the same approving
process do human drugs. I believe that a significant portion of our popula-
tion does not realize this fact. Further, they do not understand the relation-
ship of the other professionals—such as chemists, pharmacologists, physio-
logists, and so forth—to the development and approval of animal drugs or to
the extensive food inspection system that is in existence today. They should
be informed that government, industry, and public representatives who are
not only trained and experienced in generating and evaluating various kinds of complex data, but are also fellow consumers, have made the benefit/risk decisions in the past and established a good track record. They are continuing to make these decisions today, not as sterile scientists, but as conscientious human beings who, in seeking to protect the public, are protecting their own families as well.

The public does understand when there are wide reports of residues being found in some foodstuffs—that there is some potential hazard in their food supply—but they have no real means of determining for themselves whether such a potential hazard is significant to them individually, and thus a fear of the unknown is created. Therefore, we all must engage in a continuing educational process to inform the public at large of the extensive steps that are routinely taken to protect the purity and wholesomeness of their food supply and of the kinds of expertise utilized in implementing those steps.

Secondly, ways must be found to inform the public of the extensive evaluations of drug residues that are made prior to animal drug approval and the tremendous built-in margins of safety that are required by today's regulatory policies. I daresay that there are few in even this well-informed audience who fully understand this system. Do you all know that every animal drug that is approved for use in a food-producing animal today must meet these minimum standards?—

1. Metabolism studies in the target species must define the tissue distribution and amount of the parent compound and its significant metabolites.

2. Precise information must be obtained as to the “no-effect” level of the drug (and its metabolites, if they are important) in toxicological studies in laboratory animals.

3. This “no-effect” amount is then translated into sensitivity requirements for tissue residue assay methods by calculations which use, as a minimum when no finite tolerance is involved, a 2,000-fold safety factor relative to possible human food intake. This 2,000-fold factor is pertinent for a majority of animal drugs.

4. A “practical” withdrawal time is required to assure that the treated animal will not be marketed for food purposes until all residues are eliminated from its edible tissues. This withdrawal time is usually long enough, in itself, to provide an additional margin of safety so that it is more unlikely that any drug residues will be found in food.

Additionally, I have previously mentioned USDA's extensive tissue residue monitoring program. This program provides a check on the animal industry's observance of the required withdrawal times and on other forms of possible misuse of animal drugs.

FDA and the various state regulatory agencies conduct an extensive feed manufacturer registration and inspection system. This system assists in assuring that drugs administered in the feed are, in fact, used at proper concentrations and that such feeds are properly labeled so that the animal feeder is reliably informed as to the proper drug usage.

Many drugs are only available through the veterinary profession. Thus, drugs that require unusual withdrawal intervals to avoid residue problems
or have some other potential for harm are restricted in their distribution
to a responsible professional, thereby reducing the potentials for drug misuse and giving the public added assurance that their food supply will not have residues from such drugs.

Finally, the drug industry has voluntarily sponsored major efforts to assure that the livestock producer is provided with adequate drug usage information and prominent label statements calling the user’s attention to required withdrawal times and other directions for use.

A majority of these voluntary programs have been initiated by the Animal Health Institute and started with the "eye-clock" program urging people to read the label and observe label directions. This program was closely followed by the voluntary drug certification program, in which the livestock producer provided certification that appropriate withdrawal intervals had indeed been followed. Later the labeling program, which the Institute designates as the "universal arrow" program, was instituted, the purpose being to give more prominence to important warnings and the required withdrawal intervals.

Additionally, a slide program on the subject "Proper Drug Usage" has been prepared and has been made available for use by industry and others for presentation to livestock producer audiences. The Animal Health Institute has also initiated a program designed to further inform veterinary students of the complexities and details of the animal drug approval process.

For those drugs falling under the biologics classification, a regular biologics newsletter is distributed to the industry and livestock producers, and an educational film has been prepared for presentation to livestock producer audiences to demonstrate the proper usage of various biologics.

Collectively, all of these standards, restrictions, and programs, provide margins of safety for drug residues that go far beyond the calculated margins demonstrated under the minimum standards quoted above for animal drugs and drug residues.

We should note further that some drugs are so rapidly metabolized that under ordinary conditions they are not detected, even with today's advanced technology. Others are used only very early in an animal's lifespan so that they are completely eliminated by the time of slaughter. Others have been shown to be so innocuous that tolerances for various small amounts in edible tissues have been established. Even with these, large margins of safety are utilized to further assure the safety of the human food supply.

Added to all these factors is the other important factor that not all animals receive all drugs. Some diseases do not exist in some areas of the country. In other cases, disease outbreaks are so infrequent that drugs for prevention are not economical, and only therapeutic drugs are used. The animal drug management tools, such as growth promotants, are not always profitable to use. Thus not all animals receive them.

While these use considerations are obvious to the livestock producer and other professionals in the animal health business, the general public may not understand that such factors also constitute additional safety factors that serve to reduce the chances for residues to occur and, thus, should be added to the above list of factors that help create the large margins of
safety associated with the use of animal drugs and relative to the potentials for finding significant amounts of drug residues.

Admittedly, there are weaknesses in the system. Certainly, one of the more important factors is the labeling, its clarity, completeness, and so forth. Again, we should inform the public that the law requires a specific decision on the part of the approving agency that “it is reasonable to expect the labeling to be followed” and that is may not be “misleading in any particular.” The nature of this overall problem may be illustrated by the precautionary labeling now found on some simple item of commerce—if you have recently purchased an ordinary hammer you may have noted a firmly affixed label which states that this hammer is to be used for driving nails only and that any other use may be hazardous.

Such a statement is so obvious that it is humorous, but it does potentially do one thing—it may get the purchaser’s attention and thus his consideration. There is no foolproof way yet devised to guarantee absolutely that the user will read and follow label directions. Hence, the consuming public must rely on the livestock producers to accept and discharge these responsibilities. Again, the fact that our monitoring programs have disclosed a very low incidence of positive findings of drug residues and also a very, very low incidence of other adverse effects on the target species and a zero incidence of adverse effects on humans suggests that the average drug user has proven worthy of that reliance. By vigorously pursuing our current educational and enforcement programs, we will continue to get the user’s attention and consideration of the labeling for animal drugs and require that he accept his responsibilities for proper drug usage.

* In summary then I have set forth facts that illustrate that the animal health industry’s past benefit/risk decisions have been sound and that our system permitting these animal drugs and biologics to be marketed is a sound system that is unlikely to permit failures in the decision making process. Additionally, I have illustrated the need for communication with the public and suggest to you that this Association, its individual members, the livestock producers and their marketing and research organizations, the regulating agencies, and the animal drug industry all must communicate to the public how we collectively assure the purity and wholesomeness of our food supply.
REFERENCES

Antibiotics have now been used extensively in animal feeds in every major livestock producing country for over 20 years. From the beginning it was feared on theoretical grounds that the build-up of resistance to antibiotics would limit their utility. However, as it has turned out, antibiotic feed supplements have continued to be useful and in demand. While there is some fluctuation in effectiveness, which is to be expected depending on husbandry conditions, feeding practices and other variables too numerous to mention, antibiotics in general have continued to show the same degree of efficacy in animals as shown when they were first introduced as animal health drugs.

As a background for this discussion, a brief review of actions taken by regulatory bodies in the United Kingdom and the United States is in order. The topic of this paper, "Antibacterial Drugs in Food Animals — Their Environmental Impact", was first addressed by a governmental regulatory body in a formal sense in the United Kingdom when the Swann Committee was appointed. This group was asked to examine the available data and make a determination whether real or potential hazards existed from the use of antibiotics in the feed or drinking water of food animals. Does the sub-therapeutic use of antibiotics in animal feeds compromise the use of antibiotics for therapy of human and animal diseases? The evidence they examined supported either view. However, in 1969 the Swann Committee recommended that antibiotics which were used in human medicine should not be used as sub-therapeutic animal feed additives (i.e. for improved rate of growth promotion and feed efficiency or for disease prophylaxis) because of the danger that such use might lead to the development of resistance in bacteria which might be transmitted to man and which might cause illness in man. Upon adoption of this recommendation in the United Kingdom the status of antibacterials in feed became and continues today as follows:

See Slide 1

It would be interesting to know the current use pattern of antibiotics in animal feeds, the change, if any, in the resistance pattern of animal pathogens and the change in animal disease incidence in the United Kingdom. Unfortunately, hard data are not available on the effects of the Swann legislation.

In April, 1970, the Commissioner of the Food and Drug Administration established a task force to undertake a comprehensive review of the use of antibiotics in feeds. The FDA Antibiotic Task Force identified three primary areas of possible concern over the sub-therapeutic use of antibiotics in animal feeds: (1) human health hazard, (2) animal health hazard and (3) antibiotic effectiveness. Hundreds of pertinent publications were reviewed and many experts were consulted during the deliberations of the Task Force. The
complexity of the problems associated with interpreting the existing data is highlighted by the fact that fifteen scientists on the Task Force, all of whom had access to the same data, did not agree unanimously with the report. In a minority report, seven of the fifteen members of the Task Force took issue with many of the major conclusions reached, particularly a number of those relating to human and animal health hazards. Such being the case, it would be unprofitable for us at this time to revisit the same data and again debate the merits of the conclusions of the task force, particularly since this has already been done in several publications. Instead we will examine some of the evidence that has become available and is being collected since the Swann Committee and the Antibiotic Task Force deliberations.

The Task Force report raised questions but provided no modus operandi for initiating the kinds of experiments to generate the data to answer the questions. The Animal Health Institute appointed a committee which worked long and diligently with personnel from the Bureau of Veterinary Medicine of FDA in developing criteria to be satisfied in order to establish the safety and efficacy of the use of antibiotics in animal feeds. I do not intend to discuss these criteria in detail, but they have been used to guide the research carried out to respond to the questions raised by the Task Force.

In contrast to the recommendations of the Swann Committee, the Antibiotic Task Force concluded the available evidence did not allow firm decisions to be made. Instead it was recommended additional data be generated bearing on the three areas of possible concern: (1) human health hazard, (2) animal health hazard and (3) antibiotic effectiveness. We will focus only on the questions raised concerning possible hazard to human or animal health, particularly the former, resulting from the subtherapeutic use of antibiotics in animal feeds. These questions are briefly summarized on the following slide.

See Slide 2

Once the criteria were available, an Animal Health Institute committee (representatives from the major producers in the U.S. of animal health drugs) outlined protocols for experiments deemed necessary to generate the kinds of data required. In addition, a number of experts were contacted to aid in certain of the studies. The committee agreed some of the studies would be done by the company sponsoring a particular antibiotic, while others of the studies were amenable to joint sponsorship through the Animal Health Institute. A limited number of the projects are complete and the results are available, but most are still on-going, and I prefer not to discuss interim data. I will comment briefly on some of the typical projects undertaken:

1. A comprehensive review of the literature on salmonellosis was compiled and analyzed and published by the Animal Health Institute under the title "The Origins of Salmonellosis". (A.H.I.)*
2. A retrospective epidemiological study of Salmonella and Shigella infections occurring in sample years during the last three decades and

*sponsored by the Animal Health Institute
Data from Boston City Hospital indicate the increasing occurrence of bacteria and of deaths from such infections since the introduction and widespread use of modern antibacterial agents. The greatest increase in bacteremic infections occurred before broad-spectrum antibiotics became available and before antibiotic supplements were first used in animal feeds. No similar increases in salmonellosis or shigellosis have occurred at this hospital.

These findings do not support the basis for the recommendation to discontinue use of certain antibacterial agents as feed supplements for animals, nor for the concern over the hazards to human health from such use. (A.H.I.)

3. Experiments were designed to determine the effect of subtherapeutic use of antibiotics in animal feeds on the quantity, prevalence, duration of shedding and the antibiotic resistance characteristics of Salmonella in food animals. The Animal Health Institute committee has decided studies of this nature should be carried out by the individual companies for their respective antibiotics. Time does not allow a detailed technical discussion of the extensive experimentation Pfizer has carried out, but a brief description of a typical experiment and the results obtained are appropriate to this topic. As my example, I have selected one which demonstrates the effect of oxytetracycline on a Salmonella reservoir in swine.

Swine were pretreated with sub-therapeutic doses of oxytetracycline in feed for 5 days prior to colonizing the intestinal tracts of the animals with an oral dose of Salmonella typhimurium and drug dosage was continued until termination of the experiment. Similarly inoculated, but unmedicated swine served as controls. Periodic measurements of the numbers and antibiotic resistance patterns of the fecal Salmonella population were made over a 28-day period post-inoculation.

Slide 3 shows the Salmonella counts per gram of feces as a function of time for the oxytetracycline-tested and control animals.

See Slide 3

Slide 4 depicts the percent of swine continuing to shed Salmonella as a function of time for oxytetracycline medicated and control animals.

See Slide 4

Conclusions reached from the experiment are:

A. There was no increase in the quantity of Salmonella in medicated swine as compared with the non-medicated group. In fact, lesser numbers of Salmonella were detected in the medicated group than in the controls.

B. There was no increase in the prevalence of Salmonella in medicated swine as compared with the non-medicated control group. Lesser numbers of animals in the medicated group were found to contain Salmonella over the test period than in the non-medicated control group.
C. There was no increase in the duration of shedding of *Salmonella* in medicated swine as compared with the non-medicated control group. Duration of shedding was shorter in the medicated than in the non-medicated group.

D. There was no change in the susceptibility pattern of the *Salmonella* in either medicated or non-medicated groups.

Similar results were obtained when calves or chickens were used instead of swine. Similar results have also been obtained by companies sponsoring other antibiotics.

4. Studies are underway to determine the antibiotic resistance pattern of various strains of *Salmonella* isolated from poultry and from swine. (A.H.I.)

Among the studies designed to obtain additional information on the effect of antibiotics in feed on the intestinal coliform resistance pattern of animals and whether any observed changes are a health hazard to animals or man are the following:

1. A trial to determine the effect of an antibiotic upon the establishment in the enteric tract of calves of an *E. coli* carrying an identifiable R-factor; to determine whether the *E. coli* can be transferred to and can colonize in human caretakers. This study is on-going. (A.H.I.)

2. A similar study using chickens as the test animal. (A.H.I.)

3. A comparison of a swine herd raised conventionally with subtherapeutic levels of antibiotic in the diet and a herd in which no sub-therapeutic levels were used. Measurements will be made on enteric bacterial resistance patterns and health/performance of the animals. (A.H.I.)

One further study intended to be responsive to another of the criteria (Slide 2- No. 3) and sponsored by A.H.I. is the following:

Experiments designed to determine whether a plasmid for toxin production can become linked to an R-factor and whether such a factor can be transferred *in vitro* and *in vivo*. This study is in progress. (A.H.I.)

So much for the criteria and the studies required to obtain data to respond to them. But is not the key question "Is the use of antibiotics in animal feeds a hazard to the health of man?" Such a question is inherently difficult to answer with scientific facts because one is trying to prove a negative conclusion. Perhaps it is simpler to design experiments which answer this question regarding *Salmonella* than it is for coliform organisms. If one believes an increase in the animal *Salmonella* reservoir is a hazard to man, data from experiments typified by the oxytetracycline/swine study described above should allay concern. Reduction of a *Salmonella* reservoir in chickens by chlorotetracycline has also been reported. Based on our data, oxytetracycline and those antibiotics which give similar results are serving a useful purpose over and above the important properties of disease prevention and animal performance improvement for which the antibiotic is employed.

Our data (if one assumes increasing the animal *Salmonella* reservoir is a hazard to man) indicate antibiotics are actually reducing the numbers of potentially dangerous *Salmonella* organisms hence reducing the risk of human illness from animal bacteria. Also, the Finland study showed no parallelism between human salmonellosis and antibiotic use in animal feeds.
Following coliform bacteria, or more specifically *E. coli*, through the scheme shown in Slide 5 is exceedingly difficult for several reasons, and important one being *E. coli* is a normal resident of human and animal intestines.

There are only a limited amount of data and these provide only inferential evidence from stages 2 to 5 of the scheme shown. All experts agree when animals are dosed (either therapeutically or sub-therapeutically) with antibiotics, the ratio of resistance to sensitive *E. coli* increases. Does the ratio remain high, do they transfer to man, do they cause illness in man? Most of the evidence available at the time of the Task Force report was reviewed by the group, which came up with questions rather than conclusions. Another analysis of these data at this time would be purposeless. Two of the experiments described previously will attempt to detect a marked *E. coli* through steps 1-4. However, it is difficult to design reasonable experiments which clarify steps 4 to 5. Smith has recently reported an experiment in which 649 people from 148 families were studied to compare the bacterial flora of people on farms with animals with those in rural dwellings without animal contact or in urban settings. Rectal cultures were studied for the resistance of *E. coli*. The prevalence of resistance to multiple antibiotics was: farm families with animals > farm families without animals > urban dwellers. However, a comparison of hospital admission on bacterial disease diagnosis for the three groups showed no significant differences.

Obviously, there has been no explosive illness in man resulting from the use of antibiotics in animal feed or the answers to our questions would be self-evident. Good data are not available on the total numbers of human illnesses per year caused by gram-negative bacteria. However, good records are kept of deaths caused by gram-negative organisms. These are presented along with the assumption that the pattern is the same for the less serious disease occurrences. What do the data say about serious illnesses in man caused by gram-negative bacteria?

According to the United States National Center for Health Statistics (USNCHS), of the 1,930,082 deaths reported in the United States in 1970, only 250 were reported as being caused by gram-negative bacteremia. (These figures are drawn from death certificates which are filled out by physicians and filed with HEW.) If a proportional number of deaths due to “unspecified” types of septicemia is added, the USNCHS figures would indicate an estimated 1,766 deaths due to gram-negative bacteremia in 1970. Even on the unrealistic assumption that all deaths due to “unspecified” septicemias were assumed to be caused by gram-negative organisms. the USNCHS figures would indicate an estimated 3,282 deaths in 1970 due to gram-negative bacteremia.**

**These statistics are based on data gathered by the USNCHS from all death certificates filed out and filed in the United States. There are no data available on the number of deaths due to gram-negative bacteremia for the years prior to 1968, but in 1968 the USNCHS adopted the International Classification of Disease’s system of coding and began reporting the deaths due to the various types of septicemia, including those due to gram-negative organisms.
A recent hospital study of *E. coli* resistance to various chemotherapeutics revealed a decline in the incidence of resistant strains over a ten year period.\(^8\)

See Slide 6

In Slide 7 the same data are presented in terms of numbers of *E. coli* showing resistance to 0-1, 2 or more and 4 or more antibiotics. According to these data, there has been a decrease in the incidence of multiply resistant *E. coli* during the period 1959-1960 to 1968.

See Slide 7

I would like to close by suggesting a possible outcome of the banning of the use of sub-therapeutic levels of antibiotics in animal feeds. The speculation that use of antibiotics in animal feeds might lead to treatment-resistant infectious illness in man can be matched by the speculation that prohibitory action might lead to the same sort of repercussion. The use of antibiotics in feeds for the last twenty years has been accompanied by widespread decreases in animal mortality and morbidity from enteric disease.

Like the opposite speculation, this one seems unlikely, though any proposal for changes in regulations covering agricultural antibiotic use must include programs for monitoring desired or undesired effects in animals and human health. But a possible scenario involves the following sequence:

1. "Real-world" producers of meat animals cannot or do not change their husbandry practices in the direction of reducing overcrowding and intensive farming.

2. Morbidity of livestock increases precipitously, and a lot of sick animals are soon around.

3. These are hurried off to slaughter.

4. More meat comes on the market contaminated with bacterial pathogens.

5. Dysentery reappears, and epidemics of salmonellosis and shigellosis become more frequent.

6. A *real* public health problem has replaced a *suppositious* risk.

7. Regulatory bodies are condemned for permitting unsanitary food to reach the consumer.

8. Policy reversal is attempted, confusion is compounded, science policy mechanisms are further discredited, etc.

It should also be noted that there is now increasing recognition, based on advances in systems theory, of the potential dangers inherent in tinkering with complex social systems in the absence of (1) compelling, objective demonstration of a real justification for doing so, (2) understanding of how the system will react, and (3) knowledge of whether that adaptation of the system is in the public interest.
Slide 1

1. Bacitracin, virginiamycin, flavomycin and nitrovin may be purchased for use in swine and poultry feeds by the producer without veterinary prescription.

2. Flavomycin and nitrovin may be purchased by the producer for use in calf feeds without veterinary prescription.

3. Nifursol, sulfaquinoxaline and sulfanitran may be used in poultry feeds without a veterinary prescription.

4. All other antibacterial animal health drugs are restricted to use only on the order of a veterinarian.

Slide 2

1. Is the animal Salmonella reservoir increased? Is there an increase in the quantity, prevalence, duration of shedding or change in the antibiotic resistance pattern?

2. Is there an increase in resistant coliforms which are a hazard to the health of animals or man?

3. Is the pathogenicity of bacteria enhanced?

4. Are any remaining drug tissue residues harmful to man?
PERCENT OF SWINE YIELDING *S. TYPHIMURIUM*

Days (Post-Inoculation)

Oxytetracycline Treated

Infected Controls

Per cent of Swine Shedding *Salmonella Typhimurium*
1. ANIMALS + ANTIBIOTICS

2. bacteria harmful to man?

3. transfer to man?

4. colonize in man?

5. SICK MAN
Slide 6

INCIDENCE OF RESISTANT STRAINS OF *ESCHERICHIA COLI*
UNIVERSITY OF WASHINGTON HOSPITAL

Percent of strains resistant by year

<table>
<thead>
<tr>
<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>Tetracycline</td>
<td>23.4</td>
<td>23.8</td>
<td>23.4</td>
<td>28.9</td>
<td>20.5</td>
<td>26.5</td>
<td>26.9</td>
<td>16.6</td>
<td>19.1</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>5.9</td>
<td>7.0</td>
<td>3.9</td>
<td>7.8</td>
<td>4.5</td>
<td>3.5</td>
<td>2.1</td>
<td>1.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>39.0</td>
<td>17.8</td>
<td>15.2</td>
<td>21.6</td>
<td>19.7</td>
<td>21.3</td>
<td>17.8</td>
<td>13.5</td>
<td>13.1</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>4.1</td>
<td>1.8</td>
<td>1.5</td>
<td>2.4</td>
<td>0.9</td>
<td>0.5</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>13.4</td>
<td>3.1</td>
<td>0.2</td>
<td>0.9</td>
<td>0.5</td>
<td>1.6</td>
<td>2.1</td>
<td>1.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Sulfamethizol</td>
<td>50.9</td>
<td>37.2</td>
<td>25.5</td>
<td>35.0</td>
<td>24.1</td>
<td>23.2</td>
<td>25.9</td>
<td>15.6</td>
<td>12.6</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>1.8</td>
<td>2.1</td>
<td>0</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>2.9</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Total Number of Strains</td>
<td>269</td>
<td>387</td>
<td>585</td>
<td>509</td>
<td>584</td>
<td>767</td>
<td>578</td>
<td>416</td>
<td>468</td>
</tr>
</tbody>
</table>

Slide 7

ANNUAL INCIDENCE (PERCENT) OF MULTI-RESISTANCE AMONG STRAINS OF *ESCHERICHIA COLI*
UNIVERSITY OF WASHINGTON HOSPITAL

<table>
<thead>
<tr>
<th>Number of chemotherapeutics to which strains were resistant</th>
<th>1959-60</th>
<th>1961</th>
<th>1962</th>
<th>1963</th>
<th>1964</th>
<th>1965</th>
<th>1966</th>
<th>1967</th>
<th>1968</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>61.7</td>
<td>75.0</td>
<td>79.7</td>
<td>72.1</td>
<td>79.4</td>
<td>76.1</td>
<td>79.2</td>
<td>85.8</td>
<td>86.9</td>
</tr>
<tr>
<td>2 or more</td>
<td>38.3</td>
<td>25.0</td>
<td>20.3</td>
<td>27.9</td>
<td>20.6</td>
<td>23.9</td>
<td>20.8</td>
<td>14.2</td>
<td>13.1</td>
</tr>
<tr>
<td>4 or more</td>
<td>6.2</td>
<td>4.2</td>
<td>2.3</td>
<td>6.1</td>
<td>3.5</td>
<td>2.8</td>
<td>2.6</td>
<td>1.9</td>
<td>2.2</td>
</tr>
<tr>
<td>Number of strains tested</td>
<td>269</td>
<td>387</td>
<td>585</td>
<td>509</td>
<td>584</td>
<td>767</td>
<td>578</td>
<td>416</td>
<td>468</td>
</tr>
</tbody>
</table>
REFERENCES

The committee reviewed the proposal for establishment of a Peer Group Review Committee for new animal drugs. This proposal was discussed in 1973 and was supported by the Association. The Committee on Pharmaceuticals again endorses the concept of Peer Group Review as outlined in briefs prepared by a special Task Force of the Animal Health Institute. (See Page 231, Proceedings, 77th Annual Meeting of the USAHA). A letter to the Food and Drug Administration will be prepared, requesting clarification of the status of the AH1 Peer Group proposal.

The Committee continues to support and again commends the Food and Drug Administration on its expanded program to inform veterinarians of the need to use drugs in strict compliance with other measures intended to minimize persistence of drug residues in animal food products. Label features such as the “Take Time - Observe Label Directions” symbol, and the highlighting of the warning statements by the pharmaceutical industry also are commended.

The attention of the Committee was drawn to the open sale of Cap-Chur Immobilizing Drug and Equipment by a Farm and Ranch Supply Company. It is the thought of the Committee that the drug is a new animal drug for which no NADA has been approved. Because of the narrow safety margin and the pharmacologic action it also is felt the drug should be limited to use by or on the order of licensed veterinarians. Further, the labeling bears no withdrawal times when used in food animals, yet drug residues would be expected to occur. This matter is called to the attention of the Food and Drug Administration for further consideration.

The Committee also was informed of pregnant mare serum gonadatropin from a foreign source being furnished to livestock owners by extension and animal science research personnel of a state university. Because of the danger of possible importation of disease viruses with this biologic material, the situation is being referred to the USDA.

The Committee recognizes that many drugs are not made available for use in food producing animals because the limited use the drugs would
receive does not justify the cost of the research necessary to support FDA approval. This is particularly true of research to establish tissue residue studies, residue tolerances, and drug withdrawal times. In order to make such drugs available to the livestock industry (and particularly for the minor livestock species), the Committee urges Congress to provide for the development of these data by the USDA, FDA or other governmental agency. A program similar to IR-4 for pesticides seems worthy of consideration.

The Committee notes the absence of any new information suggesting that the use of subtherapeutic levels of antibiotics in feed results in a more severe Salmonella control problem. Indeed, some of the data suggest that subtherapeutic levels actually reduce the spread of Salmonella organisms. It is concluded that no Salmonella public health hazard exists from the proper use of subtherapeutic levels of antibacterial drugs.

During the past year, the following new animal drug entities were approved by the Food and Drug Administration:

Ticarbodine - Anthelmintic for roundworms, hookworms, and tapeworms in dogs.

Mafenide - Topical antibacterial

Meglumine and sodium-diatrizoate - A radio opaque agent for radiography of the gastrointestinal tract of dogs and cats.

Cuprimyxin - Topical antibacterial and antifungal antibiotic for dogs and cats.
DETECTION OF NEWCASTLE DISEASE VIRUS BY COMPLEMENT-FIXATION

W. K. Butterfield and J. H. Graves
United States Department of Agriculture,
Agricultural Research Service, Northeastern Region
Plum Island Animal Disease Center
Greenport, New York 11944

Introduction

Slightly over a year ago, scientists at the Plum Island Animal Disease Center were asked to develop a test to differentiate live virus vaccine strains of Newcastle disease virus (NDV) from exotic strains that produce viscerotropic velogenic manifestation of the disease. This report describes results obtained with a complement-fixation (CF) test in which guinea pig hyperimmune antiserum was used to differentiate lentogenic vaccine strains from velogenic field isolates.

Materials and Methods

The methodology for production of hyperimmune antiserum in guinea pigs and the standardization and use of the CF test have been submitted for publication so will be discussed only briefly here.

Hyperimmune antiserum: Antisera were produced to partly purified betapropiolactone-inactivated strains of NDV that included the lentogenic Hitchner, mesogenic Roakin, and viscerotropic velogenic California-1085 pathotypes.

Block CF titrations: Tittrations were performed with homologous antigens to determine optimal antiserum dilutions for use in the CF test.

Cross CF tests: CF tests were performed with the three antigens and 1/80 dilutions of the hyperimmune antiserums produced with these antigens. CF titer refers to that dilution of antigen producing 50% hemolysis with 5 units of complement.

CF testing of NDV strains: Initial tests were performed with the three hyperimmune antiserums and 10 NDV strains from the PIADC repository or through the courtesy of Dr. Theodore W. Chomiak, University of Connecticut, and Drs. Robert P. Hanson and Josip Spalatin, University of Wisconsin. Later tests were performed with a 1/80 dilution of Hitchner hyperimmune antiserum, nine additional strains of NDV submitted as unknowns to the testers, and 18 known velogenic strains of NDV from Dr. R. P. Hanson. Four lentogenic field isolates from geese were submitted by Dr. John K. Rosenberger, University of Delaware.

To date, 254 isolates from the 1971-72 NDV outbreak in California have been tested for complement-fixing capabilities.

Results

Block titrations: A 1/80 dilution was optimal with all three antisera for use in the CF test. Antigen, antibody, and complement controls were normal and are not listed on tables for ease of presentation.

Cross CF tests: Table 1 shows results of cross testing. Hitchner and
Roakin antigens were strong complement-fixing antigens with all three antisera, whereas the California-1085 antigen fixed complement poorly, even when tested against homologous antiserum.

CF testing of NDV strains: Table 2 shows results obtained with three hyperimmune antiserums tested with 10 NDV strains of known pathotype from the PIADC repository, the University of Connecticut, and the University of Wisconsin. All velogenic pathotypes, including Texas 219, Largo Florida, GB, Parrot 73-507, and Connecticut A4618, were poor complement-fixing antigens; CF titers ranged from 1/173 to 1/580. CF titers with mesogenic strains, including Connecticut 70726, New York Jones, and Ontario McVey, ranged from 1/240 to 1/855. The lentogenic strains, England F and LaSota, were strong complement-fixing antigens ranging from 1/1776 to 1/4793. From these data, we concluded that in future testing any one of the three hyperimmune antiserums could be used. Hitchner antiserum was selected because it could be produced with an avirulent antigen and had greater differences in extremes of fixation.

Results of typing nine viruses with pathotypes unknown to the testers by the CF test agreed with pathotypic classification by Dr. R. P. Hanson except with the California Kimber 1964 strain, which was typed as a velogenic pathotype by CF testing and was presented as a mesogenic pathotype by Dr. Hanson (Table 3). As with CF findings, the mean death time of this strain was 49 hours; These data fulfill another velogenic pathotype criterion. However, pathogenicity studies in chickens were not conducted. We can discern from these tests, as well as from earlier results, that the mesogenic strains show a wide range of complement-fixing ability.

Table 4 shows CF titers of 18 additional velogenic pathotypes submitted by Dr. Hanson, ranging from 1/267 to 1/803.

Table 5 shows CF titers of four lentogenic strains of NDV isolated from feral geese ranging from 1/1540 to 1/2370. These strains, as well as earlier tested lentogenic strains, were all strong complement-fixing antigens.

Table 6 shows the number of samples of 254 California outbreak isolates tested falling within graded 50% hemolysis endpoint ranges. Most (96.5%) of the isolates had CF titers ranging from 1/101 to 1/900. Only 14 isolates surpassed the highest dilution of 1/803 previously observed with a velogenic NDV strain. A high dilution of 1/1490 was noted, which may be due to a mixture of vaccine and field strains of NDV.

Discussion

A CF test has been extensively tested to differentiate velogenic pathotypes of NDV from lentogenic strains. All lentogenic strains and the mesogenic Roakin vaccine strain were strong complement-fixing antigens, whereas both viscerotropic and neurotropic velogenic pathotypes were poor complement-fixing antigens, as were most mesogenic strains. Use of poor complement-fixing NDV strains in poultry vaccines could mask differentiation of velogenic strains with this test.

Having applied the CF test to isolates from a recent field outbreak, it is felt that the test is an excellent, rapid, diagnostic tool for use in any laboratory. The CF test would be an excellent supplement to add to the diagnostic procedures described by McDaniel and Orsborn.3

Studies are being conducted to adapt the CF test to a micro-plate system to eliminate waste of space and reagents caused by the macro-tube method.
## TABLE 1
Cross Complement-fixation Test Results with California-1085, Hitchner, and Roakin Antigens and Hyperimmune Antiserums.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antiserum (1/80 dilution)</th>
<th>California-1085</th>
<th>Hitchner</th>
<th>Roakin</th>
</tr>
</thead>
<tbody>
<tr>
<td>California-1085</td>
<td>275*</td>
<td>410</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Hitchner</td>
<td>1390</td>
<td>1920</td>
<td>1620</td>
<td></td>
</tr>
<tr>
<td>Roakin</td>
<td>1320</td>
<td>2090</td>
<td>1570</td>
<td></td>
</tr>
</tbody>
</table>

*Reciprocal of antigen dilution producing 50% hemolysis.

## TABLE 2
Complement-fixation Test Results with California-1085, Hitchner, and Roakin Hyperimmune Antiserums and 10 Known Pathotypes of Newcastle Disease Virus.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antiserum (1/80 dilution)</th>
<th>California-1085</th>
<th>Hitchner</th>
<th>Roakin</th>
<th>Pathotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texas 219</td>
<td>173*</td>
<td>265</td>
<td>220</td>
<td></td>
<td>Velogenic</td>
</tr>
<tr>
<td>Largo Florida</td>
<td>195</td>
<td>315</td>
<td>245</td>
<td></td>
<td>Velogenic</td>
</tr>
<tr>
<td>Connecticut 70726</td>
<td>240</td>
<td>350</td>
<td>265</td>
<td></td>
<td>Mesogenic</td>
</tr>
<tr>
<td>New York Jones</td>
<td>270</td>
<td>380</td>
<td>355</td>
<td></td>
<td>Mesogenic</td>
</tr>
<tr>
<td>GB</td>
<td>275</td>
<td>465</td>
<td>355</td>
<td></td>
<td>Velogenic</td>
</tr>
<tr>
<td>Connecticut A4618</td>
<td>305</td>
<td>425</td>
<td>402</td>
<td></td>
<td>Velogenic</td>
</tr>
<tr>
<td>Parrot 73-507</td>
<td>355</td>
<td>580</td>
<td>490</td>
<td></td>
<td>Velogenic</td>
</tr>
<tr>
<td>Ontario McVey</td>
<td>580</td>
<td>855</td>
<td>806</td>
<td></td>
<td>Mesogenic</td>
</tr>
<tr>
<td>England F</td>
<td>1776</td>
<td>2748</td>
<td>2231</td>
<td></td>
<td>Lentogenic</td>
</tr>
<tr>
<td>LaSota</td>
<td>2941</td>
<td>4793</td>
<td>3572</td>
<td></td>
<td>Lentogenic</td>
</tr>
</tbody>
</table>

*Reciprocal of antigen dilution producing 50% hemolysis.
TABLE 3

Complement-fixation Test Results with Newcastle Disease Virus Strains of Unknown Pathotype Obtained from the University of Wisconsin Repository and Hitchner Hyperimmune Antiserum.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Wisconsin Classification</th>
<th>Complement-fixation Titer</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa 23 1946</td>
<td>Velogenic</td>
<td>500*</td>
<td>Velogenic</td>
</tr>
<tr>
<td>Montana 1946</td>
<td>Velogenic</td>
<td>520</td>
<td>Velogenic</td>
</tr>
<tr>
<td>California Kimber 1964</td>
<td>Mesogenic</td>
<td>520</td>
<td>Velogenic</td>
</tr>
<tr>
<td>Colombia 829 1971</td>
<td>Velogenic</td>
<td>560</td>
<td>Velogenic</td>
</tr>
<tr>
<td>Kansas Manhattan</td>
<td>Velogenic</td>
<td>700</td>
<td>Velogenic</td>
</tr>
<tr>
<td>Massachusetts MK 107</td>
<td>Mesogenic</td>
<td>780</td>
<td>Mesogenic</td>
</tr>
<tr>
<td>California RO 1944</td>
<td>Mesogenic</td>
<td>790</td>
<td>Mesogenic</td>
</tr>
<tr>
<td>Massachusetts 4F 1945</td>
<td>Mesogenic</td>
<td>830</td>
<td>Mesogenic</td>
</tr>
<tr>
<td>Michigan 46967 1946</td>
<td>Mesogenic</td>
<td>1540</td>
<td>Mesogenic</td>
</tr>
</tbody>
</table>

*Reciprocal of antigen dilution producing 50% hemolysis.
**TABLE 4**

Complement-fixation Test Results with a 1/80 Dilution of Hitchner Hyperimmune Antiserum and 18 Velogenic Pathotypes of Newcastle Disease Virus.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Complement-fixation titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>New York Parrot 70181-70</td>
<td>310 *</td>
</tr>
<tr>
<td>Trinidad 7530-64</td>
<td>315</td>
</tr>
<tr>
<td>Paraguay-You-70</td>
<td>652</td>
</tr>
<tr>
<td>Italy-Teramo C53</td>
<td>355</td>
</tr>
<tr>
<td>Italy-Teramo B50</td>
<td>345</td>
</tr>
<tr>
<td>California-1956-72</td>
<td>501</td>
</tr>
<tr>
<td>Italy-Milano-45</td>
<td>803</td>
</tr>
<tr>
<td>California-1083-71</td>
<td>478</td>
</tr>
<tr>
<td>Mexico-Sonora-1-67</td>
<td>310</td>
</tr>
<tr>
<td>England-Herts-1933-(Cormorant)</td>
<td>354</td>
</tr>
<tr>
<td>England-Herts-1933-(ARS)</td>
<td>359</td>
</tr>
<tr>
<td>England-Herts-1933-(Mod.E)</td>
<td>366</td>
</tr>
<tr>
<td>Japan-Sato-1930</td>
<td>295</td>
</tr>
<tr>
<td>New Mexico-LcF2-71</td>
<td>477</td>
</tr>
<tr>
<td>England-Lamb-DP6984-68</td>
<td>326</td>
</tr>
<tr>
<td>Illinois-Great Lake-53</td>
<td>342</td>
</tr>
<tr>
<td>Iraq-AG-68(3)</td>
<td>398</td>
</tr>
<tr>
<td>Iraq-Al Khadim-267-66</td>
<td>267</td>
</tr>
</tbody>
</table>

*Reciprocal of antigen dilution producing 50% hemolysis.
### TABLE 5
Complement-fixation Test Results with a 1/80 Dilution of Hitchner Hyperimmune Antiserum and 4 Lentogenic Pathotypes of Newcastle Disease Virus Isolated from Feral Geese.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Complement-fixation titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delaware 120</td>
<td>2180</td>
</tr>
<tr>
<td>Delaware 139</td>
<td>1830</td>
</tr>
<tr>
<td>Delaware 149</td>
<td>2370</td>
</tr>
<tr>
<td>Delaware 390</td>
<td>1540</td>
</tr>
</tbody>
</table>

*Reciprocal of antigen dilution producing 50% hemolysis.

### TABLE 6
Complement-fixation Test Results with 254 Newcastle Disease Virus Isolates from the 1971-72 California Outbreak.

<table>
<thead>
<tr>
<th>Range of antigen dilutions producing 50% hemolysis*</th>
<th>Number of isolates within range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-100</td>
<td>4</td>
</tr>
<tr>
<td>101-200</td>
<td>26</td>
</tr>
<tr>
<td>201-300</td>
<td>39</td>
</tr>
<tr>
<td>301-400</td>
<td>61</td>
</tr>
<tr>
<td>401-500</td>
<td>37</td>
</tr>
<tr>
<td>501-600</td>
<td>31</td>
</tr>
<tr>
<td>601-700</td>
<td>19</td>
</tr>
<tr>
<td>701-800</td>
<td>23</td>
</tr>
<tr>
<td>801-900</td>
<td>9</td>
</tr>
<tr>
<td>901-1000</td>
<td>4</td>
</tr>
<tr>
<td>Over 1000</td>
<td>1</td>
</tr>
</tbody>
</table>

*Reciprocal of antigen dilutions.
REFERENCES


ACKNOWLEDGEMENTS

The authors acknowledge Dr. C. H. Campbell for administrative support and Mr. W. Parrish for excellent technical assistance.
During the past year turkey producers in the United States have again felt the effects of influenza infections. Pomeroy and Bahl\(^9\) state that influenza has been recognized in turkeys in Minnesota every year since 1966, and has been identified in both meat birds and laying flocks. High mortalities may be encountered in flocks as young as 4-5 weeks of age and may reach 75% when complicated with colibacillosis. Mortality of 17% was noted in laying flocks with acute hemorrhagic pneumonia as the dominant lesion. The disease appears in the fall, and epizootiologic studies suggest that personnel and equipment used by artificial insemination crews may spread the disease. Confirmation of the spread of influenza through insemination was reported by Bahl and coworkers.\(^1\) Influenza infections were demonstrated in 12 of 13 organizational turkey flocks in Minnesota during the fall and winter of 1973. The infection caused a drop in egg production, an increase in cull eggs, and an estimated loss of 250,000 hatching eggs. The one unserviced flock in this group remained serologically negative.

Bahl and Pomeroy\(^2\) diagnosed influenza by the agar gel precipitin (AGP) test in 30 Minnesota turkey flocks from three geographically distinct areas. A/turkey/Minn. Pelican Rapids/73 and A/turkey/Minn. Kandiyohi caused mortalities of 76.9% and 66% respectively. Both flocks had complicating E. coli infections. Table 1 shows the losses in three flocks due to these influenza isolates. The first outbreak was recorded in September of the same year, although no infections were detected in surveys which began in January 1973. Lateral outbreaks continued until February 1974, and thereafter the disease seemed to "die-off". A total of six flocks were involved. Another wave of outbreaks in 1974; the first diagnosed in July, and three more flocks were found to be positive by September 15, 1974. All four flocks were managed by the same organization but the flocks were located in different areas of the state.

Pomeroy\(^8\) reported that influenza viruses, isolated from feral ducks in upper Minnesota in 1974, were of the Havg or Hvavg subtypes. His report completes acknowledgment of Influenza isolates from all of the waterfowl flyways in the United States.

McCapes and coworkers\(^7\) described acute influenza outbreaks in the winter and early spring of 1972-73 in several adult breeder flocks. The isolate was designated as A/turkey/California 6750/73. The disease had an acute onset, a sudden and precipitous drop in egg production, a concurrent rise in cull egg rate, severe depression and diarrhea. Egg production and quality were affected early in the illness and production was depressed as much as 34% for as long as 6 weeks. Morbidity approached 100%. Losses in some flocks were slight whereas in others mortality rates were 6-8%. Heavier losses appeared to be associated with flocks exposed to wind and

---

*Plum Island Animal Disease Center, Northeastern Region, Agricultural Research Service, United States Department of Agriculture, P. O. Box 848, Greenport, New York 11944.
rain in outdoor breeder pens. Clinical signs and necropsy lesions were similar to those observed with A/turkey/Ontario 7732/66 infections. The virus isolated from the flocks differed from strains previously isolated from turkeys in California and although the disease was experimentally reproducible, no mortality was noted. The mortalities experienced in the field may have been due to concurrent viral or bacterial infections. Epizootiologic studies of wild geese and swans in northern California failed to show presence of antibodies in the blood nor was virus isolated from tissues of these birds. A serologic survey of 112 turkey flocks in California was conducted in the fall of 1973 to determine the incidence and distribution of influenza infections. One flock located in the same general area as the initial outbreaks in January 1973, and closely associated with migratory birds in a nearby pond, was shown to be infected. Blood samples taken from the ducks and geese were negative in the AGP test. Bankowski reported that a serologic survey of turkey flocks in California is being conducted on a continuing basis to determine the presence and types of influenza virus in relation to his vaccine trials. He reported that prior to 1973, no significant mortalities were associated with influenza infections, but, in January 1973, a breeder flock of 2500 turkeys suffered a respiratory influenza infection with a mortality of 5% over a two-week period, and a drop in egg production from 60% to less than 20% within a four-day period. Egg production improved but did not return to predisease levels. Bankowski suggested the use of an inactivated vaccine prepared with the same antigenic strain of the virus to determine whether such a preparation would alleviate the severity of the disease and particularly protect against a drop in egg production in breeder flocks.

Preliminary studies were undertaken by Bankowski and McCapes with a formalin-inactivated vaccine utilizing A/turkey/California 6750/73. The experimental vaccines contained either the whole virus or desoxycholate split virus, with or without adjuvant. Precipitin antibodies were demonstrable by the 14th day postvaccination, but hemagglutination-inhibition (HI) antibodies were not detectable until after the 14th day. The best serologic response was observed in turkeys receiving the whole virus in Freund's complete adjuvant. When the birds were 31 weeks old, or seven weeks after a second immunization, 65% were positive to the HI test and 80% were positive to the AGP test. Groups of 20 turkeys were trap-nested to establish egg production patterns, and the immunity of the vaccinated and nonvaccinated birds was challenged intratracheally. In spite of the severity of the challenge, a marked difference was noted between vaccinated and control groups of mature breeder hens. A depression and diarrhea was observed in nonvaccinated hens beginning 9 days after challenge. Signs were less severe in the vaccinated hens. A reduction of potential eggs per hen of 14.8% in vaccinated and 40.6% in nonvaccinated birds was noted. Necropsy 7 weeks after challenge revealed marked ovarian inflammation with large accumulations of caseous exudate, regressed ova, severe inflammation of the mesentery, and moderate to marked enteritis in nonvaccinated hens. Vaccinated birds did not show these severe lesions. Although the inactivated vaccine showed promise, the Freund's complete adjuvant preparation produced a moderate to severe tissue reaction at the
site of inoculation, which would undoubtedly result in the downgrading of the carcass at time of processing. Further studies are in progress to develop a more effective vaccine without the adverse tissue reaction.

Easterday\textsuperscript{5-6} reported that since 1956, more than 100 influenza viruses have been isolated from more than 15 avian species throughout the world, and one of the most remarkable aspects of these viruses is that there is no evidence of infections among chickens in the U.S. Other oddities include an influenza infection in Muscovy ducks in Pennsylvania resulting in 10\% mortality of 10 week-old ducks in which the principal sign was sneezing. There were no signs of disease among 500 turkeys and 550 domestic geese on the same farm. Easterday maintains that relatively little effort has been or is being expended toward studying avian influenza infections in the U.S. Much of the apathy is due to lack of dramatic and devastating losses among turkeys and the fact that the infection has not been identified in chickens. It must be kept in mind, however, that heavy losses in chickens have been observed in other parts of the world.

It is possible for an avirulent influenza virus to recombine with another type A influenza to produce a highly virulent strain causing fowl plague-like infections. In the past few years, Webster, Campbell, and Granoff\textsuperscript{11,12,13} have conducted extensive studies on recombination among type A influenza viruses at the Plum Island Animal Disease Center. Recombinant or “new” influenza viruses have been isolated from the lungs of turkeys after inoculation with a mixture of antigenically distinct influenza viruses. Turkeys infected with A/turkey/Massachusetts 3740/65 (Hav\textsubscript{6}N\textsubscript{2}) and A/FPV/Dutch/27 (Hav\textsubscript{1}Neq\textsubscript{1}) resulted in reisolation of two antigenic hybrids containing the turkey hemagglutinin (Hav\textsubscript{6}) and fowl plague neuraminidase (Neq\textsubscript{1}) as well as the reciprocal recombinant. Recombinants containing fowl plague hemagglutinin (Hav\textsubscript{1}) and either swine (N\textsubscript{1}) or turkey influenza (N\textsubscript{2}) neuraminidase were found to be virulent for chicken embryos and chickens. Other antigenic hybrids produced mild infections in these hosts. When lung suspensions from turkeys infected with fowl plague and turkey influenza viruses were inoculated intratracheally into chickens immunized against turkey influenza virus hemagglutinin and fowl plague neuraminidase, recombinant influenzas with fowl plague hemagglutinin-turkey neuraminidase were selected. The production of antigenic hybrids of influenza viruses in vivo with immunity being the selective pressure adds support to the idea that new influenza viruses of man, lower mammals, and birds might arise by the same mechanism.

Recombinants isolated from turkeys infected with fowl plague virus (Hav\textsubscript{1}Neq\textsubscript{1}) and A/turkey/Wisconsin/66 (Hav\textsubscript{8}Nav\textsubscript{4}) or from turkeys infected with A/turkey/Ontario 7732/66 (Hav\textsubscript{8}Nav\textsubscript{4}) and A/turkey/Wisconsin/66 always possessed the hemagglutinin subunit of the virulent parent (Hav\textsubscript{1} or Hav\textsubscript{8}). It was shown that one influenza A virus (T/Wis) can replicate in the upper respiratory tract of turkeys while an antigenically distinct virus (T/Ont) can replicate in the lower respiratory tract. Such dual infections create an opportunity for a mixed infection of cells and hence the emergence of a recombinant. The dual infection of turkeys also caused a sparing effect in which the highly virulent virus (T/Ont) failed to kill the infected birds, or that the time required to kill the infected birds with
fowl plague virus was increased. The latter situation may create conditions that would favor the occurrence of recombination. Failure to demonstrate natural transmission of recombinant viruses to susceptible contact turkeys suggests that all hybrids do not necessarily have a pandemic potential.

Snoeyenbos\textsuperscript{10} urged that the U.S.D.A. develope a policy for an eradication program which could be automatically activated in the event that fowl plague-like influenza occurs in the U.S. It was further recommended that eradication policies and programs be subjected to periodic review by members of the animal industries involved and the related scientific and technical communities, similar to the programs adopted by Canada. Initiation of eradication efforts should not be delayed until a national emergency is declared by the Secretary of Agriculture.

<table>
<thead>
<tr>
<th>Influenza A turkey isolate</th>
<th>Age of turkeys in weeks</th>
<th>Number poults brooded</th>
<th>Number turkeys marketed</th>
<th>Total mortality</th>
<th>Percent mortality</th>
<th>Total turkeys condemned</th>
<th>Percent turkeys condemned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelican Rapids/73</td>
<td>6</td>
<td>19,500</td>
<td>4,500</td>
<td>15,000</td>
<td>76.9</td>
<td>325</td>
<td>7.2</td>
</tr>
<tr>
<td>Kandiyohi/73</td>
<td>6 &amp; 16</td>
<td>50,000</td>
<td>34,000</td>
<td>15,200</td>
<td>30.4</td>
<td>2000</td>
<td>5.9</td>
</tr>
<tr>
<td>Kandiyohi/73</td>
<td>6</td>
<td>10,000</td>
<td>3,400</td>
<td>6,600</td>
<td>66.0</td>
<td>460</td>
<td>13.5</td>
</tr>
</tbody>
</table>

\textsuperscript{*}From information obtained from Bahl and Pomeroy\textsuperscript{2}.
REFERENCES

Chairman: Dr. R. A. Bamkowski
Co-Chairman: Dr. H. E. Goldstein

Dr. W. E. Merritt, Washington, D. C.; Dr. Everett S. Bryant, Starrs, Commn; Mr. Francis G. Buzzell, Augusta, Maine; Dr. A. H. Dardiri, Greenport, L.I., New York; Dr. Floyd Golan, College Station, Texas; Dr. L. C. Grumbles, College Station, Texas; Dr. J. E. Hanley, Dade City, Fl.; Mr. R. L. Hogue, Lafayette, Ind.; Dr. A. Janawicz, Montpelier, Vt.; Dr. Thomas L. Landers, Hot Springs, Ark.; Dr. Harold E. Nader, Bath, N.Y.; Dr. W. C. Patterson, Jr., Athens, Ga.; Dr. Irvin Petersen, Beltsville, Md.; Dr. Claude J. Pfow, Hyattsville, Md.; Dr. B. S. Pomeroy, St. Paul, Minn.; Dr. James B. Roberts, Muldrow, Okla; Dr. A. S. Rosenwald, Davis, Cal; Dr. Terrell B. Ryan, Cary, N.C.; Dr. Wm. C. Schofield, St. Louis, Mo.; Dr. J. B. Thomas, Columbia, S.C.; Dr. H. Wesley Towers, Dover, Del. Mr. John A. Smiley, Augusta, Maine; Dr. A. E. Decoteau, Waltham, Mass.

Asiatic Newcastle Disease

Dr. Claude Pfow, APHIS Poultry Diseases staff gave this committee an up to the date report on the situation of Asiatic Newcastle Disease in the United States. The Committee was very pleased to be informed that the Newcastle Disease Eradication Task Force Headquarters in Riverside, California was closed on July 3, 1974 and the extraordinary emergency ended after extensive surveillance proved that Viscerotropic Velogenic Newcastle Disease (VVND) no longer existed in southern California poultry flocks. Over 11.5 million chickens were destroyed during a 19 month period and nearly $56 million dollars paid for the cost of eradication before the disease was eliminated. The committee commends the USDA and the California Department of Agriculture on their perseverance and effectiveness in their operations to have eradicated a most complex disease entity from poultry flocks in that state. The committee also commends the Poultry Industry of California for their efforts and cooperation which prevented spread of this exotic disease to other parts of the country.

Because of the economic importance of the Poultry Industry, the determination to eradicate exotic Newcastle disease from the Continental United States and to counter the risk of re-introducing VVND, the USDA set up a National Surveillance Program. The program which was authorized on October 1, 1973 is directed by the APHIS Poultry Diseases staff, headquartered at Hyattsville, Maryland. It utilizes the expertise of Poultry disease diagnosticians and epidemiologists to investigate suspicious cases of poultry disease.
Although most of the field investigations proved not to be exotic Newcastle, three outbreaks of viscerotropic, velogenic Newcastle disease were detected.

1. El Paso, Texas — This outbreak affected 86 backyard poultry flocks and one commercial farm which appeared to be two epidemics.

The cases in Epidemic No. 1 which started January 8 and ended February 28 were located in four separate areas: 8 cases were in a cluster near Fresno Drive; 3 cases occurred on North Loop about three miles east of the Fresno area; 1 case occurred on Broadway about one mile west of the Fresno area; and 2 cases occurred near Clint, which is about twelve miles southeast of the Fresno area.

The information obtained suggested that four separate outbreaks were the result of four separate introductions. There was little evidence that these four outbreaks were connected or that the disease spread from one outbreak area to another. It is probable and possible that some common factor or combination of factors produced the proper association host, agent, and environment to cause the disease. Area spread did occur in the Fresno outbreak. The close proximity of flocks, movements of people, ranging to poultry, and scavenger animals can all be listed as methods of spread in that area.

Epidemic No. 2 started March 10 and extended to April 19, began with the disclosure of two positive flocks — one at San Elizario on March 23 and one at Socorro on March 24; both of these being southeast and outside the existing quarantined area. Subsequent intensified field activity in the new area resulted in the disclosure of 24 additional positive flocks. An additional area extending southeast to the Hudspeth County Line was placed under quarantine. An isolated case was located in the northeast section of the City of El Paso. An area around this case was also placed under quarantine.

2. Hidalgo, Texas — The disease was confined to one backyard flock of mixed poultry. The disease was eradicated before it could spread.

3. Bulverde, Texas — This outbreak was also confined to a single flock of mixed poultry on one farm.

The mean number of days from infected flock investigation to depopulation was 2.13. Most of the positive flocks were investigated and depopulated the same day. The exceptions were the one large commercial flock and instances where a laboratory diagnosis was needed.

A total of 190,000 birds (87 flocks) were destroyed because of infection or exposure to the disease. Flock owners were paid $400,000 to compensate them for their losses.

Quarantines of the El Paso area were lifted June 3, 1974; the last individual quarantined premises in Texas was released on July 8.

As of September 16, there are no quarantines for exotic Newcastle disease in effect on the U.S. mainland. The Commonwealth of Puerto Rico remains under quarantine.

**VVND Found in Birds Intended for Importation into the United States**

The committee was made aware of the status and program of the USDA
Quarantine Stations.
Foreign birds can be imported if they are confined in quarantine facilities where their health status can be determined before entry. During fiscal year 1974, 14 quarantine stations were put into operations by bird importers. Of 27 lots of birds imported, 5 were found to harbor exotic Newcastle disease virus. These infected and exposed birds were destroyed or removed from the United States. The healthy lots of birds were allowed entry and shipped to pet shops and zoos.

Birds from the following countries have been included in lots of birds where VVND has been found during FY 1974 (birds from more than one country may be in a single lot): Africa (Ghana, Liberia, Zaviie, Nigeria, Kenya), Mexico, Indonesia, Japan, Spain, Paraguay, Martinique, Belgium, Columbia, Antartica, Tiawan, and India.

The following kinds of birds have been in lots where VVND has been found (more than one kind of bird may appear in a single lot): parrots, finches, parakeets, cockatoo, fighting chickens, canaries, mynah, conures, lorikeytes, penquin, starlings, crowned cranes, and sunbirds.

This committee commends the USDA, for the effort in this area, in a most realistic attempt to prevent introduction of VVND infected or exposed birds. However, the committee expressed concern that there is less than daily surveillance of the present facilities. This committee recommends that no additional facilities be approved until adequate funding is available to provide the needed personnel to supervise those presently approved every day during the quarantine period.

Dr. Grumbles reported to the committee on the workshop on VVND which was held at the Western States Poultry Health Conference in Davis, California on March 17-18, 1974. The epidemiology section of this workshop recommended that a virus characterization center be established, both, at National and International levels. This committee recognizes the need for such a virus characterization center and strongly urges USDA officials to consider establishing such a center in the U.S.

Status Report on Duck Virus Enteritis
The U.S. Department of the Interior has the responsibility for the management of the migratory birds and protection of endangered species. During fiscal year 1974, the U.S. Department of the Interior developed a “duck plague contingency plan” and has designated individuals to serve as duck virus enteritis diagnosticians for various regions of the United States. Persons were assigned in the West Region at the Bear River Migratory Bird Refuge, Birgham City, Utha; Central Region - at the Northern Prairie Wildlife Research Center, Jamestown, North Dakota; and in the East at the Patuxent Wildlife Research Center, Laurel, Maryland. These individuals have the responsibility of investigating suspicious die-offs of waterfowl occurring on Service lands. The Eastern Fish Disease Laboratory has the facilities for virus isolation and identification.

Status Report on Mycoplasmosis
The committee reaffirms its position toward the need for the eradication of mycoplasma from our nations poultry flocks.
During fiscal year 1974, Veterinary Services made 340 shipments of reference material to 71 laboratories in 24 States and Canada. Also during FY 1974, Veterinary Services conducted one school for 7 State employees, and 4 industry employees for training in techniques for conducting mycoplasma tests.

Four commercial laboratories have licenses to commercially produce and/or distribute plate and tube agglutination *M. gallisepticum* antigen in the United States. No laboratory is presently producing tube antigen. *M. gallisepticum* plate antigen is available from only two sources. At the present time, one laboratory is producing *M. synoviae* plate antigen.

This committee recommends that USDA, APHIS continue to supply the necessary reference reagents and particularly the HI antigen which is not available commercially. However, it is urged that requests for these materials be made in sufficient time in advance to ensure adequate production.

**Report of Ornithosis Outbreak - 1974**

Between May 6 and June 25, 1974, a total of 154 human cases of psittacosis (ornithosis) were reported among 560 employees of 5 turkey processing plants in Missouri (1), Nebraska (1), and Texas (3) (attack rate 28%). The highest incidence of disease was noted in the employees of the kill, pick, and eviscerating areas of the processing plants.

A turkey flock that originated from central Texas was implicated by laboratory confirmation of ornithosis as the source of infection at one of the Texas processing plants. Epidemiologic evidence from poultry inspection records from the other Texas plant as well as the Nebraska and Missouri plants implicated birds from the same area in Texas as the source. Psittacosis has been confirmed in other flocks in this general area.

Ornithosis had not been reported in turkeys by the Texas laboratories since 1966.

Epidemiological investigations did not define a definite beginning point or a route of spread. Some flocks thought to be the source of human exposure at slaughter did not have a history compatible with Ornithosis. Those flocks known to have had Ornithosis were treated with Chlortetracycline prior to slaughter.

On July 12, a field office was established in College Station, Texas, with a crew of 10 veterinary diagnosticians and a support group of laboratory and administrative personnel (20) were assembled and given one and one-half days of orientation. Personnel from the Texas Animal Health Commission, Texas A&M University, Texas State Health Department, Center for Disease Control, Meat and Poultry Inspection, and Veterinary Services were represented. A conference was held with the Texas turkey growers, and plans were made to monitor all turkey flocks going to slaughter in the near future.

The course of action was clinical inspection of all flocks and acquisition of ten random blood samples for a serological survey one week prior to slaughter. Any flock showing symptoms or a serologic titer of 1:32 or higher on the direct complement fixation test was required to go on treatment for 21 days with chlortetracycline, 200 grams per ton of feed, with a withdrawal period of 48 hours and reinspeccion and certification of the flock made before slaughter.
By August 1, 1974, 152 flocks with 1,124,840 birds had been inspected and tested. Three flocks were classified positive and retained for treatment. In addition, the seven flocks previously diagnosed as positive by the Texas A&M University were required to undergo treatment. The project started phasing out August 2, 1974; all out-of-state personnel were released and the Texas Veterinary Services personnel remained to finish the surveillance. In view of the periodic occurrence of Ornithosis in Poultry, its human involvement, and our limited knowledge of the infection, this committee recommends expansion in the areas of research, diagnosis, and epidemiology of this disease.

**Status Report on Programs of Pullorum/ Typhoid and Other Diseases of Poultry**

Several approaches to the control and eradication of Pullorum/ Typhoid and both endemic and exotic diseases of poultry were proposed as follows: A report on chicken and turkey 1974 National Plans Conference, by Dr. J. Petersen; a United States Poultry Health Proposal by Dr. R. McCapes; the AAAP Avian Disease Program Committee Report by Dr. L. E. Grumbles; and the proposal and desires of the North Eastern States by Mr. John A. Smiley. In view of the divergence in opinions expressed by the various groups, a subcommittee was appointed to study the various proposals and recommend the most effective program which would be acceptable to all interested parties. A report of the subcommittee recommendations will be expected at the 1974 meeting of the Committee on Transmissable Diseases of Poultry of the USAHA.

The members selected for this committee are:

Dr. Everett Bryant  
Dr. H. E. Goldstein  
Mr. R. L. Hogue  
Dr. Irvin Petersen  
Dr. Ben Pomeroy  
Dr. Terrell Ryan  
Dr. R. McCapes - Chairman

**Status Report on Avian Influenza**

In view of the potential danger of avian influenza virus as an emerging pathogen for poultry, a report of the present status of this infection in poultry flocks in the US and isolates from exotic birds in the Quarantine Stations was reported by Dr. W. Butterfield.

Dr. Easterday also presented the latest information on the nature and biologic characteristics of this ever changing group of viruses found in not only the avian species but in horses, swine and man.

The committee recommends that the report on Avian Influenza given by Dr. Butterfield be edited and included in the proceedings of the USAHA and that a subcommittee be appointed to keep abreast of developments during the next year.

Members selected for the subcommittee were:

Dr. W. K. Butterfield - Chairman  
Dr. R. A. Bankowski  
Dr. B. C. Easterday  
Dr. B. S. Pomeroy  
Dr. E. A. Carbray
SALMONELLOSIS - AN ENVIRONMENTAL PROBLEM AFFECTING ANIMALS AND MAN

Erskine V. Morse, D.V.M., Ph.D. and Margo A. Duncan*
Purdue University, West Lafayette, Indiana

Salmonellosis ranks as the most common and economically destructive zoonosis. The host range of the salmonellae encompasses the entire vertebrate phylogenetic scale—fish, amphibians, reptiles, birds, and mammals including man. There are over 1300 serotypes or species of these enteric pathogens. Typhoid (Salmonella typhi), paratyphoid A also C and S. sendai infections are rather strictly adapted to human beings. Other more or less restricted serotypes are S. typhisuis and S. cholerae-suis of swine, S. pullorum and S. gallinarum of poultry, S. dublin of cattle, S. abortusequiv of horses and S. abortusovis of sheep. With the exceptions of S. abortusovis and S. typhisuis the other serotypes occurs in several animals as well as man. The host adapted limitation is fast becoming a misnomer and misconception. Estimates indicated there are between 100,000 and 2 million human cases of salmonellosis per year in the United States. Annually, it is probable that 1 percent of our livestock and domesticated animals are infected. The vast majority of both human and lower animal infections are not detected, recognized nor reported. It should be realized that salmonellosis in man and livestock should be made a reportable disease. There is an apparent and critical need for more accurate and concise surveillance. It is indeed fortunate that the mortality for human salmonellosis is well under 1%—actually about 500 deaths per annum.

The inconvenience and short-term discomfort of this group of enteric infections is well-known to many North Americans. The economic manpower loss is hard to assess; however, estimates may range from $3.6 million to $72 million annually. (see table 1)

Livestock losses may be quite dramatic, e.g. high morbidity, sudden mortalities and abortion. Mortality rates are approximately 5% in the usual epizootic; however, 70% of the flock or herd may succumb in severe outbreaks. Decreased production, unthriftiness, poor feed efficiency, carcass condemnation at slaughter, therapy and related health costs may well represent 25% of the economic loss for infected livestock. The total losses may range from $30 to $120 million per year for American animal agriculture. (See table 2)

In order to reduce the losses accruing due to work cessation and absenteeism in man as well as lowered production in livestock, the public must become more concerned with salmonellosis. Fortunately, public awareness and educational campaigns are being conducted. Morton Mintz of the Washington (D.C.) Post recently had an informative news release for consumers and shoppers. He indicated that the US Congress's General Accounting

*From the Department of Microbiology, Pathology and Public Health, School of Veterinary Medicine and the Environmental Health Institute. Published as journal article 5689. Purdue Agricultural Experiment Station. The work reported in this publication was supported in part pursuant to FDS (HEW) contract 72/73, 347 and by a grant from Eli Lilly & Company, Animal Sciences Department, Greenfield Laboratories.
TABLE 1
ESTIMATED ANNUAL DOLLAR LOSS DUE TO HUMAN SALMONELLOSIS
(500 DEATHS NOT ASSESSED IN LOSSES)

<table>
<thead>
<tr>
<th>HUMAN SALMONELLOSIS</th>
<th>ASSUMING 100,000 CASES</th>
<th>ASSUMING 2 MILLION CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL INDIVIDUALS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHYSICIAN'S FEE, 1 VISIT, 50% CASES a $10.00</td>
<td>$ 500,000</td>
<td>$10,000,000</td>
</tr>
<tr>
<td>MEDICINE FOR 50% a $5.00</td>
<td>$ 250,000</td>
<td>$ 5,000,000</td>
</tr>
<tr>
<td>HOSPITALIZATION, 4 DAYS, 5% CASES a $280.00</td>
<td>$1,400,000</td>
<td>$28,000,000</td>
</tr>
<tr>
<td>MEN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAGES LOST: 50% MALES; 50% WORKING AGE, 50% ACUTELY ILL - $3.39/HR, = $81.36/WK, 3 DAY ILLNESS</td>
<td>$1,017,000</td>
<td>$20,340,000</td>
</tr>
<tr>
<td>WOMEN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAME FACTORS AS MALE, BUT 42.6% WORKING</td>
<td>$ 433,242</td>
<td>$ 8,664,840</td>
</tr>
<tr>
<td>TOTAL LOSSES</td>
<td>$3,600,242</td>
<td>$72,004,840</td>
</tr>
</tbody>
</table>

TABLE 2
LOSSES IN LIVESTOCK DUE TO SALMONELLOSIS

-1972 & 1973 ANNUAL AVERAGE CASH RECEIVED & HOME CONSUMPTION VALUE FOR LIVESTOCK AND LIVESTOCK PRODUCTS = $41,628,015,000.

-ASSUME:

<table>
<thead>
<tr>
<th></th>
<th>0.25% LIVESTOCK INFECTED IN U.S.</th>
<th>1.0% LIVESTOCK INFECTED IN U.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSSIBLE TOTAL INVOLVED</td>
<td>$100 MILLION</td>
<td>$400 MILLION</td>
</tr>
<tr>
<td>DEATH/ABORTIONS 5%</td>
<td>$ 5 MILLION</td>
<td>$ 20 MILLION</td>
</tr>
<tr>
<td>DECREASED PRODUCTION 25%</td>
<td>$ 25 MILLION</td>
<td>$100 MILLION</td>
</tr>
<tr>
<td>ESTIMATED TOTAL LOSS</td>
<td>$ 30 MILLION</td>
<td>$120 MILLION</td>
</tr>
</tbody>
</table>
Office (GAO) commissioned the Gallup Organization to conduct a survey on salmonellosis (April 1973, 816 women at least 18 years old). The findings are germane and of interest: 74% incorrectly identified the term Salmonella; 64% had no concept as to means of minimizing the spread of the disease; and 39% were certain raw meat and poultry were inspected by Federal or State agencies for salmonellae. Mr. Mintz’ news item continues and indicates that the Federal Food and Drug Administration (FDA) and the US Department of Agriculture’s Animal and Plant Health Inspection Service (APHIS) have authority for regulation of meat and poultry products entering the retail market. Internal Task Forces of FDA and USDA recommended that consumers should be alerted and be made aware of salmonellosis health hazards “associated with handling raw meat and poultry.” APHIS has a cartoon, public service, T.V. commercial which should aid in consumer education. The film strip is “eyecatching”, has humor and makes it’s point! It is hopes that is will be seen on a number of T.V. stations to the benefit of the American public.

The incidence of salmonellosis in human and animal hosts has not dropped during the last decade. There are approximately 25,000 isolations made from man and about 9,000 from non-human sources annually. Actually, these surveillance reports confirm that infections are increasing yearly or are being recognized more frequently. Accurate monitoring of the prevalence would appear to be definitely warranted for at least another decade.

Salmonella sp. are ubiquitous and opportunistic microorganisms with the widest host range of any zoonotic pathogen. Their propensities for tolerating unfavorable environmental conditions approaches that of saprophytic microorganisms. The growth range is 44°F (6.7°C) to 114°F (41°C), while the optimal range is 95°F to 99°F (35°C to 37°C). The pH range for growth is reported as 4.1 to 9.0, with an optimum of pH 6.5-7.5. The pathogens will not reproduce in brines containing 7% salt. Freezing is not markedly deleterious for the Salmonella; however a very moderate “die-off” occurs during freezing and thawing. The organisms survive in dust and thus may continue to serve as sources of infection. It will be recalled that a number of typhoid outbreaks have been traced to contaminated “lake ice” stored until summer in the old-time ice house. Evidence suggests that salmonellae may multiply in bottom sludge of rivers, lakes and streams. Sludge contains salmonellae more frequently than does the overlay of river water. Of considerable importance to the livestockman, animal scientist, agricultural engineer and veterinarian concerns salmonellae survival in an animal manure oxidation ditch. Such means of manure disposal are becoming increasingly common in confinement rearing-production units for swine. The Minnesota group found salmonellae would persist for 47 days at 2°C in the model system. Aerosols generated contained Salmonella (At 2° and 21°C) which were capable of infecting turkey poults caged above the model. Research must be continued and fostered which relates to salmonellosis and evolving, innovative confinement rearing and production facilities.

The persistence of salmonellosis in a given environment and within an infected animal population is dependent upon a number of interactions. The
infecting serotype as well as the individual strain, e.g., *typhimurium*, *dublin*, *orienburg*, etc., have definite bearing on the course, severity and duration of an epizootic.12,13,14,15 *S. typhimurium*, once considered a host adapted murine pathogen, represents the most prevalent and important cause of salmonellosis in man and animals. The significant role of this enteric can be assessed from Table 3 and 4.

Likewise, the total number of salmonellae in the infecting dose is of importance.12,15 Russian workers reported that with aerosols (respiratory and conjunctival exposure) only 27% of the number of organisms were required for ovine and bovine infections as contrasted to the number via the oral route.16 In most healthy young livestock 10⁸ salmonellae may be required to produce clinical infection.17 It should be recognized that majority of severe field cases and epizootics in livestock do not, on the contrary, involve the most healthy subjects. The clinically ill, the very young, the debilitated, the chronically diseased or stressed individuals are the victims of most outbreaks. Sanitation as well as housing with related unfavorable environmental conditions (overheating, high humidity, chilling, water or feed deprivation, fatigue and psychological stress) all may play interrelated and interdigitated roles upon the course of an epizootic.17 Specific antimicrobial therapy is of questionable value in acute or even chronic salmonellosis if the rationale is destruction of the salmonellae per se. Secondary infections, however, may be aborted and minimized by treatment. Antimicrobials may be beneficial as preventive measures to limit the spread of the infection and to protect highly susceptible stock.

Salmonellosis resistance can be genetically controlled, i.e. resistant and susceptible animal populations can be selected and developed. It is well recognized that the Leghorn chicken is three to five times more resistant to *S. gallinarum* and *S. pullorum* infections than are the heavier Rock breeds.18 Gowen cites many critical experiments in which selective breeding has increased or decreased resistance to salmonellae in mammals and fowl.19 Insects and other invertebrates should not be overlooked as sources and vectors of salmonellae.20,21,22 The fact that rodents and free flying birds serve as reservoir hosts for salmonellae is well established. The bacteria remain viable in rodent feces for over a year.23 The significance of water fowl and sea-birds as carriers of these enteric pathogens is documented. Gulls, feeding on *S. typhi* contaminated garbage at sea, fouled the water supply in an Alaskan community with their infectious feces.24 Droppings of sea gulls, off the Oregon coast, yielded (2.5% positive) *S. typhimurium*, *S. reading* and *S. enteritidis*.25 Apparently, sea birds represent a hazard to facilities processing fresh and frozen fish for human consumption.25 Duck farm effluent, draining into a commercial oyster bed, infected the oysters. Several serotypes were isolated from the effluent as well as the bivalves.24 Bats have been observed to be carriers and shedders of salmonellae.26,27

Dogs appear to rival swine as *Salmonella* carriers. Simultaneous and multiple infections involving two or more serotypes are not rare.28 Infection rates of 27.6 percent of 2252 dogs with concurrent isolation of 53 different serotypes have been reported.28 Factors influencing the persistence of salmonellosis in animal populations are summarized in Table 5.
### TABLE 3

**EPIZOOTIOLOGICAL/EPIDEMIOLOGICAL STATUS OF *Salmonella typhimurium* IN THE U.S.**

<table>
<thead>
<tr>
<th>HOST</th>
<th>1967-1973 TOTALS - USDA, NADL, Ames, Iowa</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NUMBER</td>
<td>PERCENT</td>
</tr>
<tr>
<td>CATTLE</td>
<td>2436</td>
<td>62.2</td>
</tr>
<tr>
<td>CHICKENS</td>
<td>1832</td>
<td>17.4</td>
</tr>
<tr>
<td>HORSES</td>
<td>352</td>
<td>63.4</td>
</tr>
<tr>
<td>SHEEP</td>
<td>74</td>
<td>29.9</td>
</tr>
<tr>
<td>SWINE</td>
<td>732</td>
<td>17.1</td>
</tr>
<tr>
<td>TURKEYS</td>
<td>886</td>
<td>6.9</td>
</tr>
</tbody>
</table>

**MAN (CDC REPORTS)**

<table>
<thead>
<tr>
<th>YEAR</th>
<th>NUMBER</th>
<th>PERCENT</th>
<th>RANK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>5917</td>
<td>24.4</td>
<td>1</td>
</tr>
<tr>
<td>1971</td>
<td>6878</td>
<td>26.8</td>
<td>1</td>
</tr>
<tr>
<td>1972</td>
<td>6738</td>
<td>25.8</td>
<td>1</td>
</tr>
</tbody>
</table>

### TABLE 4

**MOST COMMONLY REPORTED SEROTYPES IN ANIMALS/FEEDS AND MAN 1972/73**

<table>
<thead>
<tr>
<th>RANK</th>
<th>SEROTYPE</th>
<th>ANIMALS, FEED OR ENVIRONMENT (5123 SAMPLES)*</th>
<th>MAN (26.110 SAMPLES)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NUMBER</td>
<td>PERCENT</td>
<td>RANK</td>
</tr>
<tr>
<td>1</td>
<td>TYPHIMURIUM</td>
<td>1571</td>
<td>30.7</td>
</tr>
<tr>
<td>2</td>
<td>CHOLERAE-SUIS</td>
<td>291</td>
<td>5.7</td>
</tr>
<tr>
<td>3</td>
<td>SAINT-PAUL</td>
<td>288</td>
<td>5.6</td>
</tr>
<tr>
<td>4</td>
<td>HEIDLEBERG</td>
<td>277</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>NEWPORT</td>
<td>257</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>SAN-DIEGO</td>
<td>217</td>
<td>4.2</td>
</tr>
<tr>
<td>7</td>
<td>ANATUM</td>
<td>198</td>
<td>3.9</td>
</tr>
<tr>
<td>8</td>
<td>INFANTIS</td>
<td>134</td>
<td>2.6</td>
</tr>
<tr>
<td>9</td>
<td>SENFTENBERG</td>
<td>127</td>
<td>2.5</td>
</tr>
<tr>
<td>10</td>
<td>MONTEVIDEO</td>
<td>121</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*USDA/NADL DATA

**C.D.C. SALMONELLA SURVEILLANCE ANNUAL SUMMARY 1972
Persistence of Salmonellosis In An Animal Population Depends Upon:

- *Individual strain within serotype group.*
- Serotype involved.
- Number of salmonella in infecting dose.
- Animal serving as host (Species & genetic Pattern)
- Age of Animal Host.
- Physical condition of Host (other disease conditions)
- Type of Housing for Livestock Host (Sanitation)
- Stress Factors Affecting Host
- Therapy Initiated - individually or collectively.
- Non-vertebrate vectors, e.g. flies, cockroaches, etc.
- Infected, carrier wildlife hosts, e.g. birds, rabbits, rodents, deer, etc.

Concurrence of other chronic or acute disease conditions increases the susceptibility of the host as well as influence the severity of the infection. *S. typhimurium* infections, when superimposed upon chronic lead poisoning in mice produced greater mortality than was observed for the controls.\(^{29}\)

When aflatoxicosis was established in rats and *S. typhimurium* challenge administered, the groups receiving aflatoxin experienced greater mortality than did the non-toxic rats.\(^{30}\) While these critical experiments were conducted on laboratory animals, a comparable situation no doubt exists for livestock being fed grains contaminated with mycotoxins and then exposed to *Salmonella*.

Water has classically served as a most significant medium for the transmission of typhoid and other salmonelloses. Contaminated river water used to cool cans of beef have been responsible for at least two typhoid epidemics.\(^{24}\) Canned ham served as the medium contaminated by serotype *Wein*. The product was also cooled with contaminated water which entered the cans through microleaks.\(^{24}\)

Concern over turtle salmonellosis, possibly responsible for 280,000 cases in children annually,\(^{31}\) relate to the situation of other fresh water fauna as carriers and vectors of *Salmonella*.\(^{13,32,33}\) Recently, the authors surveyed the incidence of *Salmonella* sp. on body slime and in the gastrointestinal tract of Wabash River fish. The results are summarized in Table 6. These data further indicated that fishes may play a role in the transmission of salmonellosis.

Antimicrobial sensitivity patterns for coliforms, salmonellae and related enterics have become increasingly important with the discovery of resistance transfer factor (R factor). During 1973 and 1974 the authors compared the sensitivities of *Salmonella* sp. isolated from clinical cases with the patterns observed for 144 salmonellae isolates from an aquatic environment, i.e. fishes, mussels, river bottom sludge and river water. (see Table 7)

It will be noted that the "aquatic" salmonellae, as a group, were significantly more sensitive to *Aureomycin*, *Kanamycin*, *Streptomycin*, *Sulfonamides*, and *Ampicillin* than were the strains isolated from clinical infections. A comparison of data for coliforms isolated in two large commercial swine herds, with those for *E. coli* obtained from clinical salmonellosis cases is given in Table 8. Similar, but not identical differences in
# Table 6

**Salmonella Serotypes Isolated from the River Environment**

<table>
<thead>
<tr>
<th>Fish (10/8/73)*</th>
<th>Total Isolates</th>
<th>Serotypes - Number of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amsterdam</td>
</tr>
<tr>
<td>Body Slime: 9</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Gastro-Intestinal Tract: 23</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>20+ &quot;Gizzard&quot; Shad: 12</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>20+ Suckers: 4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>15+ Carp: 7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>3 Game Fish: 0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Water (12/5/73)**:

- **Muenchen** = 5 isolates
- **Derby** = 11 isolates
- **Typhimurium, var. copenhagen** = 3 isolates
- **Eimsbuettel** = 1 isolate
- **Infantis** = 1 isolate
- **Anatum** = 2 isolates

*Water temperature ±69F; 15 pounds Cl₂ - 5 mil. gal./da. through plant.

**Water temperature ±63F; NO Cl₂; WATER LEVEL HIGH
### Table 7
**Antimicrobial Sensitivities (Kirby-Bauer) of Salmonella Isolates, 1973-74**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Percent Cultures Sensitive</th>
<th>Mammalian Isolates* (Total)</th>
<th>Water, Bottom Sludge, Fish &amp; Mussel Isolates (Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aureomycin, 30 mcg</td>
<td>1.1% (89)</td>
<td></td>
<td>19.6% (143)</td>
</tr>
<tr>
<td>Chloramphenicol, 30 mcg</td>
<td>98.3 (180)</td>
<td></td>
<td>100 (144)</td>
</tr>
<tr>
<td>Furadantin/Macrodantin, 300 mcg</td>
<td>98.3 (165)</td>
<td></td>
<td>97.9 (144)</td>
</tr>
<tr>
<td>Kanamycin, 30 mcg</td>
<td>72.4 (116)</td>
<td></td>
<td>96.5 (143)</td>
</tr>
<tr>
<td>Neomycin, 30 mcg</td>
<td>45.3 (75)</td>
<td></td>
<td>81.9 (144)</td>
</tr>
<tr>
<td>Nalidixic Acid, 30 mcg</td>
<td>97.1 (70)</td>
<td></td>
<td>96.5 (144)</td>
</tr>
<tr>
<td>Novobiocin, 30 mcg</td>
<td>0 (125)</td>
<td></td>
<td>0.70 (143)</td>
</tr>
<tr>
<td>Gentamicin, 10 mcg</td>
<td>100 (66)</td>
<td></td>
<td>99.3 (144)</td>
</tr>
<tr>
<td>Polymyxin B, 300 u</td>
<td>100 (131)</td>
<td></td>
<td>98.6 (143)</td>
</tr>
<tr>
<td>Streptomycin, 10 mcg</td>
<td>4.2 (120)</td>
<td></td>
<td>23.8 (143)</td>
</tr>
<tr>
<td>Triple Sulfonamides, 1 mg</td>
<td>9.1 (132)</td>
<td></td>
<td>49.7 (143)</td>
</tr>
<tr>
<td>Ampicillin, 10 mcg</td>
<td>60.0 (15)</td>
<td></td>
<td>100 (113)</td>
</tr>
</tbody>
</table>

*Equine, bovine, porcine (1), canine (3), & human (1) isolates.*
### Table 8

**Antimicrobial Sensitivities (Kirby-Bauer) of Various Coliform Isolates, 1973-74**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Percent Cultures Sensitive</th>
<th>Normal &amp; Scours Swine Isolates (Total)</th>
<th>Salmonellosis Isolates* (Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aureomycin, 30 mcg</td>
<td>5.7% (70)</td>
<td>1.6% (62)</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol, 30 mcg</td>
<td>100 (72)</td>
<td>77.7 (63)</td>
<td></td>
</tr>
<tr>
<td>FURADANTIN/MACRODANTIN, 300 mcg</td>
<td>98.6 (71)</td>
<td>92.4 (66)</td>
<td></td>
</tr>
<tr>
<td>Kanamycin, 30 mcg</td>
<td>98.5 (68)</td>
<td>67.7 (62)</td>
<td></td>
</tr>
<tr>
<td>Neomycin, 30 mcg</td>
<td>89.6 (67)</td>
<td>42.9 (63)</td>
<td></td>
</tr>
<tr>
<td>Nalidixic Acid, 30 mcg</td>
<td>98.3 (58)</td>
<td>92.5 (53)</td>
<td></td>
</tr>
<tr>
<td>Novobiocin, 30 mcg</td>
<td>0 (62)</td>
<td>1.6 (63)</td>
<td></td>
</tr>
<tr>
<td>Gentamicin, 10 mcg</td>
<td>100 (63)</td>
<td>100 (50)</td>
<td></td>
</tr>
<tr>
<td>Polymyxin B, 300 u</td>
<td>100 (62)</td>
<td>98.3 (60)</td>
<td></td>
</tr>
<tr>
<td>Streptomycin, 10 mcg</td>
<td>9.6 (73)</td>
<td>12.1 (66)</td>
<td></td>
</tr>
<tr>
<td>Triple Sulfonamides, 1 mg</td>
<td>12.5 (72)</td>
<td>24.2 (66)</td>
<td></td>
</tr>
<tr>
<td>Ampicillin, 10 mcg</td>
<td>100 (11)</td>
<td>50.0 (18)</td>
<td></td>
</tr>
</tbody>
</table>

*E. coli* from bovine & equine hospital cases with salmonellosis.
antimicrobial sensitivity patterns were observed as was also the case of the aquatic versus the clinical salmonellae cultures.

Further sensitivity comparisons were made for aquatic enteric isolates, e.g. coliforms (4 strains), Enterobacter sp. (10 strains), Edwardsiella sp. (3 strains), Pseudomonas sp. (1 strain), Aeromonas sp. (3 strains), Proteus sp. (4 strains) and Citrobacter sp. (3 strains). (Table 9). These genera were uniquely sensitive to the majority of antibiotics tested.

In general, those microorganisms which were prevalent in nature, i.e. the aquatic environment, had not become resistant to the wide variety of antimicrobials. The situation would appear to indicate that "naturally occurring" enterics or "wild types" had not been in contact with antimicrobials commonly employed in human or veterinary medicine nor with those widely-used as feed additives. Further investigations should clarify the situation and are warranted.

The presence of Salmonella in animal feeds and various additives, premixes and concentrates creates a problem in the control of livestock and human infections. Ewing has reviewed the extensive literature dealing with the isolation of salmonellae from poultry and livestock feeds as well as rendering plant products, fish meal and vegetable protein concentrates.24 Epizootics due to the feeding of contaminated rations have been confirmed by numerous investigators. A recent epidemiologic study traces the course of S. agona infections from fish meal to livestock to man.34 Since fish meal is not infrequently incriminated as a source of salmonellae,24 critical surveys should indicate the role of infected fish, contaminated processing facilities and equipment in the epidemiologic-epizootiologic scheme.

Recommendations for the prevention and control of salmonellosis in livestock are:
1. Expand and support efforts to detect and recognize epizootics at the state animal disease diagnostic laboratory level.
2. Strengthen state and federal reporting services. Make salmonellosis a reportable disease!
3. Encourage establishment of Specific Pathogen Free (S.P.F.) herds and flocks.
4. Promote and improve confinement rearing and production units.
5. Counsel producers to maintain a "closed-herd system" with limited introduction of disease-free replacements.
7. Apply modified quarantines during severe epizootics.
8. Identify Salmonella sources in nature and the environment.
9. Develop more effective preventive therapies and possibly vaccines.
10. Devise and seek more accurate and rapid diagnostic procedures.
11. Accelerate rural and urban education and public information programs, i.e., through USDA-APHIS, FDA, and the USDA Cooperative Extension Service (CES).

Salmonellosis cannot be eradicated. It is too deeply entrenched in the animal-human environment. The infections can, to a degree, be prevented and controlled. More effort and support at the community, state and federal levels are necessary to reduce the incidence of and concurrent economic losses due to salmonellae infections.
REFERENCES

24. Ibid., pg. 80-81.
REPORT ON THE DILLON BEACH PROJECT COOPERATIVE AGREEMENT ON SPECIFIC PATHOGEN FREE TURKEYS

Authors: Dr. J. W. Walker, Senior Staff Veterinarian, Poultry Diseases, U.S. Department of Agriculture, Hyattsville, Maryland; Dr. R. H. McCapes, Assistant Dean-Public Programs, School of Veterinary Medicine, University of California, Davis, California; Dr. William Dungan, Nicholas Turkey Breeding Farms, Inc., Sonoma, California; Dr. R. J. Holte, Bureau of Animal Health, Department of Food and Agriculture, Sacramento, California; and Dr. H. G. Purchase, Staff Scientists, U.S. Department of Agriculture, Washington, D.C.

The Dillon Beach Project was developed in 1969 as a cooperative effort with the Nicholas Turkey Breeding Farms, Inc.; California Department of Food and Agriculture, Division of Animal Industry; the Regents of the University of California, Davis; United States Department of Agriculture, Agricultural Research Service, Animal and Plant Health Inspection Service. The objective is to determine the feasibility of establishing primary turkey breeder flocks free of Salmonella, Arizona, and Mycoplasma disease organisms. Support is provided by the California Department of Food and Agriculture, Bureau of Veterinary Laboratory Services, Bureau of Animal Health; the Animal and Plant Health Inspection Service, Veterinary Services; the Agricultural Research Service; the Southeast Poultry Disease Research Laboratory; the University of California, Davis; and by the Nicholas Turkey Breeding Farms, Inc. A quarterly review of project progress is made by members of each support group through a joint steering committee.

1974 Progress Report

I. Salmonella Program

Salmonella was detected in the feed in FY 1970 and again in FY 1972 at which time it began to be seen in various lots of turkeys and their environment. Attempts to free the premises of Salmonella in FY 1973 were not completely successful. During FY 1974, the Steering Committee carried out the following activities designed to more accurately define the scope and source of the Salmonella organisms and to initiate corrective steps.

1. The feed mill was given a thorough inspection and evaluation by an Animal and Plant Health Inspection Service (APHIS) Regional Poultry Epidemiologist, Dr. C. D. Murphy. His recommendaitions for correction were considered, and it was concluded that it would be more efficient in the long run to build a new feed mill.

2. Dr. J. E. Williams introduced the antiglobulin and microtitre testing techniques into the laboratory procedures to improve the diagnostic efficiency of the laboratory. A comparison of this test with rectal swabbing and attempted organism isolation is found in Table 2.

3. Drs. Robert L. Ziriax of the Bureau of Animal Health, California; and H. Riemann, University of California, Davis; conducted inspection tours of the entire Dillon Beach operation. They made a detailed evaluation of the sanitation and husbandry procedures utilized at the feed mill and on the
farm and submitted recommendations.

4. The Salmonella monitoring of the adult flocks and of Hatch No. 7 (the most infected) are shown in Tables 1 and 2. These results indicate the S. tennessee in the feed is the most likely source of infection and that the disease progresses erratically.

The findings of the 1974 Salmonella program were:

1. *Salmonella Contamination in Feed* — It appears that this was the primary source of Salmonella infection. The solution to correcting this problem has not yet been resolved.

2. *Breaking the Salmonella Cycle* — The destruction of Salmonella in eggs to prevent egg transmission has not been shown to be perfected to the point it can be relied upon.

3. *Definitive Means to Establish and Maintain Salmonella Negative Flocks That Have Not Been Established* — Present sanitation practices under commercial conditions at both the feed manufacturers level and at the farm level will not prevent the introduction and spread of Salmonella contamination in turkey flocks.

II. Mycoplasma Meleagridis Program

An attempt was made this year to produce *M. meleagridis* free pouls from breeding stock culturally negative to infection of the genital tract but positive to upper respiratory infection.

Culture testing was conducted in January and February 1974. The tom turkeys were tested by culturing of the phallus. This was repeated three times and each time the positive birds removed from the flock and slaughtered. There were three positive of the 131 tested on the final test.

The hens were swab cultured prior to the first insemination. Of 1,004 birds tested, 1.7 percent were found positive. Results of culturing pouls are seen on Table 3.

A summary of this work concluded that:

1. Egg treatment utilizing pressure differential or temperature differential egg dipping is not 100 percent effective but does apparently, greatly reduce transmission.

2. Artificial insemination crews appear to mechanically spread infection from infected toms and hen turkeys to negative toms and hens with contaminates through the genital tract or by the insemination equipment.

These two factors were thought to have contributed to the presence of *M. meleagridis* in pouls produced from genital tracts of culturally negative parents at the onset of production even though the eggs were dipped in Tylan.

III. Future Activities

As the current problems are mainly engineering in nature and are outside the scope of veterinary medical expertise, APHIS/VS is reducing its participation in the Dillon Beach Project beginning in FY 1975.
IV. Summary

In our opinion the establishing of Salmonella negative turkey breeder flocks will be delayed until solutions to the above problems have been made. An alternative method for establishing Salmonella and Mycoplasma free flocks by serological and cultural testing is needed. These tests serve well for flock monitoring but are not a final answer for establishing free flocks, especially from valuable pedigree breeder flocks.
### TABLE I

**SALMONELLA CULTURES ISOLATED FROM TURKEY AND ENVIRONMENTAL SAMPLES AT DILLON BEACH - FY 1974**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Pedigree House #1</th>
<th>Pedigree House #2</th>
<th>Line House 1</th>
<th>Line House 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floor Litter</td>
<td>0/108</td>
<td>0/82</td>
<td>0/108</td>
<td>0/82</td>
</tr>
<tr>
<td>Nest Litter</td>
<td>0/108</td>
<td>-</td>
<td>0/108</td>
<td>-</td>
</tr>
<tr>
<td>Environmental Swabs</td>
<td>0/12</td>
<td>0/6</td>
<td>0/12</td>
<td>0/6</td>
</tr>
<tr>
<td>Cloacal Swabs</td>
<td>0/36</td>
<td>-</td>
<td>0/36</td>
<td>-</td>
</tr>
<tr>
<td>GI Tracts</td>
<td>0/235</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eggs</td>
<td>0/175</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reproductive Tract</td>
<td>0/188</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Feed</td>
<td>1/266*</td>
<td>1/266*</td>
<td>1/266*</td>
<td>1/266*</td>
</tr>
</tbody>
</table>

Number positive/Number tested.

* S. tennessee
** S. reading

Note: These are the adult breeders hatched in 1973 and parent stock for the seven hatches in fiscal year 1974 — the breeders in Line House 1 and 2 are not included in the specific pathogen free program.
TABLE 2

SALMONELLA IN SEVEN HATCHES AT DILLON BEACH - FY 1974

<table>
<thead>
<tr>
<th>Hatch Numbers</th>
<th>I (1)</th>
<th>II (2)</th>
<th>III (3)</th>
<th>IV (4)</th>
<th>V (5)</th>
<th>VI (6)</th>
<th>VII (7)</th>
<th>V1 (8)</th>
<th>V1 (9)</th>
<th>V1 (10)</th>
<th>V1 (11)</th>
<th>V1 (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatchery Debris/2</td>
<td>5/13</td>
<td>2/12</td>
<td>1/4</td>
<td>1/3</td>
<td>1/3</td>
<td>1/4</td>
<td>1/3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poult Mortality (1st 2 weeks)</td>
<td>9/57</td>
<td>9/44</td>
<td>9/23</td>
<td>9/16</td>
<td>9/30</td>
<td>5/23</td>
<td>9/19</td>
<td>24/90</td>
<td>9/24</td>
<td>9/5</td>
<td>2/19</td>
<td>5/33</td>
</tr>
</tbody>
</table>

Rectal Swabs/3 and Microantiglobulin Serology Group C 1/20 or greater /4

<table>
<thead>
<tr>
<th>Culture</th>
<th>Serology</th>
<th>5 weeks</th>
<th>10-12 weeks</th>
<th>18 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>Serology</td>
<td>%/155</td>
<td>%/153</td>
<td>%/150</td>
</tr>
<tr>
<td>Serology</td>
<td>%/300</td>
<td>%/300</td>
<td>%/300</td>
<td>%/300</td>
</tr>
<tr>
<td></td>
<td>%/300</td>
<td>%/300</td>
<td>%/300</td>
<td>%/300</td>
</tr>
<tr>
<td></td>
<td>%/300</td>
<td>%/300</td>
<td>%/300</td>
<td>%/300</td>
</tr>
<tr>
<td></td>
<td>%/300</td>
<td>%/300</td>
<td>%/300</td>
<td>%/300</td>
</tr>
<tr>
<td></td>
<td>%/300</td>
<td>%/300</td>
<td>%/300</td>
<td>%/300</td>
</tr>
</tbody>
</table>

Number positive/Number tested.

1. Hatch 7 is not included in the SPF program.
2. S. infantis in hatches 1, 2, 3, and 4. S. heidelberg in hatches 5 and 6.
3. S. tennessee isolated on all rectal swabs.
4. Correlation between percent positive on culture and serology at a titer of 1:20 or greater is $r_s = 0.6574$, using Spearman Rank Correlation Test - $p=0.01$. 

Walker, McCapes, Duncan, and Holte

Page 304
### TABLE 3
MYCOPLASMA MELEAGRIDIS ISOLATION FROM POULTS HATCHED IN DILLON BEACH PROJECT - FY 1974

<table>
<thead>
<tr>
<th>Date of Hatch</th>
<th>Number Set</th>
<th>Number Dipped</th>
<th>Air sac Lesion</th>
<th>Cloaca</th>
<th>Oral and Tracheal</th>
<th>Air sac</th>
<th>Yolk</th>
<th>Bursa</th>
<th>Day-old</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/12</td>
<td>1946</td>
<td>none</td>
<td>34/262</td>
<td>30/56</td>
<td>39/56</td>
<td>35/56</td>
<td>34/56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/21</td>
<td>2000</td>
<td>all</td>
<td>0/82</td>
<td>ND</td>
<td>ND</td>
<td>2/82</td>
<td>0/30</td>
<td></td>
<td>28/40</td>
</tr>
<tr>
<td>7/12</td>
<td>5600</td>
<td>all</td>
<td>3/363</td>
<td>0/113</td>
<td>0/113</td>
<td>0/113</td>
<td>0/113</td>
<td></td>
<td>6/17</td>
</tr>
<tr>
<td>7/23</td>
<td>600</td>
<td>all</td>
<td>2/359</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td>45/90</td>
</tr>
<tr>
<td>7/26</td>
<td>5000</td>
<td>all</td>
<td>2/264</td>
<td>ND</td>
<td>ND</td>
<td>0/100</td>
<td>2/100</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>8/2</td>
<td>5600</td>
<td>all</td>
<td>1/125</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td>ND</td>
</tr>
</tbody>
</table>

Number positive/Number tested.

ND - Not Done.
REFERENCES

1. A Five Year Epidemiological Study of Salmonellae and Arizonae on a Commercial Primary Turkey Breeding Ranch, by Dr. B. C. Zecha, U.S. Department of Agriculture, Sacramento, California.

2. Quarterly Reports of Dillon Beach Steering Committee.

3. Personal Communications - by Dr. J. W. Walker, Senior Staff Veterinarian, Poultry Diseases, U.S. Department of Agriculture, Hyattsville, Maryland.

Presented at the 78th U.S. Animal Health Association Annual Meeting, Roanoke, Virginia, October 14-18, 1974, by J. W. Walker, Senior Staff Veterinarian, Poultry Diseases, USDA: APHIS-VS.
REPORT OF THE COMMITTEE ON SALMONELLOSIS

Chairman: H. G. Geyer, Washington, D.C.
Co-Chairman: John W. Walker, Hyattsville, Maryland; Edward R. Ames, Chicago, Illinois; William B. Bixler, Rockville, Maryland; Ralph Johnston, Washington, D.C.; E. M. Foster, Madison, Wisconsin; Marshall Fox, Atlanta, Georgia; Rube Harrington, Ames, Iowa; Conwell Johnston, Des Plaines, Illinois; E. V. Morse, Lafayette, Indiana; Daniel M. Wenger, Decatur, Indiana; Howard B. Norton, Alexandria, Virginia; Ned W. Rokey, Mesa, Arizona; Walt Salder, Davis, California; Raymond Schar, Beltsville, Maryland; Stanley A. Vezey, Athens, Georgia; Rufus Weidner, Chicago, Illinois.

Your Salmonella Committee notes with regret that no action had been taken by the U.S.A.H.A. on a Resolution submitted to the U.S.A.H.A. at the 76th Annual Meeting, November 1972, expressing "grave concern over discontinuation of the Cooperative State-Federal Salmonella Program". (See page 394 - Proceedings, 76th Annual Meeting of the U.S.A.H.A.).

Your Committee wishes to commend the Extension Service, USDA, and The National Association of Retail Grocers of the United States (NARGUS) for the development of Sanitation Guidelines for food stores and supermarkets. These Guidelines are being actively placed into operating practice at retail food outlets by several food chains. (See pages 284-290 - Proceedings 77th Annual Meeting U.S.A.H.A.)

Committee member Dr. William B. Bixler reported to the Committee on progress over the past year of the F.D.A. program for the control of Salmonella in Animal and Marine Products intended for use in animal feeds. As a result of a series of meetings between government and affected industry representatives, The Commissioner of FDA believes that there continues to be a need for a program to control Salmonella in rendered animal and industrial marine products. Plans are under way to take the appropriate steps to put into operation a cooperative program based upon Federal-State-Industry Cooperation for controlling Salmonells in rendered animal and marine products.

The Committee also noted that, even though Salmonella contamination of animal feed ingredients and complete feeds must be corrected, Salmonella transmission through feeds is only a part of the total environmental pollution problem stemming from this group of organisms. In this regard the Committee noted with interest the summary of a report from the Journal of Water Pollution Control, September, 1974, which is as follows:
Salmonella in Water

<table>
<thead>
<tr>
<th>Component</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home Cisterns</td>
<td>59</td>
</tr>
<tr>
<td>Suburban Cisterns</td>
<td>40</td>
</tr>
<tr>
<td>Farm Wells</td>
<td>0</td>
</tr>
<tr>
<td>Septic Tank Sludges</td>
<td>50</td>
</tr>
<tr>
<td>Ohio River</td>
<td>45</td>
</tr>
<tr>
<td>Ohio River</td>
<td>76</td>
</tr>
<tr>
<td>Mississippi River</td>
<td>75</td>
</tr>
<tr>
<td>Wabash River</td>
<td>75</td>
</tr>
<tr>
<td>Creek: 1 Mile Below Packing Plant</td>
<td>100 and 43</td>
</tr>
<tr>
<td>Streams (31)</td>
<td>58</td>
</tr>
<tr>
<td>Storm Water Run-off</td>
<td>78</td>
</tr>
<tr>
<td>Waste Water (Municipal)</td>
<td>100 and 79</td>
</tr>
<tr>
<td>Packing Plant</td>
<td>66 and 46.7</td>
</tr>
<tr>
<td>Stockyards</td>
<td>100</td>
</tr>
<tr>
<td>Storm Run-offs</td>
<td>78 and 50</td>
</tr>
<tr>
<td>Sludge Effluents</td>
<td>79 and 72.5</td>
</tr>
<tr>
<td>Trickling Filter</td>
<td>76 and 57.7</td>
</tr>
<tr>
<td>Sludge</td>
<td></td>
</tr>
<tr>
<td>Raw Primary</td>
<td>83</td>
</tr>
<tr>
<td>Primary Activated</td>
<td>87</td>
</tr>
<tr>
<td>Aerobic Digester</td>
<td>83</td>
</tr>
<tr>
<td>Aerobic Digester (28 days)</td>
<td>33</td>
</tr>
<tr>
<td>Activated Secondary</td>
<td>82</td>
</tr>
<tr>
<td>Septic Tank</td>
<td>50</td>
</tr>
</tbody>
</table>

Your Committee also regrets the disappointing response from the various states to a questionnaire, submitted through this Association, to the appropriate state officials on the Voluntary Cooperative Salmonella Program. (Insert Attachment No. 1)

Attachment No. 1

Voluntary Cooperative Industry-State-Federal Program for the Control of Salmonella in Animal and Marine Products Intended for Use in Animal Feed
### QUESTIONS:

1. Did you participate in the former USDA Program?  
   - Yes (34)  
   - No (4)

2. Are you now participating in a salmonella program of your own for:  
   (a) feed ingredients (animal or marine origin)  
   - Yes (11)  
   - No (24)  
   (b) mixed animal feeds  
   - Yes (3)  
   - No (28)

3. Do you have a law or act for regulatory support of a Salmonella Control Program?  
   - Yes (15)  
   - No (21)

4. Can you take regulatory action on violations to obtain compliance?  
   - Yes (15)  
   - No (20)

5. Are you interested in participating in a Voluntary Cooperative Salmonella Control Program? (It is to be understood, at the present time, no Federal funds will be available.)  
   - Yes (17)  
   - No (21)

   If no, explain  
   - No (14) unavailable funds and/or personnel  
   - No (7) not interested

6. What is the name, title, and organization of the person who would directly manage State participation in this program?  
   - Name  
   - Title  
   - Organization

7. (a) Do you have legislation requiring product registration or licensing of animal and marine renderers and blenders?  
   - Yes (23)  
   - No (6)  
   (b) Please attach separate lists of the known names and addresses of:  
   - Out of 38 States — 21 States enclosed lists totaling:  
   - Renderers 353 renderers  
   - Blenders 25 blenders  
   (A blender is defined as a firm that does not cook the product but simply blends previously cooked products from other sources.)

8. Do you have laboratory facilities to test samples in accordance with the enclosed UMAR?  
   - Yes (33)  
   - No (3)

   If yes, will you conduct the testing?  
   - Yes (23)  
   - No (7)

9. Would your State or your present law allow and recognize certified private laboratory testing?  
   - Yes (20)  
   - No (9)  
   (Some replies: present law does not state so; or State has no law)

10. Do you believe there is a need to certify private laboratories for conducting the testing?  
    - Yes (14)  
    - No (17)

   If yes:  
    (a) Could they be certified by your State?  
    - Yes (9)  
    - No (5)  
    (b) Could you maintain surveillance over them?  
    - Yes (9)  
    - No (5)

11. Would you contemplate a regulatory posture as part of your State Activities?  
    - Yes (21)  
    - No (14)

   If so, would it include any or all of the following:  
    (a) Inspection?  
    - Yes (20)  
    - No (3)  
    (b) Reinspection?  
    - Yes (19)  
    - No (3)  
    (c) Regulatory letters?  
    - Yes (18)  
    - No (3)  
    (d) Regulatory actions such as embargoes, prosecutions, injunctions, etc.?  
    - Yes (14)  
    - No (3)
12. Would you need to require a fee for conducting the laboratory testing?  
   Yes (14)  No (14)  
   If yes, (a) Could you legally require a fee?  Yes (14)  No (4)  
   (b) What would you charge for each method?  
      (1) ARS-98-68-1  Average $9.40/10 per sample  
      (2) BAM/AOAC  $5.00 to $15.00  

13. If you participate in the program will you submit periodical reports of findings to FDA?  Yes (26)  No (3)  

14. Do you think some formal recognition should be given to Phase III “In Compliance” plants?  Yes (22)  No (7)  
   If yes, would you suggest a form of recognition?  Published Lists; Certificates; Patent Advertising; FDA Approval.  

15. Do you believe it would be desirable and would you participate in a workshop, for responsible State officials, to plan implementation of the program by the States?  Yes (24)  No (5)  

Name, Title and Organization of the individual completing these questions  

Name  

Title  

Organization
State response regarding participation is reflected in Attachment No. 2.

Attachment No. 2

The Following States Responded to Item No. 5 of the Salmonella Questionnaire

<table>
<thead>
<tr>
<th>YES (17)</th>
<th>NO (7)</th>
<th>MAYBE (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaska</td>
<td>Florida</td>
<td>Alabama</td>
</tr>
<tr>
<td>Hawaii</td>
<td>Iowa</td>
<td>Arkansas</td>
</tr>
<tr>
<td>Idaho</td>
<td>Kentucky</td>
<td>Arizona</td>
</tr>
<tr>
<td>Illinois</td>
<td>Mississippi</td>
<td>California</td>
</tr>
<tr>
<td>Maine</td>
<td>North Dakota</td>
<td>Colorado</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>Texas</td>
<td>Georgia</td>
</tr>
<tr>
<td>Minnesota</td>
<td>West Virginia</td>
<td>Indiana</td>
</tr>
<tr>
<td>Montana</td>
<td>Florida</td>
<td>New Jersey</td>
</tr>
<tr>
<td>Nebraska</td>
<td>Iowa</td>
<td>North Carolina</td>
</tr>
<tr>
<td>New York</td>
<td>Idaho</td>
<td>Pennsylvania</td>
</tr>
<tr>
<td>Ohio</td>
<td>Illinois</td>
<td>South Carolina</td>
</tr>
<tr>
<td>Oregon</td>
<td>Iowa</td>
<td>Tennessee</td>
</tr>
<tr>
<td>South Dakota</td>
<td>Iowa</td>
<td>Utah</td>
</tr>
<tr>
<td>Vermont</td>
<td>Iowa</td>
<td>Washington</td>
</tr>
<tr>
<td>Virginia</td>
<td>Iowa</td>
<td></td>
</tr>
<tr>
<td>Wisconsin</td>
<td>Iowa</td>
<td></td>
</tr>
<tr>
<td>Wyoming</td>
<td>Iowa</td>
<td></td>
</tr>
</tbody>
</table>

The Following States Have Not Responded to the Salmonella Questionnaire (12)

| Connecticut | Maryland | New Hampshire |
| Delaware    | Michigan | New Mexico   |
| Kansas      | Missouri | Oklahoma     |
| Louisiana   | Nevada   | Rhode Island |

Veterinary Services, APHIS, has continued the compilation of Salmonella serotyping statistics from Agricultural Sources for FY 1974. This compilation was initiated as a result of a recommendation of the U.S.A.H.A. They are detailed as follows:
This is a report of the serotyping of cultures of Arizona and Salmonella isolated from livestock and poultry and their environment by the cooperating animal diagnostic laboratories in the United States during fiscal year 1974.*

We call attention to the deficiency in recording statistical data on the request form. It is most urgent that the request form (VS Form 10-3), especially Item 9 (number in the flock or herd) and Item 10 (number dead), be filled in as accurately as possible. If more than one submission is made from the same case, this should be noted in Item 15 (comments), e.g., 1 of 3 cultures from this case; 2 previous submissions have been made; etc. Also, submissions from nonclinical cases should be noted in Item 15. This information will help to prevent multireporting of single cases and lessen the chance of reporting nonclinical cases as salmonellosis.

---

*The Salmonella Serotyping in this report was carried out at the National Animal Disease Center, Veterinary Services Laboratories, Ames, Iowa, and in cooperation with state and private animal disease laboratories.
### TABLE I

**ARIZONA SEROTYPES REPORTED FROM DOMESTIC ANIMALS OF THE UNITED STATES**

**FISCAL YEAR 1974**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Turkey</th>
<th>Chicken</th>
<th>Other Animal</th>
<th>Swine</th>
<th>Cattle</th>
<th>Other Animal</th>
<th>Feed or Environment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arizona 7A, 7B:1, 7, 8</td>
<td>322</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>342</td>
</tr>
<tr>
<td>Arizona 26:30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Arizona 26:29-30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Arizona 13:1, 2, 5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Arizona 24:24-28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Arizona 9A, 9B:24-31</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Arizona 15:22-21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Arizona 29:33-31</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Arizona 5:26-31</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Arizona 1, 33:24-25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Arizona 30:31-32</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Arizona 26:32-21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Arizona 7A, 7B:23-31</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Arizona 9A, 9B:29-31</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Arizona 7A, 7C:27-28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Arizona 26:24-25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Arizona 24:22-25</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Arizona 5:24-28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Arizona 29:32-31</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Arizona Unknown</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

**TOTAL** 324 4 6 8 5 68 4 419
## TABLE I - (Continued)

**SALMONELLA SEROTYPES REPORTED FROM DOMESTIC ANIMALS OF THE UNITED STATES**

**FISCAL YEAR 1974**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Turkey</th>
<th>Chicken</th>
<th>Other</th>
<th>Avian</th>
<th>Swine</th>
<th>Cattle</th>
<th>Other</th>
<th>Animals</th>
<th>Feed or Environment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. adelaide</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. agona</td>
<td>35</td>
<td>21</td>
<td>0</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>S. alachua</td>
<td>0</td>
<td>23</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>S. albany</td>
<td>12</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>S. amager</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>S. amsterdam</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>23</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>S. anatum</td>
<td>120</td>
<td>26</td>
<td>6</td>
<td>11</td>
<td>48</td>
<td>31</td>
<td>81</td>
<td>323</td>
<td>0</td>
<td>323</td>
</tr>
<tr>
<td>S. iberia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>S. arechavaleta</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. bareilly</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>S. bere</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>S. bern</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>S. binza</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>S. bareilly var. 14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>S. iberi</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. blockley</td>
<td>9</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>29</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>S. bornum</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>17</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>S. bovis-morbillans</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>S. brenderup</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>S. bredeney</td>
<td>18</td>
<td>18</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>44</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>S. california</td>
<td>7</td>
<td>32</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>46</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>S. carrau</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S. cerro</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>14</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>S. challey</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. chester</td>
<td>15</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>S. cholerae-suis</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>S. cholerae-suis var. kunzendorf</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>387</td>
<td>11</td>
<td>4</td>
<td>0</td>
<td>404</td>
<td>0</td>
<td>404</td>
</tr>
</tbody>
</table>

**Total** 323

**Note:** The table represents the distribution of Salmonella serotypes reported from domestic animals of the United States during fiscal year 1974.
<table>
<thead>
<tr>
<th>Serotype</th>
<th>Turkey</th>
<th>Chicken</th>
<th>Other Avian</th>
<th>Swine</th>
<th>Cattle</th>
<th>Other Animals</th>
<th>Feed or Environment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. corvallis</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. cubana</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>S. degana</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. derby</td>
<td>13</td>
<td>9</td>
<td>2</td>
<td>23</td>
<td>0</td>
<td>7</td>
<td>28</td>
<td>82</td>
</tr>
<tr>
<td>S. chameleon</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>S. drypool</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>S. dublin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>134</td>
<td>3</td>
<td>0</td>
<td>141</td>
</tr>
<tr>
<td>S. duesseldorf</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. eimsbuettel</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>S. enteritidis</td>
<td>6</td>
<td>24</td>
<td>2</td>
<td>10</td>
<td>11</td>
<td>19</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>S. gallinarum</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>S. georgia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. good</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>S. gee</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>S. habana</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>S. hartford</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>142</td>
<td>151</td>
<td>3</td>
<td>13</td>
<td>4</td>
<td>9</td>
<td>15</td>
<td>337</td>
</tr>
<tr>
<td>S. houten</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. hvititingfoss</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>S. illinois</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>S. indiana</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>S. infantis</td>
<td>21</td>
<td>130</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>21</td>
<td>8</td>
<td>194</td>
</tr>
<tr>
<td>S. irumu</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. java</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>S. javlana</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>S. johannesburg</td>
<td>2</td>
<td>31</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>26</td>
<td>59</td>
</tr>
<tr>
<td>S. kentucky</td>
<td>4</td>
<td>13</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>43</td>
<td>62</td>
</tr>
<tr>
<td>S. kottbus</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>S. lexington</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>S. litchfield</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>S. lomita</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>S. london</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>S. marina</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S. manhattan</td>
<td>11</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>28</td>
</tr>
</tbody>
</table>
TABLE I - (Continued)

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Turkey</th>
<th>Chicken</th>
<th>Other Avian</th>
<th>Swine</th>
<th>Cattle</th>
<th>Other Animals</th>
<th>Feed or Environment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. manila</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S. menhaden</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. meleagrids</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>S. miami</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>S. minneapolis</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. minnesota</td>
<td>12</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>S. mississippi</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>26</td>
</tr>
<tr>
<td>S. molade</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. montevideo</td>
<td>8</td>
<td>82</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td>S. muenchsen</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>8</td>
<td>8</td>
<td>130</td>
</tr>
<tr>
<td>S. muenster</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>S. new-brunswick</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>S. newington</td>
<td>13</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>55</td>
</tr>
<tr>
<td>S. newport</td>
<td>24</td>
<td>11</td>
<td>3</td>
<td>10</td>
<td>117</td>
<td>23</td>
<td>0</td>
<td>188</td>
</tr>
<tr>
<td>S. nenstedten</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S. nima</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>S. norwich</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. ohio</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>S. oranienburg</td>
<td>4</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>9</td>
<td>8</td>
<td>36</td>
</tr>
<tr>
<td>S. orion</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. ordonez</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>S. oslo</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S. panama</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>S. poona</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>S. portsmouth</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. pullorum</td>
<td>6</td>
<td>55</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>S. reading</td>
<td>121</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>147</td>
</tr>
<tr>
<td>S. rubislaw</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>11</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>S. sachsenwald</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. saint-paul</td>
<td>202</td>
<td>32</td>
<td>3</td>
<td>18</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>275</td>
</tr>
<tr>
<td>S. san-diego</td>
<td>113</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>13</td>
<td>133</td>
</tr>
<tr>
<td>S. schwarzenburg</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>S. senttenberg</td>
<td>54</td>
<td>15</td>
<td>2</td>
<td>11</td>
<td>3</td>
<td>3</td>
<td>10</td>
<td>98</td>
</tr>
</tbody>
</table>
### TABLE I - (Continued)

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Turkey</th>
<th>Chicken</th>
<th>Other Avian</th>
<th>Swine</th>
<th>Cattle</th>
<th>Other Animals</th>
<th>Feed or Environment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. siegburg</td>
<td>2</td>
<td>25</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td>S. simsbury</td>
<td>1</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>29</td>
<td>41</td>
</tr>
<tr>
<td>S. singapore</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. sundsvall</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. taksony</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>S. tallahassee</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. tennessee</td>
<td>3</td>
<td>19</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>31</td>
</tr>
<tr>
<td>S. thomasville</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>S. thompson</td>
<td>5</td>
<td>73</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>S. tuindorp</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>46</td>
<td>221</td>
<td>92</td>
<td>193</td>
<td>1079</td>
<td>197</td>
<td>9</td>
<td>1837</td>
</tr>
<tr>
<td>S. typhimurium var. copenhagen</td>
<td>19</td>
<td>33</td>
<td>49</td>
<td>46</td>
<td>345</td>
<td>66</td>
<td>12</td>
<td>570</td>
</tr>
<tr>
<td>S. typhi-suis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>S. urbana</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. wassenaar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. worthington</td>
<td>8</td>
<td>47</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>47</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>Contaminated or Untypable Groups</td>
<td>11</td>
<td>27</td>
<td>5</td>
<td>26</td>
<td>17</td>
<td>17</td>
<td>19</td>
<td>122</td>
</tr>
<tr>
<td>Unknown or Not Salmonella or Ariz.</td>
<td>56</td>
<td>34</td>
<td>9</td>
<td>34</td>
<td>35</td>
<td>45</td>
<td>17</td>
<td>230</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1230</td>
<td>1335</td>
<td>212</td>
<td>857</td>
<td>1869</td>
<td>653</td>
<td>584</td>
<td>6740</td>
</tr>
<tr>
<td>State</td>
<td>Number of Reports</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>
</tr>
<tr>
<td>Alabama</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alaska</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arizona</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arkansas</td>
<td>214</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>California</td>
<td>946</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connecticut</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delaware</td>
<td>218</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dist. of Col.</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida</td>
<td>166</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Georgia</td>
<td>612</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hawaii</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idaho</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illinois</td>
<td>490</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indiana</td>
<td>589</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iowa</td>
<td>339</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kansas</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kentucky</td>
<td>165</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Louisiana</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maine</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maryland</td>
<td>187</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Massachusetts</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Michigan</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minnesota</td>
<td>861</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mississippi</td>
<td>113</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missouri</td>
<td>225</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montana</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nebraska</td>
<td>103</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nevada</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Hampshire</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Jersey</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Mexico</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New York</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Carolina</td>
<td>128</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Dakota</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohio</td>
<td>162</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oklahoma</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oregon</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhode Island</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Carolina</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Dakota</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tennessee</td>
<td>111</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texas</td>
<td>462</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Utah</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vermont</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virginia</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washington</td>
<td>129</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>West Virginia</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wisconsin</td>
<td>493</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wyoming</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State</td>
<td>Turkey</td>
<td>Chicken</td>
<td>Other Avian</td>
<td>Swine</td>
<td>Cattle</td>
<td>Other Animals</td>
<td>Feed or Environment</td>
<td>Total</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
<td>---------</td>
<td>-------------</td>
<td>-------</td>
<td>--------</td>
<td>---------------</td>
<td>---------------------</td>
<td>-------</td>
</tr>
<tr>
<td>California</td>
<td>281</td>
<td>50</td>
<td>59</td>
<td>15</td>
<td>364</td>
<td>162</td>
<td>15</td>
<td>946</td>
</tr>
<tr>
<td>Minnesota</td>
<td>397</td>
<td>12</td>
<td>20</td>
<td>34</td>
<td>120</td>
<td>90</td>
<td>188</td>
<td>861</td>
</tr>
<tr>
<td>Georgia</td>
<td>40</td>
<td>450</td>
<td>2</td>
<td>4</td>
<td>45</td>
<td>30</td>
<td>41</td>
<td>612</td>
</tr>
<tr>
<td>Indiana</td>
<td>72</td>
<td>40</td>
<td>0</td>
<td>272</td>
<td>96</td>
<td>84</td>
<td>24</td>
<td>588</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>148</td>
<td>12</td>
<td>10</td>
<td>13</td>
<td>276</td>
<td>32</td>
<td>2</td>
<td>493</td>
</tr>
<tr>
<td>Illinois</td>
<td>112</td>
<td>63</td>
<td>2</td>
<td>122</td>
<td>139</td>
<td>34</td>
<td>13</td>
<td>489</td>
</tr>
<tr>
<td>Texas</td>
<td>150</td>
<td>22</td>
<td>7</td>
<td>21</td>
<td>213</td>
<td>41</td>
<td>8</td>
<td>462</td>
</tr>
<tr>
<td>Iowa</td>
<td>25</td>
<td>5</td>
<td>2</td>
<td>180</td>
<td>110</td>
<td>15</td>
<td>2</td>
<td>339</td>
</tr>
<tr>
<td>Missouri</td>
<td>107</td>
<td>18</td>
<td>5</td>
<td>20</td>
<td>59</td>
<td>9</td>
<td>7</td>
<td>225</td>
</tr>
<tr>
<td>Delaware</td>
<td>13</td>
<td>84</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>118</td>
<td>218</td>
</tr>
<tr>
<td>Arkansas</td>
<td>60</td>
<td>144</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>214</td>
</tr>
<tr>
<td>Maryland</td>
<td>3</td>
<td>89</td>
<td>4</td>
<td>30</td>
<td>6</td>
<td>2</td>
<td>50</td>
<td>184</td>
</tr>
<tr>
<td>Florida</td>
<td>0</td>
<td>47</td>
<td>41</td>
<td>1</td>
<td>38</td>
<td>39</td>
<td>0</td>
<td>166</td>
</tr>
<tr>
<td>Kentucky</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>20</td>
<td>103</td>
<td>36</td>
<td>0</td>
<td>165</td>
</tr>
<tr>
<td>Ohio</td>
<td>4</td>
<td>13</td>
<td>0</td>
<td>2</td>
<td>10</td>
<td>43</td>
<td>84</td>
<td>156</td>
</tr>
<tr>
<td>Washington</td>
<td>42</td>
<td>21</td>
<td>2</td>
<td>2</td>
<td>53</td>
<td>9</td>
<td>0</td>
<td>129</td>
</tr>
<tr>
<td>North Carolina</td>
<td>36</td>
<td>56</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>32</td>
<td>1</td>
<td>128</td>
</tr>
<tr>
<td>Mississippi</td>
<td>4</td>
<td>82</td>
<td>2</td>
<td>11</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>113</td>
</tr>
</tbody>
</table>
**TABLE 4**

**DISTRIBUTION OF THE TEN MOST FREQUENTLY REPORTED SEROTYPES IN STATES WITH 100 OR MORE REPORTS - FY 1974**

<table>
<thead>
<tr>
<th>STATE</th>
<th>Typhimurium</th>
<th>Arizona 7A,7B v. copenhagen</th>
<th>Typhimurium v. kundendorf</th>
<th>Cholera-suis St. paul</th>
<th>Heidelberg</th>
<th>Newport</th>
<th>S. boydii</th>
<th>Anatua</th>
<th>Infantis</th>
<th>Percent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>105</td>
<td>168</td>
<td>180</td>
<td>0</td>
<td>34</td>
<td>33</td>
<td>60</td>
<td>12</td>
<td>41</td>
<td>12</td>
</tr>
<tr>
<td>Minnesota</td>
<td>130</td>
<td>67</td>
<td>46</td>
<td>9</td>
<td>80</td>
<td>20</td>
<td>17</td>
<td>222</td>
<td>106</td>
<td>8</td>
</tr>
<tr>
<td>Georgia</td>
<td>201</td>
<td>5</td>
<td>9</td>
<td>0</td>
<td>16</td>
<td>48</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>37</td>
</tr>
<tr>
<td>Indiana</td>
<td>170</td>
<td>52</td>
<td>196</td>
<td>129</td>
<td>14</td>
<td>6</td>
<td>1</td>
<td>32</td>
<td>9</td>
<td>77</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>294</td>
<td>61</td>
<td>7</td>
<td>0</td>
<td>17</td>
<td>45</td>
<td>25</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Illinois</td>
<td>164</td>
<td>19</td>
<td>12</td>
<td>69</td>
<td>11</td>
<td>6</td>
<td>50</td>
<td>6</td>
<td>13</td>
<td>72</td>
</tr>
<tr>
<td>Texas</td>
<td>150</td>
<td>26</td>
<td>27</td>
<td>5</td>
<td>9</td>
<td>3</td>
<td>25</td>
<td>9</td>
<td>56</td>
<td>9</td>
</tr>
<tr>
<td>Iowa</td>
<td>88</td>
<td>34</td>
<td>34</td>
<td>108</td>
<td>24</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Missouri</td>
<td>51</td>
<td>22</td>
<td>12</td>
<td>4</td>
<td>23</td>
<td>16</td>
<td>6</td>
<td>9</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Delaware</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Arkansas</td>
<td>21</td>
<td>11</td>
<td>13</td>
<td>0</td>
<td>5</td>
<td>21</td>
<td>10</td>
<td>1</td>
<td>17</td>
<td>33</td>
</tr>
<tr>
<td>Maryland</td>
<td>30</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>29</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Florida</td>
<td>58</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>15</td>
<td>8</td>
<td>57</td>
</tr>
<tr>
<td>Kentucky</td>
<td>81</td>
<td>0</td>
<td>45</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>Ohio</td>
<td>12</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Washington</td>
<td>31</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>24</td>
<td>1</td>
<td>14</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>North Carolina</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Mississippi</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
</tbody>
</table>

*The percentage these top ten represent of the total serotypes isolated in the State.

It has been recommended that control programs should begin with an attack against the most prevalent serotypes. The "percent of total" column indicates that there is some variation in the geographic distribution of the ten most frequent serotypes. Perhaps control measures that would be for Salmonella in general would be more appropriate on the national level.
TABLE 5

SUMMARY OF THE TEN MOST FREQUENTLY REPORTED SALMONELLA SEROTYPES AND PULLORUM-GALLINARUM ISOLATED

<table>
<thead>
<tr>
<th>FY 1974</th>
<th>TURKEYS</th>
<th>Number Reports Showing Mortality</th>
<th>Number Turkeys</th>
<th>Average Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>saint-paul</td>
<td>202</td>
<td>37</td>
<td>493354</td>
<td>1.9</td>
</tr>
<tr>
<td>heidelberg</td>
<td>142</td>
<td>24</td>
<td>248741</td>
<td>2.1</td>
</tr>
<tr>
<td>anatum</td>
<td>120</td>
<td>16</td>
<td>118093</td>
<td>5.6</td>
</tr>
<tr>
<td>san-diego</td>
<td>113</td>
<td>11</td>
<td>110679</td>
<td>2.4</td>
</tr>
<tr>
<td>newington</td>
<td>13</td>
<td>8</td>
<td>117410</td>
<td>1.0</td>
</tr>
<tr>
<td>reading</td>
<td>121</td>
<td>7</td>
<td>65406</td>
<td>5.8</td>
</tr>
<tr>
<td>typhimurium</td>
<td>46</td>
<td>7</td>
<td>139594</td>
<td>1.8</td>
</tr>
<tr>
<td>chester</td>
<td>15</td>
<td>5</td>
<td>29055</td>
<td>5.9</td>
</tr>
<tr>
<td>senftenberg</td>
<td>54</td>
<td>5</td>
<td>46800</td>
<td>2.5</td>
</tr>
<tr>
<td>derby</td>
<td>13</td>
<td>4</td>
<td>33475</td>
<td>5.1</td>
</tr>
<tr>
<td>pullorum-gallinarum</td>
<td>6</td>
<td>3</td>
<td>42231</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Reporting efficiency 15.0%

<table>
<thead>
<tr>
<th>FY 1974</th>
<th>CHICKENS</th>
<th>Number Reports Showing Mortality</th>
<th>Number Chickens</th>
<th>Average Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>infantis</td>
<td>130</td>
<td>53</td>
<td>1014903</td>
<td>2.9</td>
</tr>
<tr>
<td>typhimurium</td>
<td>221</td>
<td>48</td>
<td>102941</td>
<td>4.0</td>
</tr>
<tr>
<td>heidelberg</td>
<td>151</td>
<td>45</td>
<td>876059</td>
<td>2.3</td>
</tr>
<tr>
<td>thompson</td>
<td>73</td>
<td>24</td>
<td>406827</td>
<td>2.2</td>
</tr>
<tr>
<td>saint-paul</td>
<td>32</td>
<td>16</td>
<td>211150</td>
<td>2.6</td>
</tr>
<tr>
<td>california</td>
<td>32</td>
<td>15</td>
<td>247400</td>
<td>5.4</td>
</tr>
<tr>
<td>montevideo</td>
<td>82</td>
<td>12</td>
<td>134659</td>
<td>1.6</td>
</tr>
<tr>
<td>reading</td>
<td>18</td>
<td>12</td>
<td>304200</td>
<td>2.8</td>
</tr>
<tr>
<td>agona</td>
<td>21</td>
<td>9</td>
<td>123260</td>
<td>1.9</td>
</tr>
<tr>
<td>typhimurium var.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>copenhagen</td>
<td>33</td>
<td>8</td>
<td>107632</td>
<td>4.7</td>
</tr>
<tr>
<td>pullorum-gallinarum</td>
<td>65</td>
<td>22</td>
<td>169025</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*Reporting efficiency 30.7%

*Reporting efficiency is the percentage of the requests for serotyping that included the number in the flock or herd and the number dead.
**TABLE 5 - (Continued)**

**SUMMARY OF THE TEN MOST FREQUENTLY REPORTED SALMONELLA SEROTYPES ISOLATED**

<table>
<thead>
<tr>
<th>FY 1974</th>
<th>SWINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype</td>
<td>Number Reports</td>
</tr>
<tr>
<td><strong>Salmonella cholerae-suis var kunzendor</strong></td>
<td>388</td>
</tr>
<tr>
<td><strong>typhimurium</strong></td>
<td>193</td>
</tr>
<tr>
<td><strong>typhimurium var copenhagen</strong></td>
<td>46</td>
</tr>
<tr>
<td><strong>derby</strong></td>
<td>23</td>
</tr>
<tr>
<td><strong>enteritidis</strong></td>
<td>10</td>
</tr>
<tr>
<td><strong>heidelberg</strong></td>
<td>13</td>
</tr>
<tr>
<td><strong>dublin</strong></td>
<td>4</td>
</tr>
<tr>
<td><strong>habana</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>newport</strong></td>
<td>10</td>
</tr>
<tr>
<td><strong>saint-paul</strong></td>
<td>18</td>
</tr>
</tbody>
</table>

*Reporting efficiency* 22.5%

<table>
<thead>
<tr>
<th>FY 1974</th>
<th>CATTLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype</td>
<td>Number Reports</td>
</tr>
<tr>
<td><strong>Salmonella typhimurium</strong></td>
<td>1079</td>
</tr>
<tr>
<td><strong>typhimurium var copenhagen</strong></td>
<td>345</td>
</tr>
<tr>
<td><strong>newport</strong></td>
<td>117</td>
</tr>
<tr>
<td><strong>anatum</strong></td>
<td>48</td>
</tr>
<tr>
<td><strong>cholerae-suis var kunzendor</strong></td>
<td>11</td>
</tr>
<tr>
<td><strong>dublin</strong></td>
<td>134</td>
</tr>
<tr>
<td><strong>bovis-morbillicans</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>enteritidis</strong></td>
<td>11</td>
</tr>
<tr>
<td><strong>heidelberg</strong></td>
<td>4</td>
</tr>
<tr>
<td><strong>java</strong></td>
<td>4</td>
</tr>
</tbody>
</table>

*Reporting efficiency* 22.0%

*Reporting efficiency is the percentage of the requests for serotyping that included the number in the flock or herd and the number dead.*
### SUMMARY OF THE MOST FREQUENTLY REPORTED SALMONELLA SEROTYPES ISOLATED

#### FY 1974

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. Reports Showing Mortality</th>
<th>Number Sheep &amp; Goats</th>
<th>Average Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>typhimurium</em></td>
<td>26</td>
<td>2</td>
<td>270</td>
</tr>
<tr>
<td><em>cholerae-suis</em> var</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>kunzendorf</em></td>
<td>3</td>
<td>2</td>
<td>640</td>
</tr>
<tr>
<td><em>typhimurium</em> var</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>copenhagen</em></td>
<td>11</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Reporting efficiency* 12.5%

#### FY 1974

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. Reports Showing Mortality</th>
<th>Number Horses</th>
<th>Average Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>typhimurium</em></td>
<td>87</td>
<td>5</td>
<td>357</td>
</tr>
<tr>
<td><em>dublin</em></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>typhimurium</em> var</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>copenhagen</em></td>
<td>37</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>anatum</em></td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Reporting efficiency* 4.2%

---

*Reporting efficiency is the percentage of the requests for serotyping that included the number in the flock or herd and the number dead.*
TRANSMISSION OF HOG CHOLERA VIRUS BY FLIES:
RECOVERY OF VIRUS FROM FLIES FOLLOWING EXPOSURE
TO INFECTIVE BLOOD

L. D. Miller, D.V.M., Ph.D.
D. R. Downing, B.S.
N. O. Morgan, Ph.D.

In 1919, Dorest et al.² reported successful transmission of hog cholera virus (HCV) by house flies and stable flies. With the inception of the State-Federal Hog Cholera Eradication Program in 1962, interest in the role of insects in transmission of HCV has increased.

Observations made during a hog cholera (HC) epizootic in north central Indiana in 1972 prompted additional inquiry of insect vectors. It was observed that flies disappeared rapidly from premises where infected swine had been depopulated. Immediate questions were: How long would contaminated flies harbor the virus and were they effective vectors of HCV, thereby, contributing to the spread of HC in this epizootic?

This report concerns an investigation of the persistence of HCV in or on house flies (Musca domestica), stable flies (Stomoxys calcitrans) and face flies (Musca autumnalis).

Materials and flies.
Flies — All flies tested were received at the laboratory as pupae.(a) On arrival, the pupae (150-200) were apportioned to observation/maintenance cages (Fig. 1). These were clear, plastic containers measuring 140 mm in depth and 118 to 127 mm in diameter. The open end was fitted with a screened cone for ventilation. For maintenance, house flies and face flies were provided with a dry diet consisting of equal parts of granulated sugar and powdered milk. Stable flies were offered warm heparinized bovine blood on cotton pads twice daily. Water was provided by wetting sponges and placing them on the screens. Sponges were resoaked as necessary.

After exposure to the infective blood meal, separate groups of flies were maintained at 2, 12, 24 and 37°C. Flies maintained at 2, 12 and 37°C were returned to room temperature environment (24°C) each day for 1 to 2 hours at feeding time. The 2, 12 and 37°C environments were provided by a walk-in cooler, a program controlled growth chamber(b), and a dry heat incubator.(c)

a. Supplied by the Chemical and Biophysical Control Laboratory, Beltsville, Maryland.

From Veterinary Services Laboratories (Miller, Downing), Animal and Plant Health Inspection Service, U.S. Department of Agriculture, P.O. Box 70, Ames, Iowa 50010, and the Chemical and Biophysical Control Laboratory (Morgan), Agricultural Environmental Quality Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.

The authors acknowledge Drs. E. A. Carbrey and W. C. Stewart for contributing to the experimental design and Mr. J. F. Beattie for technical assistance.
Exposure of Flies — Weanling pigs were inoculated intramuscularly with 2 ml of blood containing the virulent Ames strain of HCV. The titer of the virus was $10^6$ plaque forming units/0.2 ml of blood. After 5 to 7 days, blood was collected in tubes containing 143 units of sodium heparin(a) to provide the infective blood meal. The blood was poured onto sponges which were placed in a fly cage for 1 to 4 hours.

Virus Isolation from Flies — Thirty to 35 flies were collected from each group at various intervals after removal of the infective blood meal. The number of viable flies remaining in each group dictated the time span for which samples could be obtained. Collections were made while the flies were temporarily immobilized by exposure to CO$_2$. On occasion, the flies were transferred to clean cages before collection of samples.

The flies were ground in 10 ml of Earle's balanced salt solution(b) with Ten Broeck tissue homogenizers.(c) The mixture was centrifuged at 16000 g for 20 minutes and the supernatant fluid decanted. Aliquots (1 ml) of the supernatant fluid were inoculated on monolayer cultures of a procine kidney cell line (PK 15) for virus isolation and identification by the fluorescent antibody cell culture technique (FACCT). Fluids not used immediately after preparation were stored at -70°C.

Selected samples were later inoculated into susceptible pigs to assay for HCV.

Results

The quantity of HCV recovered from each species of fly is recorded in Table 1. Similarities were noted between stable flies and house flies in regard to time of survival and quantity of HCV harbored (Fig. 2,3). Face flies tended to live longer and harbor greater quantities of virus at 2, 12 and 24°C (Fig. 4). At 37°C all face flies were dead within 24 hours.

Four samples of stable flies (2° at 96 hours, 12° at 96 hours, 24° at 96 hours, 37° at 48 hours) and 1 sample of house flies (24° at 72 hours) were toxic to the tissue culture cells and were recorded as "no tests".

A portion of each of 6 samples (stable flies — 2° at 96 hours, 12° at 96 hours, 24° at 48 hours, 37° at 24 hours; house flies — 24° at 48 hours, 37° at 24 hours) were inoculated in HC susceptible pigs. All 6 pigs became acutely ill and developed signs and lesions of HC. Hog cholera virus was isolated by the FACCT from blood specimens collected from the pigs 4 or more days postinoculation.

Discussion

The persistence of HCV at high titer in the face flies was unexpected. In these experiments, the face flies were noticeably less active than either house flies or stable flies. During such periods of inactivity, a longer time would probably be required to clear ingested HCV from the body. The amount of light in the room may have been a factor. Face flies require more light to become active than some other species of flies.

The quantity of HCV available to the flies in the infective blood meal may have been different even though the donor pigs were inoculated with the

---

same strain of virus. Blood from 1 pig was given to house flies and stable
flies and a different donor pig was used in the face fly trial.

The recovery of HCV from flies clearly illustrates their role as potential
vectors and is consistent with other work on insect vectors. Transmission of
HCV from infected to susceptible pigs by house flies, stable flies, face flies, horse
flies and mosquitoes has been reported. In successful trials, the interval between contact with infected pigs and exposure to susceptible pigs has ranged from a few minutes to 48 hours. Another example of a potential vector was illustrated by recovery of HCV from mosquitoes collected on a farm 3 days after all swine were depopulated.

Noteworthy in the present study was survival of HCV for 3 to 7 days in the
flies. During this interval, flies could spread HCV for considerable distances
by natural movements or accidentally by being carried on vehicles such as
autos and trucks.

It is apparent that insect vectors may be a significant factor in the area
spread of HC. Reduction of large insect populations in close contact with
swine is an important consideration in preventing dissemination of HC.

**Summary**

Stable flies, house flies and face flies were allowed to feed on blood from
hog cholera infected pigs. Groups of flies held at 2, 12, 24 and 37°C were
tested for HCV at intervals after removal of the infective blood meal. Stable flies and house flies were found to harbor HCV for at least 72 hours
by the FAACT. Face flies harbored large quantities of HCV through 168
hours.

The results indicate that these species of flies can become vectors of
HCV. Flies may be an important means of area spread of HC.
**TABLE 1.** Titration of Hog Cholera Virus by the Fluorescent Antibody Cell Culture Technique From Flies Fed Infective Swine Blood

<table>
<thead>
<tr>
<th>Holding Temperature</th>
<th>Kind of Fly</th>
<th>Hours Between Removal of Infective Blood Meal and Sample Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>2°C</td>
<td>Stable</td>
<td>4.30&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>House</td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td>Face</td>
<td>4.83</td>
</tr>
<tr>
<td>12°C</td>
<td>Stable</td>
<td>4.25</td>
</tr>
<tr>
<td></td>
<td>House</td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td>Face</td>
<td>4.99</td>
</tr>
<tr>
<td>24°C</td>
<td>Stable</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>House</td>
<td>2.85</td>
</tr>
<tr>
<td></td>
<td>Face</td>
<td>3.08</td>
</tr>
<tr>
<td>37°C</td>
<td>Stable</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>House</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Face</td>
<td>0.0</td>
</tr>
</tbody>
</table>

1/ Expressed as log<sub>10</sub> plaque forming units/ml

2/ No test due to toxic effect of sample on tissue culture cells

3/ No sample; all remaining flies collected at 96 hours
FIG. 1 — Plastic containers with screened inserts used as observation/maintenance cages for flies.

STABLEFLIES: TITRATION OF HCV AFTER INFECTIVE BLOOD MEAL

FIG. 2 — Titration of hog cholera virus by the fluorescent antibody cell culture technique from stable flies fed infective swine blood.
FIG. 3 — Titration of hog cholera virus by the fluorescent antibody cell culture technique from house flies fed infective swine blood.

FIG. 4 — Titration of hog cholera virus by the fluorescent antibody cell culture technique from face flies fed infective swine blood.
REFERENCES


THE STATUS OF THE STATE-FEDERAL HOG CHOLERA ERADICATION PROGRAM

R. E. Thompson, D.V.M.

Introduction

Fiscal year 1974 was a vintage year for the State-Federal Hog Cholera Eradication Program. Following a year of disappointment, many goals were realized and the nation’s swine industry enjoyed the longest period of time without the disease since it first appeared on the continent in the early 1830’s.

Program Status

(Figure 1)

On July 1, 1974, 50 States were in hog cholera “free” status and Puerto Rico was in Phase III.

During FY 1974, the goal of having all States and Puerto Rico in hog cholera “free” status was attained and then lost. Texas progressed to Phase IV in August 1973, the same month that the State of Virginia regained hog cholera “free” status. Indiana, which suffered a severe outbreak during FY 1973, regained “free” status in December 1973.

Starting in January 1974, three States and Puerto Rico celebrated having gone one year without hog cholera by being declared hog cholera free: New Jersey and North Carolina in January, Puerto Rico in March, and Texas on May
This was a red-letter day — all States were considered hog cholera free. This elation was short-lived as Puerto Rico was set back into Phase III on June 5, 1974. Puerto Rico returned to Phase IV during August and barring further cases could again attain “free” status during November 1974.

The net phase progress during FY 1974 was the gaining of five hog cholera free States.

Incidence

Hog cholera incidence decreased dramatically during FY 1974. Only two cases were officially diagnosed during the year compared to 163 during FY 1973. The first case diagnosed occurred in Grenada County, Mississippi, on February 7, 1974, and ended a 225-day period of freedom from the disease (the last previous case had occurred in Indiana in June 1973). The only other case was diagnosed in the Commonwealth of Puerto Rico on May 4, 1974.

The reporting and investigation of sick swine decreased considerably during the past fiscal year in accordance with the decrease of incidence (figure 2). There were 1,506 investigations conducted in FY 1974 compared with 4,183 the previous year. This decrease is comparable with that seen in FY 1972 when the incidence of the disease also decreased.

All of the hog cholera diagnosed during FY 1974 was found in two counties located in one State and Puerto Rico (figure 3). Last fiscal year the disease was reported in 77 counties in 18 States and Puerto Rico.
Epidemiology

A considerable amount of epidemiology was conducted on both of the positive cases without pinpointing a source of the virus. One herd was depopulated as exposed to the Mississippi case and 29 herds were depopulated because of exposure to the Puerto Rico case. Tissues and serum were taken from swine from all infected and exposed herds at the time of depopulation. Hog cholera virus was isolated from three of the 29 exposed herds in Puerto Rico. One of these herds had a history of long-standing chronic illness. A number of very high hog cholera serum neutralization antibody titers were also demonstrated in the animals of the herd. It was surmised that this herd was the probable source of infection of the positively diagnosed case.

The Southeastern Regional Emergency Animal Disease Eradication Organization (READEO) was activated to conduct eradication activities in both Mississippi and Puerto Rico.

The infected herd in Mississippi had received swine from several sources including a livestock auction market in the Western portion of Tennessee. Mississippi immediately placed a quarantine on all counties in the upper one-half of the State. The State of Tennessee also took swift action to prevent possible spread of the disease by placing a stop movement order on all swine in the Western one-third of the State. Tracing revealed no additional infection and the stop movement orders were rescinded. The positive response by Mississippi and Tennessee regulatory officials has been termed highly commendable. If hog cholera had still been present, and the stop movement orders had not been placed, considerable damage could have been inflicted on the swine population of these and other States through movements of their sizeable feeder pig industries.

More than 1,700 farm inspections were conducted by State and Federal regulatory veterinarians in nine States as a result of the case in Mississippi. The Puerto Rican task force conducted approximately 2,000 inspections in addition to almost 200 swine disease investigations.

Surveillance

Reduced numbers of swine disease investigations have made it necessary to place more emphasis on other forms of surveillance.

The last few hog cholera cases diagnosed in the Nation have re-emphasized the need for adequate surveillance for the detection of hog cholera. These cases have been of a nonapparent, insidious nature and were not easily detected. This low virulent type of virus often times does not exhibit classic symptoms and lesions in a swine herd and may not become virulent until it has passed through one or more additional herds. The extended incubation periods evidenced in these cases have made determination of the source of the virus highly speculative.

Veterinary Services and Meat and Poultry Inspection Programs have set up two field trials aimed toward surveillance for the chronic type hog cholera.

Research on chronic hog cholera using low virulent virus has pointed out that chronic carriers can harbor the virus for long periods of time. During intermittent periods these carriers shed virus and show low hog cholera antibody titers. Typical symptoms and lesions are not observed in these animals, but a generalized depletion of lymphoid tissues in the body does take place, which
lowers the animals' resistance and renders them more susceptible to a variety of bacterial and parasitic conditions. It was therefore decided to collect tonsils from all swine condemned for septicemia and toxemia at slaughter and to randomly collect serum samples from slaughter swine on a 3-month trial basis. The collection of tonsils has been continued for an additional 3 months. They are being screened by the fluorescent antibody (FA) test and none have been positive for hog cholera. The serum survey is completed and being evaluated. Inability to trace a large percentage of the slaughter animals back to their source because of lack of identification has been a problem in this surveillance activity.

State laboratories capable of conducting the hog cholera FA test have been encouraged to screen tissues from all swine submitted, for hog cholera. State and Federal veterinarians have been asked to inspect swine herds when they are on farms for other reasons and to help and encourage practicing veterinarians to submit swine tissues from questionable herds to diagnostic laboratories.

Statistics show that we are inspecting a sizeable number of swine in this country each year for health reasons (figure 4). From April 1 through June 30, 1974, Meat and Poultry Inspection Programs performed both ante-mortem and post-mortem inspections on almost 20 million swine. During this period, over 2½ million swine were inspected on over 43,000 farms. These were examined to place or release quarantines for interstate or intrastate movement, for garbage inspection or other reasons. Over three million head were also inspected in markets during the 3-month period. Laboratory screening for hog cholera during this period consisted of running the FA test on swine tissues from 1,209 herds and the serum neutralization test on swine sera from over 5,000 herds. These statistics do not reflect the swine disease surveillance conducted by individual herd owners, practicing veterinarians, county agents, etc.

**Outlook**

Certainly we must stress the need to continue the program activities which have brought us to this point in the program. First, we must continue to encourage the reporting of sick swine and publicize to owners that the hog cholera last seen in the country was not the disease we were accustomed to. It is also important to follow program standards in markets, enforce regulations requiring the proper heat treatment of all garbage fed to swine, and increase border surveillance for hog cholera.

We are justly proud of the record of achievement in this eradication program. Hog cholera, which 10 years ago was the most dreaded swine disease in this country, is now almost nonexistent. Death losses, losses of production and vaccine costs due to the disease have essentially been eliminated. The one case diagnosed within the continental United States during the past 15½ months occurred over 8 months ago. We hope this was the last.

Barring further cases, the country will be officially declared hog cholera free by the time this association meets next year in Portland, Oregon. It is planned that a 3-year surveillance period of decreasing program activity follows this declaration.

Our outlook on the disease has to be one of optimism, however, we cannot be complacent. We still must "suspect hog cholera first" and continue our efforts until the job is complete.
HOG CHOLERA REPORTED
Fiscal Year 1974

Figure 3

HOG CHOLERA SURVEILLANCE
(April - June, 1974)

<table>
<thead>
<tr>
<th></th>
<th>HERDS</th>
<th>SWINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON-THE-FARM INSPECTION</td>
<td>43,690</td>
<td>2,511,689</td>
</tr>
<tr>
<td>MARKET INSPECTION</td>
<td>122,485</td>
<td>3,028,560</td>
</tr>
<tr>
<td>SLAUGHTER INSPECTION</td>
<td>-</td>
<td>19,847,000</td>
</tr>
<tr>
<td>LABORATORY SCREENING:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florescent Antibody (FA) Tests</td>
<td>1,209</td>
<td>34,589</td>
</tr>
<tr>
<td>Serum Neutralization (SN) Tests</td>
<td>5,338</td>
<td>16,805</td>
</tr>
<tr>
<td>TOTAL</td>
<td>172,722</td>
<td>25,438,643</td>
</tr>
</tbody>
</table>

Figure 4
1974 REPORT OF THE USAHA COMMITTEE ON NATIONWIDE ERADICATION OF HOG CHOLERA

Chairman: D. L. Smith, Indianapolis, Ind.
Co-Chairman: J. B. Taylor, Montgomery, Ala.


The Committee reaffirmed the importance of crediting the industry for outstanding achievement in the hog cholera eradication program and in conjunction with this recommends the nation be declared officially hog cholera free 18 months after the last positive case followed by a three year surveillance period.

The committee again recommends that surveillance should be emphasized and increased where possible in the following areas:

1. Surveillance along the US-Mexico border, possibly the most important recommendation.
2. Reporting and swine disease investigation. Increased personal contact with all segments of the industry is again stressed.
3. Screening all swine tissue submitted to State diagnostic laboratories for hog cholera by the fluorescent antibody technique.
4. Adequate enforcement of State laws and regulations pertaining to garbage feeding.

The committee reaffirms its prior recommendations concerning the need for a national swine identification program; the need to re-institute and maintain Phase II market standards as contained in Memorandum 561.1 of July 23, 1971; and designation of all states as either "infected" or "free."

The committee recommends the orderly withdrawal of all live hog cholera virus from the hands of researchers and state diagnostic laboratories by 18 months after the last positive hog cholera case is diagnosed, possibly by this time next year. It is thought that an adequate substitute for live virus, positive control tissues, will be available by that time so that states which wish to continue screening tissues for hog cholera during the three year surveillance period may do so. The committee recommends that the Veterinary Services Laboratory, National Animal Disease Center, Ames, Iowa continue to screen for hog cholera utilizing live positive control tissues during the three year surveillance period. It may also act as a reference center for states no longer wishing to continue to screen tissues. After the surveillance period, it is recommended that all the live virus in the nation be destroyed or shipped out of the country. The Plum Island Animal Disease Center will maintain FA hog cholera diagnostic capability.

The committee recommends that the inspection of garbage feeding premises be continued after the hog cholera eradication program ends in order to prevent
re-infection with hog cholera or other foreign animal diseases through this avenue; or until such time the practice has been converted to an approved garbage dehydration type process.

The committee's last recommendation is that increased information and publicity be disseminated concerning the danger of the introduction and spread of foreign animal diseases by foreign visitors and other persons leaving the country and returning. The dangers from such things as semen and vaccines should be pointed out along with cautions on footwear, clothing, etc. for people having been on animal premises in foreign countries.
EXPERIMENTAL TRANSMISSION OF CONGENITAL TREMORS IN SWINE

D. P. Gustafson, D.V.M., Ph.D.
C. L. Kanitz, D.V.M., Ph.D.
School of Veterinary Medicine
Purdue University
West Lafayette, Ind. 47907

Congenital tremors (CT) in swine have been called myoclonia congenita, shaker pigs, dancing pigs, and by many other names. The disease occurs in baby pigs and, if they survive, tremors may last throughout life in diminished intensity. It has been recognized in many parts of the world. The onset may occur as soon as the baby pig takes over control of its locomotion at birth or may be delayed for as much as four or five days after birth. Baby pigs die within the first week of life, during the most intense phase of the disease. Death appears to be due to starvation because of an inability to suckle effectively while enduring rather violent tremors of the head, trunk, and limbs. We have not observed lateral spread of the disease among baby pigs nor found any reports of them. Except for hypomyelination at the periphery of the spinal cord at all levels, histopathologic changes of the central nervous system or in organs are virtually non-existent. The hypomyelination is not of an order which would impair functions crucial to life during the first few, but vital, days after birth. It has been inconsistently correlated with the severity of the myoclonus observed. Piglets that survive the first four or five days of life essentially recover unless the disease is complicated by tonic muscular spasms and splayed limbs. These few clinical and histopathologic characteristics separate this disease rather definitively from tremors seen in pseudorabies, hog cholera, or enterovirus infections. Confusion with other diseases may occur when the syndrome is not strongly pronounced or may occur when a sublethal infection, one of the viral infections which causes an inflammatory response in the central nervous system, is superimposed on a syndrome earlier recognized as congenital tremors.

The cause of the disease has been thought by some to be due to poor nutrition, hyperthyroidism, muscle or fiber abnormality. The two most popular theories propose an hereditary factor or a transplacental viral infection. There has been considerable thought and effort given to the possible association of CT with hog cholera tremors in swine although cerebellar hypoplasia has not been associated with CT. More recently, it was found that pseudorabies virus (PrV) was present in a pig from each of two litters of three tested, which resulted in the suggestion that PrV may be involved in CT.

In 1964, in conjunction with studies of the pathology of CT, a subculturable cell line (PK-Kessler) was established from the kidneys of a day-old piglet affected with CT. The cell line did not appear to have any unusual cultural or morphological characteristics. It was thought to consist of typical, normal, epithelioid cells. Because of its desirable cultural features, the cell line was used for testing reagents and standardizing a protocol for a fluorescent antibody test for PrV in suspected materials. The inconsistent results of these tests led to the conclusion that some peculiarities existed in the PrV:PK-Kessler cell line.
cell line relationship. A general observation was made that, although some individual cells in the culture were susceptible to infection with PrV and underwent cytopathic changes leading to death, the culture as a whole was highly resistant to infection and did not support massive replication of PrV. Since the cell line was derived from a pig affected with CT, an effort was made to determine the nature of the relationship between the resistance of the cell line and CT. This report is to provide evidence that CT is an infectious transmissible disease caused by a filterable agent and, at present, associated with a small cuboidal virus.

MATERIALS

Cell culture media

The nutrient medium was Eagle's minimum essential medium prepared as a complete, serum supplemented medium containing 100 units/ml of penicillin and 100 mg/ml of streptomycin. It was sterilized by filtration and stored at -30 C. The medium was supplemented with fetal bovine serum at rates of 10, 5, or 2% of the final volume.

Cell lines

1. PK-Kessler. Kidneys were removed from a day-old pure-bred Hampshire pig affected with CT. Primary cell cultures were prepared by trypsinization and subsequently passed many times establishing a cell line. For these studies, four cell lines were started from single cells of PK-Kessler and designed Pk-KC1, PK-KC2, PK-KC3, PK-KC4.

2. Wilcox. The three cell lines were established from a day-old cross-bred baby pig. They were prepared from primary cell cultures of trypsin-dispersed skeletal muscle cells (WSM) and testicle cells (WST). The Wilcox swine muscle)testicle (WSMT) cell line was initiated by mixing the WSM and WST cell suspensions as a third primary cell culture from which a subculturable cell line evolved.

3. Myers. Cell cultures were developed from the kidneys and from the testicles of two crossbred pigs affected with CT. Three serial passages were made of these cultures.

4. CNS-CT 137/3. A cell line was developed from the brain of an affected pig in the litter born to gilt #137.

5. PK-15. A culture of the widely used PK-15 swine kidney cell line was obtained from the National Animal Disease Laboratory at Ames, Iowa.

Viruses

1. Pseudorabies virus. The Sullivan strain, isolated in Carroll County, Indiana in 1963, was used after it had been plaque-cloned in a low passage mouse spleen cell line. After three serial passages of the cloned PrV in mouse cells, the virus was frozen in 2.0 ml aliquots and stored at -30 C. as the stock virus was an average activity in PK-15 cells of about 3x10^7PFU/ml.

2. Vesicular stomatitis virus. The Indiana laboratory strain of vesicular stomatitis virus (VSV) was obtained from the American Type Culture Collection and passed one time in a PK-15 culture. The fluid harvested at 33 hours was diluted with 10 volumes of phosphate buffered saline and filtered prior to storage in 5.0 ml aliquots of frozen and lyophilized virus at -30 C. The reconstituted virus had an infectivity titer of slightly under 10^5 PFU/ml in PK-15 cells.
Swine

1. Pregnant gilts. The gilts were farrowed and raised in isolation facilities. The stock from which they were derived were among the disease evaluated herd on the Veterinary Research Farm and were at least five generations removed. They were bred by related boars of similar background. No evidence of CT had ever been observed in any litter of pigs farrowed from this breeding stock during the six years that the isolated breeding program had been in operation.

2. Weanlings. Weaned pigs about six weeks of age were of the stock farrowed in the isolation facilities.

Inoculums

All inoculums used were cell free fluids of cell cultures. The inoculum for sow #137 was clarified by centrifugation at 1000 x gravity for 15 minutes. All other inoculums were passed through 200 nm filters.

PROCEDURES

Fluorescent antibody tests

1. Pseudorabies. A specimen of pooled serum from several PrV immune swine was used as a source of antibody for the preparation of anti-PrV fluorescent antibody (FA). A crude globulin fraction was separated from the serum by precipitation with concentrated ammonium sulfate [(NH₄)₂SO₄]. Twenty ml were labeled with fluorescein isothiocyanate (FITC) using a procedure comparable to that described by Spendlove. The final preparation was clarified by filtration and stored at -30 C.

2. Hog cholera. The anti-HC FA used was obtained from the National Animal Disease Laboratory at Ames, Iowa.

Electron microscopy

Collodion membrane pseudoreplicas were prepared from cell culture fluids, using a modification of Sharp's technique. Solutions to be examined were placed on the surface of 2% agarose discs, and the fluids allowed to flow into the gel. Particulate matter remaining on the surface was entrapped in a thin film of collodion, which was, subsequently, floated off of the gel surface, stained, mounted, and examined in a Philips, EM-20 electron microscope.

Swine inoculations

All transmission trials were initiated with subcutaneous inoculation of cell-free cell culture fluids.

EXPERIMENTAL OBJECTIVES AND RESULTS

1. Resistance to viral infections by PK-Kessler cells.

Tests using PK-Kessler cultures for various virus assay procedures suggested that this cell line was unusually resistant to infection with several viruses which normally grow well in swine kidney cells. For example, an enterovirus, isolated from a field case of swine polioencephalomyelitis and replicated in PK-15 cells, failed to produce CPE in PK-Kessler cells. In another trial, parallel titrations of two specimens of virulent HCV were performed in PK-15 and PK-Kessler cultures. Infectivity was demonstrated by the FA test 48 hours after inoculation. The PK-Kessler cultures were FA negative at all dilutions and including undiluted material, while PK-15 cultures were FA positive through 10⁻³.
dilution of one specimen and the $10^{-4}$ dilution of the second specimen.

Similar results were obtained when PK-Kessler cells were exposed to PrV in many trials concerned with the replication and assay of PrV in vitro. The inconsistent results of such assays led to the opinion that another agent possibly associated with CT was present in the PK-Kessler cells. The resistance of these cells to PrV was manifested in the following ways:

1. Low susceptibility to infection.
2. Slow rate of viral synthesis.
3. Slow viral spread from cell to cell.
4. Low viral yield.
5. Altered cytopathic effects.
6. Spontaneous abortion of infection.

The susceptibility of some of the cell lines derived from pigs affected with CT to vesicular stomatitis virus (VSV) was tested. This RNA virus has a broad host spectrum, both in vivo and in vitro. It multiplies rapidly in PK-15 cells and is highly cytocidal. In PK-15 cells, VSV produces plaques 2 to 4 mm in diameter within 24 to 48 hours.

Petri dish cultures of PK-15, PK-Kessler, PK-KC¹, and PK-KC² cells were used for a comparative plaque assay titration of VSV. Virus dilutions of $10^{-4}$, $10^{-5}$, and $10^{-6}$ were each inoculated onto four cultures of each cell line. The cultures were overlaid with nutrient agar, incubated 26 hours, and stained with neutral red dye. The average number of plaques in the PK-15 cultures inoculated with the $10^{-5}$ dilution of VSV was 115 per dish. No plaques were detected in any of the PK-Kessler cell lines at that time or after an additional 34 hours of incubation. Even the PK-Kessler cultures inoculated with $10^{-4}$ dilution (1150 potential PFU) failed to show any evidence of infection.

2. Resistance to viral infections by Wilcox CT cell lines.

The cell lines WSM, WST, and WSMT were found to be partially resistant to PrV infection. Although all three cell lines were derived from a single myoclonic pig, they vary in their susceptibility to infection with PrV. When inoculated with the stock virus, cultures of cell lines WSM and WST reacted in a fashion similar to the PK-Kessler cell cultures. Only a small fraction of the total infectious virus in the inoculum was able to initiate infection in the cells. Pathogenesis was characterized by slowly spreading, focal areas of cell degeneration. The WSMT cell line, on the other hand, underwent a more generalized infection characterized by degenerative rounding of cells.

A plaque assay of VSV was applied to WSM and WST cultures. Plaques were only found in the cultures inoculated with the lowest dilution of virus used in the test. In these, out of 3000 potential PFU, only 20 plaques developed in the WST cultures and 3 in the WSM cultures.

3. Fluorescent antibody tests.

The PK-Kessler, WSM, WST, and WSMT cell lines were tested for the presence of PrV and HCV prior to the use of cell free fluids in swine transmission trials. No FA staining was found in the cultures.

4. Transmission of CT to baby pigs through inoculation of pregnant gilts.

Direct evidence of the presence of a virus in the cell lines derived from pigs affected CT was difficult to obtain. However, the chance that one was present seemed reasonably strong because of their resistance to infection with PrV, and VSV.
A. Kessler source. A gilt (#137) was inoculated with 20 ml of fluids harvested from the PK-Kessler cell line 44 days prior to farrowing. Eighteen pigs were farrowed, three were macerated, one was stillborn, and fourteen were living. Four had severe tremors of the head and poor coordination of limb movements — especially of the pelvic limbs. These could not suckle successfully, consequently, two were dead on the morning of their second day and the remaining two were much weaker by the late afternoon. One was killed at that time and primary cell cultures of kidney tissue were prepared, eventually giving rise to cell line PK-CT6190. The remaining shaking pig was killed on the third day, as it was unable to suckle and becoming moribund. There were no significant findings at necropsy.

Of the remaining ten pigs born alive, one died during the first night. Six of the nine, alive on the second day, had varying degrees of tremor and incoordination, especially in the pelvic limbs. They moved with difficulty and seemed to place as much weight as possible on the thoracic limbs. The affected pigs did not grow as well as the more normal littermates. One of the more severely affected pigs was killed at six days of age. There were no significant findings at necropsy. Trypsin-dispersed cells from the cerebrum and brainstem were used to initiate cell culture line, CNS-CT 1137/3. Fluids from the first passage were used to inoculate a pregnant gilt (#24) at approximately the 35th day of gestation. Fourteen pigs were farrowed 80 days later. Nine of these were killed by the gilt within hours after birth and two were found dead on the second day. Two of the three remaining had muscle tremors and were incoordinated. This was the first experimental subpassage of CT in swine.

Sow #137 was rebred and farrowed 25 pigs; fourteen alive and one dead. The pigs were normal at birth; however, the sow did not take care of them and all were lost in the first three days of life.

Another gilt (#143) was inoculated with the same fluids as #137 and farrowed 12 pigs 78 days later. These were not as severely affected as those of #137. After killing two of the pigs, she accepted ten. Several of these showed pelvic limb incoordination and hypertonicity of the musculature.

Two gilts were inoculated with fluids from PK-KC1 cell lines. One (#227) farrowed twelve living pigs 42 days afterwards. These had mild head tremors and over half of the litter had pelvic limb incoordination and muscle tremors of varying intensities. The second gilt (#228), inoculated with fluids from the 25th cell culture passage, farrowed sixteen pigs 92 days later. One pig was killed at birth and three other were either killed at birth or born dead. Of the remaining twelve, one was found dead on each of the second and third mornings. All of the living pigs had some pelvic limb incoordination and most showed muscle tremors.

B. Wilcox source. A gilt was inoculated with 5.0 ml of WST cell culture fluids 14 hours before farrowing six live pigs. At birth, the pigs all appeared to be normal, but by the fifth day, all were affected. The pigs had muscular tremors, incoordination, and a strong inclination to bear weight on the thoracic limbs. At nineteen days, the coordination had improved but an altered mincing gait remained in all.

C. Myers source. A gilt was inoculated with 2.0 ml of a mixture of fluids from the kidney and testicle cell culture. Eight days later twelve pigs were farrowed,
one was dead and one pig was killed after one day for cell culture production. The remaining nine pigs were all affected with mild head shaking and muscular tremors of the body and limbs. They had a propensity to sit like dogs apparently to avoid bearing weight on the pelvic limbs. All were weaned at six weeks. The clinical signs had diminished noticeably.

None of the pregnant gilts in these experiments showed any clinical effects of the inoculations. Body functions, appetite, demeanor, and body temperature remained unchanged and normal during the experiments.

5. Attempted transmission to weanling pigs.

Six weanling pigs were used to test their susceptibility to CT. Four were each inoculated with 5.0 ml of fluids from the PK-Kessler cell line and two were maintained as contact controls. No clinical reaction to the inoculation was observed in a three month observation period.


A preparation from the PK-KC² cell line which had been exposed to PrV was examined. The cell line had become a carrier of PrV. PrV particles were readily distinguished in the uranyl acetate preparation. They were seen as naked nucleocapsids, slightly over 100 nm in diameter with densely stained cores. The envelopes were, for the most part, removed in the preparation process or remained only as small fragments or tags of material on the surface of the capsid. In addition to the PrV particles, other small virus particles were observed. They were generally found in small clusters; most of them were observed in phosphotungstate preparations. Although the very small size of these particles (ca. 20 nm) rendered the resolution of structural detail somewhat difficult, regularly arranged capsomere could be seen.


The details of the examination of six baby pigs and five mature swine affected with CT from the Kessler case as compared with controls have been presented elsewhere. In general terms, there was a mild reaction in both vascular and central nervous system (CNS) tissues. There is no apparent constant change in a specific region of the CNS that might explain the tremor. In the mature swine (about 2 years of age), there were vascular inflammatory changes suggestive of a virus infection or possibly an immunopathologic response. However, these changes are considered with caution because of the time involved.

Findings in the CNS of baby pigs in experimental transmissions with Kessler-origin materials were consistent with those found in the naturally occurring disease. There was virtually no reaction in the cerebrum, cerebellum, brain stem, or spinal ganglia of those examined.

Tissues from the Myers case were examined and no significant findings were recorded for brain, heart, liver, or lungs of baby pigs.

DISCUSSION

The resistance of the PK-Kessler, WSM, WST, and WSMT cell lines to PrV and VSV viruses led to the speculation that these cell lines, derived from pigs affected with CT were infected with a non-cytopathogenic virus which was the etiological agent of CT in swine. It was theorized that the virus present in the cell lines came from the tissues of the pigs affected with CT. This suggested that it would have been an in utero infection stemming from the sow. While the in vitro
Evidence was suggestive of the presence of an adventitious virus, the most important evidence that such was the case came through swine inoculation trials. The credibility of the proposal was substantiated when the inoculation of pregnant gilts with fluids from the Kessler, Wilcox, and Myers cell lines resulted in the birth of pigs affected with CT. That the agent was distinct from PrV and HCV was substantiated by negative FA tests for PrV and HCV. The validity of the transmission studies are supported by the conditions under which they were conducted and the number of trials, including a serial passage through cell culture fluids obtained from a culture derived from an experimentally infected pig. Transmissions were achieved using fluids from cell cultures derived from kidneys, muscle, testicle, and brain indicating a rather wide distribution of the virus in the tissues of baby pigs. The character of the experimentally induced disease, preserved in motion pictures, was that of the natural disease in nearly every respect. It was observed that splayed limbs seemed to be a significant expression of the disease and that muscular spasms vary widely from severe tremors to very mild tremors easily missed on casual observation.

A small virus (ca. 20 nm diameter) of cuboidal symmetry was found in fluids from PK-KC² cell cultures which had become a PrV carrier cell line during experiments concerning its resistance to PrV. On the basis that the small virus originated from a latent infection of the PK-Kessler cell line and the successful transmission studies in pregnant gilts, it is tentatively proposed to be the etiological agent of CT in swine.

Hypomelinization has been the only microscopic change observed with sufficient consistency to be considered a lesion of the disease. It is so inconstant and variable that the degree of hypomyelinization does not appear to be related to the severity of the clinical disease. The disease is noninflammatory and lateral spread among baby pigs does not appear to occur or be significant if it does. Epizootiological evidence among mature animals suggests that lateral spread occurs readily. It is common to find that early in an outbreak, the baby pigs are severely affected while later farrowings during the period result in milder disease or normal pigs. Furthermore, sows or gilts having affected litters rarely, if ever, have diseased pigs again.

The CT virus, rather than being cytocidal in vivo or in vitro, may effect enzyme systems necessary in myelinization. The frequency of the tremors seem to be too great for the effects to be centered in the cerebellum. It is more likely that viral effects are centered in motor areas of the cerebrum. The fact that some individuals are noticeably affected with tremors for life and have retarded rate of growth suggests a potential latent infection or a persistent biochemical lesion.

**CONCLUSIONS**

1. Congenital tremors of swine is an acute, infectious, noninflammatory, neuropathy of neonatal swine, with life-long disability manifested by mild tremors and/or diminished growth rate.

2. The etiological agent is tentatively identified as a virus of cuboidal symmetry about 20 nm in diameter.

3. The infectious agent is non-cytotoxic in cell cultures.

4. Mature animals are not clinically affected by inoculation with the virus; however, pregnant individuals give birth to diseased pigs. In one trial, six-week-
old weanling pigs did not react clinically to inoculation with infectious cell culture fluids, tentatively suggesting that the age resistance factor in CT is exceptionally significant.

5. Splayed or spraddled legs are a common expression of the disease and may be a more frequent sign than the violent head shaking tremors. Therefore, it is likely that the disease has a much higher incidence than has been believed.

REFERENCES

PSEUDORABIES IN INDIANA: CURRENT STATUS, LABORATORY CONFIRMATION, AND EPIZOOTIOLOGIC CONSIDERATIONS

Charles L. Kanitz, D.V.M., Ph.D.*
Roy B. Hand, D.V.M., M.P.V.M.**
Sara M. McCrocklin, B.S.***

INTRODUCTION

Pseudorabies virus (PrV) infections usually cause an acute and fatal disease of wild and domestic animals — with the exception of swine in which the disease is often mild or subclinical. Natural infections have been reported in swine, cattle, sheep, dogs, cats, and at least seven genera of wild animals. Experimental infection with PrV has been successful in nearly all warm-blooded animals in which it has been tried.

In the United States, early reports of pseudorabies in swine indicated that it was a subclinical disease except in suckling pigs. In 1962 pseudorabies occurred as a serious disease affecting swine of all ages on several farms in northwestern Indiana. Since that time virulent infections of swine have been recognized in areas scattered across the country. The growing importance of pseudorabies is arousing an increasing concern by swine producers and practitioners over means to control or prevent the disease.

In Indiana, the Animal Disease Laboratory (ADDL), Purdue University, received few requests for laboratory confirmation of PrV infections of swine during 1972 and 1973, the first 2 years diagnostic virology services were being offered. Starting in January of 1974, however, an increasing number of specimens from animals with a clinical diagnosis of pseudorabies were submitted to the laboratory. Most of the specimens in which PrV infections were confirmed came from an intense swine producing area located in and adjacent to Carroll County, Indiana.

This report concerns: 1) a summary of known PrV infections in Indiana during the first 9 months of 1974, 2) a presentation of laboratory procedures used at the Purdue-ADDL for diagnosis and survey studies of PrV infections, and 3) a summary of preliminary findings of an epizootiologic study in progress in Indiana.

MATERIALS AND METHODS

Viruses. A laboratory strain of virulent PrV, designated PrV-KC152D, was used as a reference virus for serum antibody determinations. A field isolate, designated PrV-P8251, was used to infect animals used as positive controls in the wildlife studies. This virus was isolated in BT/5705 cell cultures from a tonsil.

*Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana 47907.
**USDA-APHIS-VS, (Regional Hog Cholera Epidemiologist, Northern) Wytheville, Virginia 24382.
***Department of Forestry and Conservation, Purdue University, West Lafayette, Indiana 47907.
submitted to the Purdue)ADDL through the “Dead-Pig-Pick-Up” hog cholera surveillance program. It had been passed 1 time in PK-15 cells to prepare a stock of virus.

Cell Cultures and Media. Eagle’s minimum essential medium with non-essential amino acids in Earle’s salt solution containing 10mM. NcHCO(3) and 15mM. HEPES* (MEM) or a highly modified MEM³ containing 0.25% lactalbumin hydrolysate and 15mM. HEPES (KSM) were used as basic media for cell cultures. They were supplemented with fetal bovine serum at a 10% level for use as growth media, and at a 2% level for use as maintenance media. Gentamycin was added to all media at a level of 50 ug/ml.

A bovine turbinate cell line, BT/5705**, was used for virus isolation and serum neutralization studies. A bovine leukocyte cell line, BBC/5705**, was also used for virus isolation studies.

Fluorescent Antibody (PrV-FA). A crude globulin fraction was prepared from a pool of serums from PrV-immune swine. Separation was accomplished by precipitation with 1.77M ammonium sulfate at 4° C. The globulin was labeled with fluorescein isothiocyanate using a procedure comparable to that described by Spendlove.8.

Tissue Specimens. During the first 3½ months of 1974 only tissues submitted to the Purdue-ADDL with a tentative diagnosis of pseudorabies were examined for PrV. In addition to swine, these submissions included tissues from dogs and cats which had died or were killed in the vicinity of infected swine herds. By the middle of April it was apparent that most of the outbreaks were occurring in and around Carroll County, Indiana. At that time it was decided to screen for pseudorabies the swine tonsils which were being submitted from the Carroll County area through a State-Federal cooperation “Dead-Pig-Pick-Up” hog cholera (HC) surveillance program. In this program tissues from all eartagged swine, dying at any unit of a large multiple farm swine feeding corporation, were delivered to the Purdue-ADDL. At Purdue these tissues were examined for HC virus and cultured for bacterial pathogens.

Since the beginning of May, 1974, all swine tonsils submitted to the Purdue-ADDL for any reason have been examined for PrV infection.

Serums. Serum samples for antibody survey studies were obtained at slaughter from packing plants in Indiana and Ohio. They were collected in groups and identified by the state of origin of the swine — Indiana, Illinois or Ohio. The serums were stored frozen for varying periods of time until they could be tested.

Procedure for Fluorescent Antibody Tissue Section (FATS) Test. Tissues were trimmed and frozen on cryostat specimen holders using a carbowax embedding medium***. Sections 8 mu thick were cut in a cryostat at -20° C., picked up on glass slides, and allowed to dry at room temperature. The sections were fixed in acetone at room temperature for 10 minutes, drained, dipped in PBS containing 3% Tween-80**** and allowed to air dry. PrV-FA was placed on

---

*N-2 Hydroxyethylpiperazine-N'-ethanesulfonic acid.
**Cell lines started at Purdue ADDL.
****Difco Laboratories, Detroit, Michigan 48232.
the sections in a moist chamber (waterbath) at 37° C. for 30 minutes. At the end of the incubation period the slides were washed for 10 minutes in PBS, rinsed briefly in distilled water and air dried. After coverslipping with PBS-glycerine (1:1, pH 8), the sections were examined for specific immunofluorescence.

Procedure for Virus Isolation Tests. Tissues were homogenized in MEM without serum in laboratory tissue blenders for 2 minutes. The homogenates were centrifuged at 3000 X g for 15 minutes and the supernatant fluids filtered through 0.45 mu membrane filters.* The filtrates were inoculated onto Leighton tube coverslip cultures of BT/5705 and BBC/5705 cells, 0.2 ml. per tube. The cultures were examined daily for the appearance of cytopathic effects (CPE). PrV infection was confirmed by removing coverslips from the tubes and staining them with PrV-FA. Staining procedures used for coverslip cultures were essentially the same as those used for the FATS tests.

Procedure for Neutralization Tests. Serum neutralization tests were performed in a microtiter system** in the following manner.

Serums were diluted 1:2 in PBS and inactivated at 60° C. for 20 minutes. Tissue culture medium, KSM with 10% fetal bovine serum, was used as a diluent for serum dilutions and for virus and cell suspensions. Two-fold serum dilutions were prepared in triplicate in 96-well, flat bottom microtiter plates in 0.025 ml. volumes. A 0.025 ml. drop of virus suspension, containing 100-1000 50% tissue culture infective doses, was added to each well of 2 of the 3 serum dilution series to give a final serum dilution scheme of 1:4 to 1:32. An equal drop of diluent was added to each well of the third series which served as a serum toxicity control. The plates were covered and incubated at room temperature for 1 hour. After the incubation period a 0.050 ml. drop of BT/5705 cell suspension was added to each well and the plates sealed with adhesive film. The plates were incubated at 37° C. and examined for CPE after 2 or 3 days. Serum antibody titers were recorded as the highest dilution of serum completely inhibiting CPE.

Approach to Wildlife Surveillance Studies. In order to study the possible role of indigenous wildlife in herd to herd spread of PrV during epizootics of the disease, one of the authors (S.M.M.) set about establishing a program of trapping and testing various species found on and around swine producing premises.

Preliminary trapping experience was gained by catching animals on an isolated Purdue Wildlife Area. The animals trapped in this area were used as positive and negative controls to develop protocols for handling the animals and to determine the tissues of choice for testing in future surveillance studies. All animals were live-trapped and brought to the laboratory in the traps. They were either killed immediately in a carbon dioxide chamber or placed in semi-isolation facilities for inoculation. The positive control animals were inoculated orally with ca. 10⁸ tissue culture infective doses of PrV-P8251.

Following inoculation they were observed periodically for appearance of clinical signs of pseudorabies. Animals which developed the disease were allowed to die without interference.

Selected tissue specimens were collected from animals which were killed or

**Linbro Chemical Co., New Haven, Connecticut.
which died following infection with PrV. These specimens were stored frozen until they could be examined by the FATS test. Cell culture virus isolation trials have also been conducted on tissues from positive control animals.

An initial survey has been started with animals trapped on 2 Carroll County swine breeding establishments. One of these had experienced an outbreak of pseudorabies early in 1974. The other had pseudorabies in the swine herd at the time trapping was intiated.

Serums from some of the animals trapped in Carroll County and from the animals surviving exposure to PrV were tested for neutralizing antibody.

**RESULTS**

*Fluorescent Antibody Tissue Section Tests.* It was found that tonsil is the tissue of choice for diagnosis of PrV infections in swine by the FATS test. In specimens submitted from herds experiencing acute outbreaks of the disease, specific fluorescence was detected in most of the brains examined, but not in all sections. Small foci of fluorescent cells were also seen in some kidney sections. Extensive areas of specific fluorescence were seen in the tonsils of all confirmed infections of swine.

The nature of the areas of fluorescence in tonsils from acute swine differed from those seen in the positive tonsils detected in swine from the HC surveillance program. In the acute tonsils lesions were often extensive and involved all cell types at all levels of the tonsil from the base to the surface epithelium. In the surveillance program tonsils, where signs of acute pseudorabies had not been reported, fluorescence was usually confined to necrotic cell debris in the lumen, and epithelial cells surrounding the base of the tonsillar crypts.

In dogs and cats specific fluorescence was detected only in the upper thoracic and cervical spinal cord, and in the lower midbrain, medulla and cerebellum.

PrV infections have been confirmed in 55 accessions submitted to the Purdue-ADDL during the first 9 months of 1974. A monthly breakdown of these accessions and the numbers and types of animals found positive is presented in Table 1. A quarterly summary of the total number of swine screened for PrV infection and the number found positive is presented in Table 2. Figure 1 illustrates the geographic distribution of pseudorabies in Indiana.

All of the tissues examined from the HC surveillance program were from swine identified by ear tags. Of the 38 PrV positive tonsils, the states represented on the ear tags were: Tennessee — 21, Kentucky — 6, Missouri — 6, Arkansas — 3, and Alabama — 2.

*Virus Isolation Tests.* Three classes of specimens were subjected to virus isolation tests. These were: 1) tissues from clinically suspicious pseudorabies submissions which were found to be negative by the FATS test, 2) tonsils from the HC surveillance program which were positive for PrV by the FATS test, and 3) tissues from the positive control wildlife surveillance project animals.

PrV was not isolated from any tissues of clinically suspicious pseudorabies submissions which had been found negative by the FATS test. Twelve of the FATS positive tonsils from HC surveillance swine have been tested to date. PrV was isolated from 5 of these. Virus isolation trials have been completed on tissues from 2 of the positive control wildlife project animals (1 raccoon and 1 opossum). PrV was isolated from 6 of 21 FATS positive tissues, and from none of 10 FATS
negative tissues.

Neutralizing Antibody Survey. Of 3336 serums collected from packing plant swine, 2262 have been tested for PrV neutralizing antibody. A total of 101 of these were too cell toxic to test and 184 were not identified as to the state of origin. These are not included in the results shown in Table 3. The 69 positive serums had antibody titers ranging from 1:4 to 1:32, with 2 serums having titers of 1:64.

Wildife Surveillance Studies. A program for wildlife surveillance has been devised and tested. Skills have been gained in trapping and protocols for handling and testing the animals are established.

The numbers and species of animals examined for PrV using the FATS test are presented in Table 4. All tissues tested were FATS negative except certain tissues from the animals which died following oral inoculation with PrV (Table 5). Not all of the inoculated animals developed pseudorabies. Raccoons appeared to be most susceptible to infection with the virus. All 3 inoculated animals died on the 3rd day post inoculation (PI). One died without exhibiting detectable signs of disease. The other 2 showed various signs of central nervous system disturbance. Both inoculated opossums also developed clinical pseudorabies, but they did not die until the 5th and 7th days PI. Only 1 of 3 inoculated rats developed the disease. It died 4 days PI. Neither of the inoculated woodchucks developed pseudorabies.

Neutralizing antibody was not detected in serums from any of the animals which survived oral inoculation with PrV.

DISCUSSION

The FATS test for pseudorabies was found to be a more sensitive diagnostic procedure than attempting to isolate virus from PrV suspicious tissues. High titers of infectious virus are not always produced in infected tissues and may be lost due to post mortem autolysis. The swine tonsils obtained through the HC surveillance program were considered to be somewhat better than average field specimens. Of the 12 FATS positive tonsils which were tested for viable virus, only 5 yielded virus — less than 50%. Virus isolation attempts were even less successful with the more carefully handled wildlife project tissues. Virus was isolated from only 35% of the FATS positive tissues. It would seem that many PrV infections would not be detected in the laboratory if diagnosis depended on the isolation of viable virus. This would be especially true of specimens which were not handled properly or were from species other than swine.

The microtiter system was found to lend itself well to measurement of serum antibody levels in the large number of samples tested in this study. The only technical problem encountered was the sensitivity of the BT/5705 cells to toxic factors in some of the serums.

Since the original report of virulent pseudorabies in Indiana swine in 1962, sporadic outbreaks of the disease have occurred in several areas of the state. To the knowledge of the authors, none reached the epizootic proportions of the outbreak occurring during the first 6 months in 1974. The data presented in this report includes only those infections which were confirmed in the laboratory. It is known, however, that pseudorabies was diagnosed on the basis of clinical signs...
on several other farms in the epizootic area. Drs. John Bush and Lawrence Stauffer, practitioners in Carroll County, reported* a total of 35 outbreaks of pseudorabies within their areas of practice between 1 January 1974 and 30 April 1974. Thirty-three of these were in Carroll County and 2 in neighboring Cass County. During the same period of time only 12 outbreaks in Carroll County were confirmed in the laboratory.

The geographic distribution of the field diagnosed pseudorabies outbreaks in Carroll County appear to be associated with natural drainage systems (Figure 2). Since it is known that wild animals often travel along such drainage systems, some speculation has arisen as to the possible role of wildlife in herd to herd spread of pseudorabies. To add to this speculation is the fact that during the winter months, the time of occurrence of the recent epizootic, wild animals are found in close association with swine herds as they inhabit farm buildings seeking refuge from severe weather and limited natural food sources.

While it is known that most animals are susceptible to infection with PrV, evidence is lacking concerning their possible role in disseminating the virus. It is hoped that the wildlife surveillance program established in this study will provide such evidence in the event an anticipated epizootic of pseudorabies reoccurs in Carroll County with the advent of colder weather.

In studying the epizootiology of pseudorabies in the Carroll County one must consider the fact that there are a large number of continuous-production swine feeding operations dispersed among many closed breeder or breeder-feeder herds. It was the closed herd type of operation that experienced most of the outbreaks of virulent pseudorabies during the recent epizootic. While increased losses due to pseudorabies were not reported from the continuous production operations, the presence of PrV in these herds was confirmed by screening tonsils submitted through the HC surveillance program.

At the present it is thought that pseudorabies remains enzootic in Carroll County by the continual influx of feeder pigs from at least 10 states**, as indicated by ear tag identification of swine tested in the HC surveillance program. When conditions are appropriate infections occur in closed, presumably highly susceptible breeder herds and death losses ensue. The means by which virus is transmitted from herd to herd is not yet known. If wildlife is in fact involved, raccoons and oppossums are likely suspects. Both of these omnivorous species were found to be quite susceptible to infection with PrV by oral inoculation. Both species also move about freely and are not adverse to close cohabitation with man and domestic animals.

The intent of the survey of PrV neutralizing antibodies in serums from packing plant swine was to compare the incidence of exposed swine in Indiana with that of adjacent states. Although it was found that the percentages of positive serums from Indiana and Illinois were comparable, the percentage of positive serums from Ohio swine was disproportionately higher. This disparity is believed to be a result of sampling inadequacies. Since many of the serums collected in Ohio were cell toxic they could not be tested. The total number of serums tested was, therefore, too low. Also, the relatively large number of positive swine were found mainly in a group from a single source.

*Personal communication.
**Tenn., Kan., Ark., La., Ind., Ala., Wis., Miss., I..
The data presented in this report provides evidence that pseudorabies is a disease of increasing concern to the swine production industry. While the need for a control and prevention program exists, it is believed that additional information on the actual incidence of PrV infections in all segments of the swine population should be accumulated prior to initiating such a program. This information could be gathered through serologic surveys and more widespread use of the FATS test.

Table 1

Number of Pseudorabies Infections Confirmed in Indiana Between January 1 and September 30, 1974

<table>
<thead>
<tr>
<th>Month</th>
<th>Accessions</th>
<th>Swine</th>
<th>Others*</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>3</td>
<td>2</td>
<td>2-C, 1-F</td>
</tr>
<tr>
<td>February</td>
<td>4</td>
<td>5</td>
<td>1-C</td>
</tr>
<tr>
<td>March</td>
<td>7</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>April</td>
<td>18 (14)**</td>
<td>20 (18)</td>
<td>2-C</td>
</tr>
<tr>
<td>May</td>
<td>10 (9)</td>
<td>13 (12)</td>
<td>-</td>
</tr>
<tr>
<td>June</td>
<td>4 (3)</td>
<td>4 (3)</td>
<td>-</td>
</tr>
<tr>
<td>July</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>August</td>
<td>3 (1)</td>
<td>5 (1)</td>
<td>-</td>
</tr>
<tr>
<td>September</td>
<td>2 (2)</td>
<td>4 (4)</td>
<td>-</td>
</tr>
<tr>
<td>Totals</td>
<td>55 (31)</td>
<td>62 (38)</td>
<td>6</td>
</tr>
</tbody>
</table>

* C = Canine, F = Feline

** Figures in parentheses indicate the number of positives from the surveillance program swine.
### Table 2

Results of Fluorescent Antibody Tissue Section Tests of Swine During the First Three Quarters of 1974

<table>
<thead>
<tr>
<th>Quarter</th>
<th>Number Tested</th>
<th>Number Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>47</td>
<td>14</td>
</tr>
<tr>
<td>2nd</td>
<td>236</td>
<td>37</td>
</tr>
<tr>
<td>3rd</td>
<td>222</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>532</td>
<td>62</td>
</tr>
</tbody>
</table>

### Table 3

Incidence of Neutralizing Antibodies to Pseudorabies Virus in Serums From Packing Plant Swine (Listed by State of Origin)

<table>
<thead>
<tr>
<th>State</th>
<th>Number Tested</th>
<th>Number Positive</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indiana</td>
<td>1254</td>
<td>18</td>
<td>1.4</td>
</tr>
<tr>
<td>Illinois</td>
<td>924</td>
<td>12</td>
<td>1.3</td>
</tr>
<tr>
<td>Ohio</td>
<td>199</td>
<td>39</td>
<td>19.6</td>
</tr>
<tr>
<td>Total</td>
<td>2377</td>
<td>69</td>
<td>2.9</td>
</tr>
</tbody>
</table>
Table 4

Wild Animals Examined by Fluorescent Antibody Tissue Section Test for Pseudorabies Virus

<table>
<thead>
<tr>
<th>Species</th>
<th>Control Animals</th>
<th>Survey Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trapped from Purdue</td>
<td>Trapped in Carroll Co., Ind.</td>
</tr>
<tr>
<td></td>
<td>Wildlife Area</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uninoculated</td>
<td>Inoculated</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Control Animals</th>
<th>Survey Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raccoon</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Opossum</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Woodchuck</td>
<td>2</td>
<td>1*</td>
</tr>
<tr>
<td>Rat</td>
<td>1</td>
<td>2*</td>
</tr>
<tr>
<td>Wild Dog</td>
<td>-</td>
<td>2**</td>
</tr>
</tbody>
</table>

* The woodchuck and 1 rat did not develop pseudorabies following inoculation.

** The wild dogs were shot out of an indigenous pack by a cooperative swine producer.
Table 5

Tissues Found Positive in Fluorescent Antibody Tissue Section Tests of Pseudorabies Inoculated Wild Animals

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Raccoons</th>
<th>Opossums</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrum</td>
<td>3**</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Medulla</td>
<td>3</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tonsil</td>
<td>3</td>
<td>2</td>
<td>ND</td>
</tr>
<tr>
<td>Salivary Gland</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mandibular Node</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Adrenal Gland</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

* Number of animals tested

** Number of animals positive, - = none positive, ND = not tested.
Figure 1
Geographic Distribution of Pseudorabies Virus Infections
Confirmed Between January 1 and September 30, 1974

(P = Porcine, c = Canine, F = Feline)
Figure 2
Geographic Distribution of Field Diagnosed Pseudorabies in Carroll County, Indiana (Jan.-Apr., 1974)
(Black dots indicate infected premises)
ACKNOWLEDGEMENTS

The authors wish to thank the following practicing veterinarians for their cooperation in this study: Dr. John A. Bush, Flora, Indiana and Dr. Lawrence Stauffer, Delphi, Indiana.

We also thank Dr. Hussein I. Ibrahim for his assistance in performance of the fluorescent antibody tests.

REFERENCES

RECOVERY AND CHARACTERIZATION OF A CORONAVIRUS FROM MILITARY DOGS WITH DIARRHEA

D. L. Huxsoll, D.V.M., Ph.D., R. H. Marchwicki, B.S., and
A. J. Strano***, M.D.

Division of Veterinary Medicine
Walter Reed Army Institute of Research
and the Armed Forces Institute of Pathology
Washington, D.C. 20012

INTRODUCTION

Coronaviruses are important agents of respiratory and gastrointestinal diseases of poultry, swine, and man.1 Experimental infections and serological surveys have provided evidence that dogs may play a role in the epizootiology of the swine coronavirus, transmissible gastroenteritis virus (TGE).5,6,8,9 In 1972, Cartwright and Lucas4 reported that TGE antibody titers had significantly increased in the sera of dogs after an outbreak of vomiting and diarrhea. As the virus was not isolated, it was concluded that TGE or a serologically related virus produced disease in these dogs.

During February and March of 1970 an epizootic of diarrheal disease occurred in 60 of 63 dogs at the USAF Patrol Dog Training School in Weisbaden, Germany. Attempts in Germany to determine the responsible agent were unsuccessful and a presumptive diagnosis of "viral gastroenteritis" was made.7 In January 1971, a reoccurrence of the diarrheal disease occurred and specimens were sent to the Walter Reed Army Institute of Research (WRAIR) for virus studies.

This report summarizes studies of a coronavirus, designated 1-71, recovered from these dogs and its relationship to TGE virus of swine.

MATERIALS AND METHODS

Specimens. Fecal specimens were collected from 3 of 5 dogs with gastrointestinal disease. Convalescent sera were obtained from blood specimens collected approximately 1 month after onset.

Cell Cultures. Primary dog kidney (PDK) cells and the Walter Reed canine cell (WRCC) line were prepared and maintained as previously described.2 Canine thymus (CT) 8156 cells were obtained from the Naval Biomedical Research Laboratory (NBRL), Oakland, CA and the canine synovium DEN *Deceased.

**Present Address: Department of Veterinary Microbiology, School of Veterinary Medicine, University of California, Davis, Ca. 95616.

***Present Address: St. John's Hospital Laboratory, Springfield, Ill. 62701.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences — National Research Council.
cells were obtained from the Bureau of Biologics (BOB), FDA, Bethesda, Md. The remaining cell cultures were obtained from commercial sources (Flow Laboratories, Inc., Rockville, Md., or HEM Research, Inc., Rockville, Md).

**Virus Recovery and Characterization.** The procedures used for virus isolation, purification, and chemical and physical characterization have been previously described, except as noted. Inocula for cell cultures contained 50 μg of DEAE-dextran* per ml. A virulent TGE strain and cell culture adapted TGE strain were obtained from Dr. M. Ristic, University of Illinois, Urbana, Ill.

**Electron Microscopy.** Control and infected cell cultures for electron microscopic examination were washed with Hanks balanced salt solution, and then fixed in 1.5% gluteraldehyde in phosphate buffer, pH 7.2. Specimens were post fixed in 1% osmium tetroxide, dehydrated in increasingly graded alcohol solutions, and embedded in Epon 812.

**Experimental Infections.** Pregnant beagles, free of neutralizing antibodies against TGE virus and the 1-71 canine isolate, were obtained from Dr. Earl, Food and Drug Administration Laboratories, Beltsville, Md. Swine that were free of neutralizing antibodies to TGE virus and the canine virus isolate were obtained from the Department of Laboratory Animal Medicine, Division of Veterinary Medicine, WRAIR. The pregnant dogs and pigs were kept in isolation and allowed to give birth and nurse their offspring. At 3 or 5 days of age the dogs and pigs were fed the virus preparation or uninfected cell culture media. In addition, 4 pigs, 2.5 months of age, were given the virus by the oral-nasal route and 2 pigs were inoculated by the intravenous route. The challenge doses of virus was determined by titration in PDK cell cultures.

Following inoculation the dogs and pigs were examined daily for clinical signs of infection. Throat and rectal swab specimens for virus isolation were taken each day for 10 days post inoculation from the dogs and rectal swab specimens obtained from the pigs. Blood for serologic tests was obtained from the pregnant animals and 2.5-month-old swine prior to inoculation, and from all the animals 3 weeks post exposure.

Three weeks after exposure of the baby pigs to 1-71 virus, the pigs were fed 10,000 infectious dose(50) of the TGE virus. Twenty days after TGE virus challenge the surviving pigs were bled for antibody determinations.

**Coronavirus Antiserums.** Antiserums against established coronaviruses were obtained from the sources listed in Table I. Neutralization tests with the canine isolate were done in PDK cells and TGE virus in primary swine kidney cell cultures as previously described.

**RESULTS**

A transmissible agent was recovered from one of 3 fecal specimens in PDK and CT cell cultures. The agent, designated 1-71, was first detected in PDK cells 6 days after inoculation. On subculture in PDK, cytopathic effects (CPE) were evident on the second day. Infected cell cultures contained enlarged, bizarre shaped cells, with distinct cytoplasmic membranes, which subsequently detached from the glass surface. Multinucleated giant cells were evident in infected cells stained with hematoxylin and eosin. Many of the giant cells contained condensed

---

*Molecular weight 2 million obtained from Pharmacia, Uppsala, Sweden
basophilic “materials” without organized nuclei. Viral cytoplasmic and nuclear inclusion bodies were not evident in infected cells. In PDK cell cultures, the extent of viral CPE was somewhat variable and the addition of DEAE-dextran to the inocula increased the CPE and viral titer. The infected cell cultures did not hemadsorb guinea pig erythrocytes.

The 1-71 agent was reisolated in PDK cells from the original fecal specimen. A serum neutralizing antibody titer of 1:4 was present in the convalescent serum of the dog from whom the agent was obtained. Sera from 3 of 4 other convalescent dogs also had 1:4 to 1:16 neutralizing antibody titers.

The isolate produced CPE only in dog cell cultures (Table 2). Cytopathic effects were not evident in pig, bovine, feline, human, monkey or WRCC cultures. The 1-71 agent was not pathogenic for suckling or weanling mice.

Studies on the chemical and physical properties of the virus are summarized (Table 3). Chloroform treatment markedly reduced the infectivity of the virus. At pH 3.0, the virus was stable for 3 hours at room temperature. The effect of 5-iodo-2-deoxyuridine (IUDR) on the growth of the virus was measured to determine the type of viral nucleic acid. The IUDR markedly inhibited the growth of the infectious canine hepatitis (ICH) virus, the reference DNA virus, but had no effect on SV5, the reference RNA virus, or the 1-71 isolate. To estimate the agent’s size, the viral preparation was passed through a series of graded pore size membrane filters. The 1-71 agent readily passed through the HA (450 nm) and GS (220 nm) filters. A 100-fold reduction in virus titer occurred when the virus was passed through a VC (100 nm) filter, and the virus did not pass through a VM (50 nm) filter. On the basis of these chemical and physical studies the 1-71 isolate appears to be a RNA enveloped virus slightly smaller than 100 nm.

Ultrathin sections of infected German shepherd embryo cell cultures were examined in the electron microscope. Forty-eight hours after inoculation enveloped virus particles were seen in the cytoplasm of infected cells. Virions were not evident in uninfected cultures. The virions were round or oval and ranged from 59 to 90 nm in diameter, with an inner core of 35 to 45 nm (Fig. 1). The virus appeared to form by budding into cytoplasmic vacuoles, similar to coronaviruses. These findings indicate that the 1-71 isolate is a member of the coronavirus group.

Serum neutralization tests were performed to determine the relationship of the 1-71 virus to other coronaviruses. Antisera against the CT and MA strains of infectious bronchitis virus, mouse hepatitis virus, rat coronavirus, calf coronavirus, human 229E virus, and hemagglutinating encephalomyelitis virus of swine did not neutralize the 1-71 agent. Swine anti-TGE sera, however, neutralized the 1-71 isolate at one-sixteenth or less than the homologous titer.

Studies on the pathogenicity of the 1-71 agent for puppies and swine are summarized (Table 4). Three or 5 days after birth, all but 1 or 2 pups in each of 2 litters were fed 1-71 virus. The remaining pups became infected by contact. Three to 5 days after feeding, the pups of the younger litter had clinical signs of acute gastroenteritis and dehydration. The signs persisted for 5 to 6 days. The puppy infected by contact also developed signs of diarrhea which occurred on days 4 and 5. The agent was recovered from fecal swab specimens from each puppy during an 8 to 9 day period but only on 1 day from throat swab specimens of 2 puppies. Presence of the virus in the feces occurred within 48 hours of onset of
clinical signs. The response of the second litter was similar to the first, except the clinical signs were milder. Three weeks after feeding, all the puppies had low levels of 1-71 neutralizing antibody and the majority had similar titers to TGE virus. The bitches had an inapparent infection, as they developed antibody without overt signs of disease.

In contrast, 5-day-old and 2.5-month-old pigs were resistant to 1-71 virus infection. Following inoculation, clinical signs were not evident, the virus could not be recovered, and neutralizing antibody was not present in the 21 day post inoculation serums. The baby pigs were not protected against virulent TGE virus challenge 3 weeks after feeding of 1-71 virus. Three days after TGE virus challenge all 6 pigs had signs of acute gastroenteritis, manifested by vomiting, diarrhea, weight loss, and anorexia. On the sixth day one of the pigs died and 2 other pigs were killed for histopathological examination. One of the 2 pigs had histopathological lesions compatible with TGE virus infection. Twenty days after TGE virus challenge, each of the 3 remaining pigs had developed TGE virus neutralization antibody. The titers ranged from 1:2 to 1:32, but antibody to the 1-71 canine isolate was not found.

DISCUSSION AND SUMMARY

A previously unrecognized canine virus was recovered from the feces of a military dog with diarrheal disease. Reisolation of the agent and the presence of neutralizing antibody in the convalescent serums of affected dogs provided further evidence of the canine origin of the isolate.

The recovered virus had many properties in common with the coronaviruses which were evident in electron micrographs of ultrathin sections of infected cell cultures. With the exception of TGE virus antiserum, the 1-71 isolate was not neutralized by antiserum against other coronaviruses. Hyperimmune anti-TGE virus swine serum neutralized the 1-71 agent, but at a significantly lower titer than the homologous TGE virus. Puppies fed 1-71 virus developed similar antibody titers to 1-71 and TGE viruses; therefore, the presence of TGE virus neutralizing antibody in dogs may result from infection with either virus. The resistance of pigs to 1-71 virus infection clearly distinguishes this virus from TGE virus of swine. Further studies are required to define the antigenic relations between these two viruses.

Puppies fed 1-71 virus developed a diarrheal disease. The virus could be readily recovered from their feces and antibody developed. These findings indicate that 1-71 virus can produce a gastrointestinal disease in young puppies. Further studies will be required to determine the pathogenicity of this virus for older dogs and the extent that similar agents are responsible for canine viral gastrointestinal disease.
TABLE 1. SOURCE OF CORONAVIRUS ANTISERUMS

<table>
<thead>
<tr>
<th>Viral antiserum to: (prepared in)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious bronchitis</td>
<td></td>
</tr>
<tr>
<td>CT strain (avian)</td>
<td>Spafas, Inc., Norwich, CT</td>
</tr>
<tr>
<td>NA strain (avian)</td>
<td>Spafas, Inc., Norwich, CT</td>
</tr>
<tr>
<td>Polyvalent mouse hepatitis (mouse)</td>
<td>Microbiological Associates, Inc., Bethesda, MD</td>
</tr>
<tr>
<td>Transmissible gastroenteritis of swine (swine)</td>
<td>Dr. M. Ristic, U. of Illinois, Urbana, IL</td>
</tr>
<tr>
<td>Hemagglutinating encephalitis (swine)</td>
<td>Dr. G. Lambert, National Animal Disease Laboratory (NADL), Ames, IA</td>
</tr>
<tr>
<td>Rat coronavirus (rat)</td>
<td>Dr. J. Parker, Microbiological Associates, Inc., Bethesda, MD</td>
</tr>
<tr>
<td>Human coronavirus 229E (guinea pig)</td>
<td>Dr. A. Z. Kapikian, National Institute of Health, Bethesda, MD</td>
</tr>
<tr>
<td>Calf coronavirus (calf)</td>
<td>Dr. Kemeny, NADL, Ames, IA</td>
</tr>
<tr>
<td></td>
<td>Norden Laboratories, Lincoln, NB</td>
</tr>
</tbody>
</table>

TABLE 2. CYTOPATHIC EFFECTS BY THE 1-71 CANINE VIRUS ISOLATE IN CELL CULTURES

<table>
<thead>
<tr>
<th>Cell culture (source)</th>
<th>Cytopathic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary dog kidney (WRAIR)</td>
<td>+</td>
</tr>
<tr>
<td>Canine thymus 8156 (NBRL)</td>
<td>+</td>
</tr>
<tr>
<td>German shepherd embryo (commercial)</td>
<td>+</td>
</tr>
<tr>
<td>Canine synovium DEN (BOB)</td>
<td>+</td>
</tr>
<tr>
<td>Walter Reed canine cell line (WRAIR)</td>
<td>0</td>
</tr>
<tr>
<td>Primary swine kidney (commercial)</td>
<td>0</td>
</tr>
<tr>
<td>Pig Kidney PK15 (commercial)</td>
<td>0</td>
</tr>
<tr>
<td>Primary bovine embryonic kidney (commercial)</td>
<td>0</td>
</tr>
<tr>
<td>Primary feline kidney (commercial)</td>
<td>0</td>
</tr>
<tr>
<td>Human embryonic lung WI-38 (commercial)</td>
<td>0</td>
</tr>
<tr>
<td>Primary rhesus monkey kidney (commercial)</td>
<td>0</td>
</tr>
<tr>
<td>Primary african green monkey kidney (commercial)</td>
<td>0</td>
</tr>
<tr>
<td>African green monkey kidney, Vero (commercial)</td>
<td>0</td>
</tr>
</tbody>
</table>
### TABLE 3. CHEMICAL AND PHYSICAL PROPERTIES OF THE 1-71 CANINE VIRUS ISOLATE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Virus (strain)</th>
<th>Not treated (A)</th>
<th>Treated (B)</th>
<th>Change (A-B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>1-71</td>
<td>5.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>ICH (Cornell)</td>
<td>5.8</td>
<td>6.3</td>
<td>+0.5</td>
</tr>
<tr>
<td></td>
<td>Canine herpes (D004)</td>
<td>4.3</td>
<td>&lt;1.0</td>
<td>&gt;3.3</td>
</tr>
<tr>
<td>pH 3.0</td>
<td>1-71</td>
<td>5.8</td>
<td>5.3</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>ICH (Cornell)</td>
<td>5.3</td>
<td>5.4</td>
<td>+0.1</td>
</tr>
<tr>
<td></td>
<td>Canine herpes (D004)</td>
<td>4.3</td>
<td>&lt;2.0</td>
<td>&gt;2.3</td>
</tr>
<tr>
<td>IUDR (10⁻³.₅M)</td>
<td>1-71</td>
<td>4.2</td>
<td>4.6</td>
<td>+0.4</td>
</tr>
<tr>
<td></td>
<td>ICH (Cornell)</td>
<td>5.3</td>
<td>1.8</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>SV5 (3X84)</td>
<td>6.2</td>
<td>6.3</td>
<td>+0.1</td>
</tr>
<tr>
<td>Filtration HA*</td>
<td>1-71</td>
<td>6.5</td>
<td>6.3</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>GS</td>
<td></td>
<td>5.5</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>VC</td>
<td></td>
<td>4.5</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>VM</td>
<td></td>
<td>&lt;1.0</td>
<td>&gt;5.5</td>
</tr>
</tbody>
</table>

*Millipore Filter Corp., Bedford, MA
**TCID₅₀ = Tissue culture infectious dose 50**

---

### TABLE 4. EXPERIMENTAL INOCULATION OF PUPPIES AND SWINE WITH THE 1-71 CANINE VIRUS ISOLATE

<table>
<thead>
<tr>
<th>Animal (Age)</th>
<th>Route of Inoculation</th>
<th>Dose of Virus (TCID₅₀)*</th>
<th>No. of Animals</th>
<th>No. Animals with Diarrhea</th>
<th>No. Animals with Neutralizing Antibodies</th>
<th>No. Animals with Neutralizing Antibodies in Serum</th>
<th>Throat</th>
<th>Rectum</th>
<th>1/11</th>
<th>J/11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog (3 days)</td>
<td>Oral</td>
<td>30,000</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1/3</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td>Contact**</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog (5 days)</td>
<td>Oral</td>
<td>2,000,000</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Contact</td>
<td>-</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig (5 days)</td>
<td>Oral</td>
<td>250,000</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig (2.5 mos)</td>
<td>Oral-Nasal</td>
<td>25,000</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>25,000</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Tissue culture infectious dose 50
**Same cage or pen.
Fig. 1. Forty-eight hours post 1-71 infection of German shepherd embryo cell cultures (X50,000). Arrows indicate the location of budding forms.
ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of the many investigators who generously made antiserums and cell cultures available. We also express our thanks to Dr. Earl of the Food and Drug Administration Laboratories at Beltsville, Md., for providing the pregnant beagles and to Dr. Ristic of the University of Illinois for the virulent and tissue culture adapted TGE viruses and anti-TGE serums. The canine thymus 8156 cells were provided by contract E-73-2001-NO1 within the Special Virus-Cancer Program, NIH, PHS, through the courtesy of Dr. Nelson-Rees of the Naval Biomedical Research Laboratory, Oakland, Ca.

REFERENCES

TRANSMISSIBLE DISEASES OF SWINE COMMITTEE

Chairman: E. A. Butler, Des Moines, Iowa
Co-Chairman: R. D. Ledgerwood, West Plaines, Mo.

There is probably no other species that is affected with so many different diseases and so it is impossible in the course of two afternoons to consider all the transmissible diseases of swine. Therefore, there were discussions on selected diseases, those that have been particularly troublesome to the swine industry either this year or throughout the last several years.

There were a great many outbreaks of pseudorabies in swine in the Midwest during the past year.

Dr. C. L. Kanitz of Purdue finds that the outbreak in Indiana tended to follow along drainage ditches. He also suspects that wildlife of the area are involved as reservoirs or carriers. Laboratory inoculation of oppossums, raccoons and rats have produced the onset of the disease and subsequent death. There is a need for further serologic surveys.

Dr. John Kluge of Iowa State in discussing the pseudorabies outbreaks in Iowa concur with the need for further serologic studies. He discussed the effect of the disease on reproduction. Experimental infection of pregnant gilts produced fetal reabsorption, macerated fetuses, mummified fetuses and abortion.

He has obtained some K varient vaccine from Hungary which product he is using experimentally. This product is used routinely in large swine operations in Hungary and Ireland.

Dr. Robert Glock of Iowa State gave a very interesting report on Bloody Dysentery of swine. A great deal of progress has been made. A spirochaete seems to be the primary organism involved, however, that does not preclude the involvement of other organisms playing a secondary role.

There are many questions. What can be done to eliminate the disease from our swine herds? Is the quarantine system as used in Wisconsin and Iowa feasible? Carrier swine cannot at present be detected. It is doubtful that a good vaccine can be developed.

Drugs showing great promise in the treatment of the disease have not been cleared by FDA. They are, however, being used commonly. The Committee urges the FDA to speed up their work on these products.

Because of the tremendous financial loss to the swine industry the National Pig Dealers Association asked that an eradication program be undertaken at this time for Bloody Dysentery. Discussion by those in attendance questioned the feasibility of such a program at this time.

Dr. Mengeling reported on reproductive diseases of swine associated with parvo virus infection. Pregnant sows have been experimentally infected through

367
the uterus. When the fetuses in one horn are infected they die and become mummified or macerated while those in the other horn are produced as normal pigs.

Dr. L. N. Binn, Phillip Sprino and Dr. Robert Parks gave information on work accomplished relative to TGE. This information is attached as a TGE Subcommittee report.

Dr. D. P. Gustafson showed some slides and films on congenital tremors in swine.

The Committee realizes that there are many other diseases that deserve attention of this Committee.

The Committee reiterates its position of last year on swine identification. Identification of all swine is most desirable for disease eradication purposes.

Respectfully submitted,

E. A. Butler
Chairman

REPORT OF THE TRANSMISSIBLE GASTROENTERITIS SUBCOMMITTEE

Recent research has indicated that the TGE coronavirus can be isolated from the lungs and respiratory tract of clinically normal swine. The lungs can act as reservoirs of TGE virus infection and this helps to explain some of the season to season carry-over on some premises. Intermittent shedding of the virus in nasal secretions can spread infection to susceptible swine.

The paper presented at this meeting on the effect of the route of vaccine administration in production of immunity to TGE indicates that better protection is afforded by the intranasal route of vaccine administration rather than by the intramuscular route. This study agrees with earlier published data on the enhanced levels of immunoglobulin A detected in the colostrum of orally and intranasally vaccinated sows as compared to intramuscularly vaccinated sows. It also confirms the importance of immunoglobulin A in the colostrum in protecting baby pigs from challenge with virulent TGE virus. Levels of immunoglobulin G predominate in the colostrum of intramuscularly vaccinated sows and this immunoglobulin appears to be least protective for baby pigs. The Subcommittee urges research to determine ways of further enhancing levels of protective immunoglobulins in the colostrum of TGE vaccinated sows.

The report of a Coronavirus isolated from an outbreak of gastroenteritis in military dogs which is serologically related to the TGE virus may help to explain the high incidence of TGE neutralizing antibodies in dogs in this country.

The Subcommittee has noted a significant increase in the efficacy of TGE virus isolation from infected tissues by use of cell monolayers aged 5 to 9 days as opposed to use of younger cell monolayers. They recommend routine use of the older monolayers for diagnostic purposes.

The Subcommittee also recommends research on the pathogenesis of enzootic TGE in piglets aged 2 to 3 weeks. Additional research is needed to determine the significance of viral neutralizing antibody titers and their
relationship to either a carrier status or an immune status.

G. Lambert, Chairman
M. Ristic
E. H. Bohl
T. W. Tamoglia
B. C. Easterday
E. O. Hoelterman
CURRENT STUDIES ON HYDATID DISEASE IN UTAH

by
Ferron L. Andersen, Ph.D.1
James R. Everett, M.D.2
Alan G. Barbour, M.D.3
F. James Schoenfeld, D.V.M.4

BACKGROUND

Hydatid disease is a zoonosis (Fig. 1) which occurs in limited foci throughout the world. Since dogs and sheep are the predominant hosts in the pastoral cycle, endemic conditions involving humans generally exist only where sheep raising is a major industry. The adult tapeworms which occur in the small intestine of the carnivore host (dogs, wolves, coyotes) are approximately 4 to 6 mm in length when mature (Fig. 2). Embryonated eggs containing 6-hooked oncospheres develop in the terminal proglottid approximately 40 days post infection of the dog or related carnivore (Smyth, 1969). The eggs are microscopic (30-40 μ) and may be contained in intact terminal proglottids passed with the feces or may be free in the fecal sample if the proglottid has been broken or disintegrated during passage.

In order for the life cycle to continue, the eggs must be ingested by a suitable intermediate host, ordinarily an herbivore, which ingests the eggs while grazing on vegetation contaminated with feces passed from an infected carnivore host. After ingestion, the eggs hatch in the duodenum where the escaping oncospheres penetrate the intestinal wall, enter the mesenteric venules and become lodged generally in the liver or lungs. A wall forms around the entrapped oncosphere and a small cyst-like structure begins to develop. Daughter cysts and brood capsules within the cyst asexually produce numerous invaginated larval stages termed protoscolices (Fig. 3), which are the infective stage for the definitive or carnivore host. Fluid accumulates inside the hydatid cyst as it grows and the structure appears as a “watery blister” (Fig. 4), which is readily recognizable at necropsy. The word “hydatid” is taken from the Greek “hydatis”, meaning watery vesicle. During the development of the hydatid cyst many of the protoscolices (100-160 μ) are liberated into the fluid and feel like sand particles when rubbed between the thumb and finger tips; hence the term “hydatid sand” for the contents of such a cyst. If the infected visceral organs are consumed by a member of the Canidae family, the protoscolices evaginate inside the gut and attach to the mucosal lining of the small intestine. The worm begins to segment in approximately 2 weeks and the second and third proglottids appear soon afterward.

Humans become accidentally infected by handling parasitized dogs or by

1Department of Zoology, Brigham Young University, Provo, Utah.
2Former EIS Officer, Center for Disease Control, U.S. Public Health, located in the Utah State Division of Health. Currently at Boston V.A. Hospital.
3EIS Officer, Center for Disease Control, U.S. Public Health, located in the Utah State Division of Health.
4State Veterinarian, Department of Agriculture. Salt Lake City, Utah.
This study was supported in part by P.H.S. grant, AI-10588, and by the Utah State Division of Health, Dr. Lyman J. Olsen, Director.
ingesting food or water contaminated with canine feces containing the infective ova. Presumably the development of hydatid cysts in humans is similar to that in herbivore hosts. Most cysts develop in either the liver (75%) or the lungs (15%), but may develop in nearly any organ of the body (10%) including the brain or in the long bones.

CURRENT STUDIES IN UTAH

Several aspects of the disease in Utah are being investigated through the combined efforts of personnel from the Utah State Division of Health, National Center for Disease Control at Atlanta, Utah State Department of Agriculture, and Department of Zoology, Brigham Young University at provo.

Humans:

In order to determine the prevalence of asymptomatic hydatid disease in humans where either infected dogs or parasitized sheep are located, a series of human clinics are being held throughout central Utah. All persons attending the clinic over approximately 5 years of age are injected intradermally (in the volar surface of the forearm) with 0.05 ml of antigen prepared by Dr. Irving G. Kagan (CDC). The reaction is examined 15 minutes later and the ink-outlined border of the wheal is imprinted on an alcohol-impregnated paper for a permanent record. The reaction is designated as positive if the area of the wheal equals or exceeds 1.2cm$^2$, or appears twice the size of a control injected simultaneously in the opposite arm. A positive 15-minute reaction for this skin test is shown in Fig. 5. In addition, blood is drawn and sent to the Parasitology Section at CDC, Atlanta, for serological determination of *Echinococcus* antibodies by the indirect hemagglutination (titer $\geq 1:128$ considered positive and bentonite flocculation (titer $\geq 1:5$ considered positive) tests.

After the results from both the skin tests and the serological tests are analyzed, individuals suspected of having hydatid cysts are referred to the University of Utah Medical Center at Salt Lake City for complete physical examination, chest x-rays and radioisotope liver scans. If confirmed there, surgery is scheduled as soon as mutually convenient for the patient and the hospital staff. Figure 6 shows an x-ray of an hydatid cyst in the left lower lobe of a human prior to surgical removal of the cyst, and Figure 7 shows a histological section of a cyst removed from the lung of an infected individual.

Table 1 gives the combined results to date for all cases identified through screening clinics only. In addition, records have also been maintained for all surgical cases which have come to our attention in the state since the initiation of the present program. In 1970, a review of the records of 12 hospitals within central Utah identified 41 cases of hydatid disease which had occurred since 1944, of which 17 could be classified as having originated within the state (Spruance et al., 1974). Since that time 6 possible cases have been identified through the hydatid clinics which have been held (3 of which have now been confirmed through surgery), and 9 other cases have occurred at hospitals either within Utah Valley or Salt Lake Valley, all of which would be considered autochthonous. This makes a total of 56 cases of hydatid disease which have been diagnosed within the state in the last 30 years, with at least 32 (57%) considered to have originated within Utah. Data for 1974 alone give an annual rate of 7.3 per 100,000 for Sanpete county specifically, and rank that area with other highly
endemic areas of the world. Spruance et al., (1974) recently reported on the endemicity of hydatid disease in Utah and summarized many factors which have contributed to the spread and distribution of the disease within the state. It is of interest to note that the exact time of the introduction of hydatid tapeworms to this area is not known, but Dr. Albert W. Grundmann, Professor of Zoology, University of Utah, Salt Lake City, believes that tapeworms could have been introduced in approximately 1938 when a large sheep farm in the state imported sheep dogs from Australia. The disease has been a serious problem in that part of the world for a long time (Schantz and Schwabe, 1969) and his theory fits well with the timing of the first known surgical case recorded for Utah in 1944.

Dogs:
Since 1970, 435 dogs have been examined at field clinics held in central Utah for *E. granulosus* tapeworms. Of this total, 67 (15.4%) have been found infected. Table 2 shows the total number of dogs examined in 3 different counties since the present program started, and the number and percent positive at each community. In Sanpete County alone, 359 dogs have been checked, of which 65 (18.1%) harbored this parasite. Table 3 shows the gradual average decline in infected dogs seen in that county during the past 4 years, and hopefully suggests that dog owners in that area are instigating recommended control measures.

At the time of the clinics, letters are sent to sheep owners in the vicinity and appropriate announcements are also placed in local newspapers. In the past, civic and church leaders have also given support to the clinics and encouraged attendance for all concerned. Owners are instructed to withhold food from their dogs on the day of the clinic and to bring the dog on a leash so that it can be tied separately during the examination. A questionnaire is filled out by each dog owner in order to furnish information on the degree of sheep raising engaged in by the family. The dogs are given 1 ml of 1.5% arecoline hydrobromide orally per 10 lbs. of body weight. The time is recorded for each purging and a second (and rarely third) purge is given when deemed feasible by the attendant veterinarian. A beef bouillon cube is dissolved into each 100 ml of purging fluid in order to enhance palatability of the agent. In case of reaction to the arecoline hydrobromide, atropine sulfate is injected subcutaneously as an antidote. After 5 to 10 minutes the dogs invariably vomit (Fig. 8), followed within 15 to 20 minutes by the purge. Generally the initial purged material is not examined, since the final mucous material passed has proven to be the best portion in which to detect the small tapeworms. The purge also removes other tapeworms found in this geographic region (principally *Taenia pisiformis* contacted by the dogs having eaten jackrabbits abundant in this area, and *T. krabbei* contacted from having eaten deer meat fed to them after the annual fall hunting season). The clear mucous material is carefully scraped from the ground with a tongue depressor (Fig. 9), placed in a disposable sample cup, and labeled for examination. A portion of the collected mucus is transferred to a large 150 mm disposable petri dish, mixed with a few ml of tap water and examined for *Echinococcus* microscopically against a black background and microscopically under a dissecting microscope at 7 to 30 X (Fig. 10). Any dogs found to be infected are given tablets of bunamidine hydrochloride at 50 mg/kg of body weight. When
feasible, a second treatment is given within one month in order to remove worms that may have been in the immature stages at the time of the first treatment. Persons attending the dog clinics are shown pictures and samples of the hydatid cysts, and given instructions in the basic aspects of the most common transfer route of the tapeworms to dogs with recommendations as to how they might improve their method of disposal of dead sheep in the future. Fig. 11 depicts a typical field clinic.

**Sheep:**

During the past 4 years a weekly surveillance for hydatid cysts has been conducted at 4 abattoirs within central Utah. Through arrangements with the state meat inspectors at those sites, any visceral organs containing suspect cysts are refrigerated and retained at the abattoir until collected. The material is taken to the parasitology laboratory at Brigham Young University where it is subsequently examined. All cysts which prove to be hydatid are categorized as either fertile (containing viable protoscolices) or sterile. To date, of 5,631 sheep over approximately 1 year of age which have been killed at these abattoirs, 687 (12.2%) have harbored hydatid cysts (Table 4). Of that number of samples 85% have been fertile and 15% sterile. Some of the protoscolices removed from fertile cysts have been used to experimentally infect dogs for laboratory controlled chemotherapeutic trials. Other protoscolices have been used in experiments to determine the ability of these larval stages to survive after exposure either as freed protoscolices or within intact cysts to a wide range of storage temperatures. Of significance to this report has been the finding that protoscolices in intact cysts die immediately if frozen or if subjected to an upper extreme of 50°C, but survive up to 2 days at 40°C, 4 days at 30°C, 8 days at 20°C, and 16 days at 1°C or 10°C. These data show the possible survival of the infective larval stages under field conditions, and the potential for transfer of the parasite to stray or roving carnivores when sheep carcasses or viscera are improperly discarded (Fig. 12).

A metal pit cover similar to that widely used in Sanpete County for disposal of dead turkeys (Fig. 13) has been proposed for the disposal of sheep carcasses. However, it is not likely that sheep which die on the range would be taken to the community animal pit, so such a device is far from the total solution to the disposal problem.

**Control measures implemented or planned:**

Personnel from our combined project are meeting periodically with officials in communities in central Utah where hydatid disease is most prevalent in an attempt to develop a control program feasible for that region. Control measures now in progress or planned for the future include:

1. Additional field clinics for the examination of dogs for hydatid tapeworms in order to continually monitor the trend of cooperation by animal owners.
2. Treatment of all dogs in the region twice each year with bunamidine hydrochloride.
3. Continued surveillance for hydatid cysts in sheep slaughtered at the local abattoirs.

4. Encouragement of all ranchers to not feed the viscera of slaughtered mutton to their dogs.
5. Fencing or covering of animal pits to prevent stray or roving dogs from eating discarded sheep carcasses.
6. Additional screening clinics for possible detection of asymptomatic cases of hydatid disease in humans from communities where infected sheep or parasitized dogs are identified.

DISCUSSION

Thus far, a major effort has been made in time and expense in screening human beings to detect hydatid disease and determine its epidemiology in Utah (Klock et al., 1973). However, on a cost-effective basis, the main thrust in the future should definitely be directed towards the identification of *E. granulosus* in sheep and dogs, with emphasis on breaking the cycle of transmission. Hydatid disease has been eradicated from Iceland (Dungal, 1957), and is being brought under effective control in such areas as New Zealand, the Australia state of Tasmania (Schantz and Schwabe, 1969), and the Island of Cypress (Kagan, 1974). In these areas, important measures which have been implemented have included: (1) preparation and dissemination of instructional brochures to the general populace, (2) registration, examination, and treatment of all dogs maintained in the region, (3) eradication of stray or roving dogs, and (4) legislative action or community cooperation which discouraged feeding raw sheep viscera to dogs. In central Utah we feel these same measures will be needed to effectively control and hopefully eradicate hydatid disease. At the present time leash laws in some of the small rural communities are either nonexistent or not enforced. Community officials, however, are currently reviewing regulative policy concerning these matters and may embark on an enforcement program in the future if deemed advisable.

A final important factor which must eventually be elucidated before a feasible control program can be designed relates to the possibility of a reservoir cycle of *Echinococcus* in wild hosts such as deer and coyotes in central Utah. If foci for such a sylvatic cycle are eventually found here, as has been determined for California (Brunetti and Rosen, 1970; Liu et al., 1970; and Romano et al., 1974), the next step would be to determine the possibility of cross transmission between the sylvatic reservoir cycle to the pastoral cycle in sheep and dogs. In Utah, sheep and deer frequently graze on the same rangelands and coyotes are important predators of both domestic and wild ruminants in this region (Wagner, 1972). Irrespective of whether or not cross transmission between cycles could occur, the existence of hydatid tapeworm in coyotes would pose a health hazard to federal or state trappers employed in predator control programs.

To date, infections in coyotes or deer in Utah have not been seen, but this does not preclude the possibility of such a finding in the future.
TABLE 1
SCREENING TESTS FOR HUMAN HYDATID DISEASE IN CENTRAL UTAH, 1969-1974

<table>
<thead>
<tr>
<th>Test</th>
<th>+/-total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin Test</td>
<td>17/1479</td>
</tr>
<tr>
<td>Serology*</td>
<td>12/1209</td>
</tr>
<tr>
<td>Chest X-ray</td>
<td>0/589</td>
</tr>
<tr>
<td>Liver Scan</td>
<td>6/20**</td>
</tr>
</tbody>
</table>

*Bentonite flocculation and indirect hemagglutination.
**Three cases confirmed at surgery, 1 awaiting surgery, 2 being followed.

TABLE 2
PREVALENCE OF ECHINOCOCCUS GRANULOSUS IN DOGS EXAMINED IN THREE UTAH COUNTIES, 1971-1974

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Dogs Examined</th>
<th>Number Infected with E. granulosus (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt Lake County</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herriman</td>
<td>55</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>Utah County</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benjamin</td>
<td>21</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>Sanpete County</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fountain Green</td>
<td>96</td>
<td>23 (24.0%)</td>
</tr>
<tr>
<td>Mt. Pleasant</td>
<td>83</td>
<td>11 (13.3%)</td>
</tr>
<tr>
<td>Fairview</td>
<td>48</td>
<td>12 (25.0%)</td>
</tr>
<tr>
<td>Moroni</td>
<td>68</td>
<td>6 (8.8%)</td>
</tr>
<tr>
<td>Spring City</td>
<td>64</td>
<td>13 (20.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>435</td>
<td>67 (15.4%)</td>
</tr>
</tbody>
</table>
### TABLE 3

**PREVALENCE OF ECHINOCOCCUS GRANULOSUS IN DOGS EXAMINED IN SANPETE COUNTY 1971-1974**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fountain Green</td>
<td>14/44 (32%)</td>
<td>3/13 (23%)</td>
<td>6/39 (15%)</td>
<td></td>
</tr>
<tr>
<td>Mt. Pleasant</td>
<td>6/26 (23%)</td>
<td>5/57 (9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fairview</td>
<td>8/24 (33%)</td>
<td></td>
<td>4/24 (17%)</td>
<td></td>
</tr>
<tr>
<td>Moroni</td>
<td></td>
<td>4/24 (17%)</td>
<td>2/44 (5%)</td>
<td></td>
</tr>
<tr>
<td>Spring City</td>
<td>13/64 (20%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 4

**PREVALENCE OF HYDATID CYSTS IN SHEEP KILLED IN FOUR UTAH ABATTOIRS, 1971-1974**

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Sheep Killed</th>
<th>Percent with Hydatid Cysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ephraim</td>
<td>1071</td>
<td>2.1</td>
</tr>
<tr>
<td>Nephi</td>
<td>383</td>
<td>13.1</td>
</tr>
<tr>
<td>Spanish Fork</td>
<td>1893</td>
<td>20.0</td>
</tr>
<tr>
<td>Springville</td>
<td>2284</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>5631</strong></td>
<td><strong>12.2</strong></td>
</tr>
</tbody>
</table>
CURRENT STUDIES ON HYDATID DISEASE

DEFINITIVE (carnivore) HOSTS

PASTORAL and SYLVATIC
LIFE CYCLES OF
Echinococcus granulosus

INTERMEDIATE (usually herbivore) HOSTS

Humans may ingest eggs accidentally and develop hydatid cysts

Pastoral and sylvatic life cycles of Echinococcus granulosus.

Fig. 1.
Figure 2. Adult tapeworm of *Echinococcus granulosus*.

Figure 3. Invaginated protoscolices from an hydatid cyst.
Figure 4. Sheep liver heavily infected with hydatid cysts.

Figure 5. A positive skin test reaction.
Figure 6. X-ray showing a cyst in the lower left lobe.

Figure 7. Histological section of an hydatid cyst showing germinal lining and cross section of protoscolices.
Figure 8. Initial vomiting reaction following administration of arecoline hydrobromide.

Figure 9. Mucous portion of purge collected for examination of *Echinococcus granulosus*. 
Figure 10. Examination of collected purged material for *Echinococcus granulosus*.

Figure 11. Field clinic examination of dogs for *Echinococcus granulosus*. 
Figure 12. Unfenced animal pit where stray or roving dogs may feed on discarded carcasses.

Figure 13. Type of metal cover proposed for animal pits.
ACKNOWLEDGEMENT

The authors appreciate the valuable assistance of:

Mr. Raymond M. Loveless, Research Associate, Department of Zoology, BYU.

Dr. Robert K. Hedelius, Federal Veterinarian, Sanpete Co., Utah.

Dr. M. John Ramsay, Resident Veterinarian, Sanpete Co., Utah.

Drs. S. L. Spruance and L. E. Klock, former EIS Officers assigned to Utah State Division of Health.

Dr. T. Fukushima, Director, Bureau of Disease Prevention, Utah State Division of Health.

Dr. I. G. Kagan, Chief, Parasitology Branch, Center for Disease Control, Atlanta.

REFERENCES


Essentially, all research on foot rot in sheep during the past decade has been done in the major sheep producing countries of Australia and New Zealand. Significant progress in understanding the etiology, pathogenesis and prevention of this perplexing disease has been made. A review of the current status of this research is particularly appropriate since the progress of these investigators has been reported in the New Zealand, Australian and British scientific journals which are not all routinely read by American veterinarians and livestock men.

PATHOLOGY OF OVINE FOOT ROT

A clear understanding of the pathogenesis and pathology of foot rot is dependent on familiarity with the normal ovine foot. An excellent description of the normal anatomy and histology of the ovine hoof has been published.\(^5\)

**Gross Pathology:** In early cases there is a mild interdigital dermatitis characterized by moistness, slight edema and paleness followed by erosion of the epidermis. In more advanced cases there is slight detachment of the hoof at the skin-horn junction. In advanced cases necrosis progressively undermines the corneum (hoof) of the periople of the bulb, the bulbar area of the axial surface, the bulb proper, the axial wall, the sole and the distal part of the lamellar part of the abaxial wall. The corneum remains attached along the proximal part of the abaxial wall.

Over affected areas the corneum is loosened and detachable. The necrotic tissue beneath the undermined corneum is soft, moist, friable and malodorous. Some viable but swollen epithelium remains deep to the necrosis. All pathological changes are limited to the integument.\(^6\)

**Histopathology:** In early lesions, tissue changes include swelling, cytoplasmic vacuolization and necrosis of epithelial cells of the stratum granulosum and superficial layers of the stratum spinosum. Cytoplasmic vacuoles of adjacent cells coalesce to form larger cavities which become infiltrated with leukocytes. Papillae of the corium are edematous and infiltrated with leukocytes.

Cellular changes of advanced lesions are similar to changes in early lesions. Liquifactive degeneration and necrosis occur in epithelial cells of the strata

---

Supported by the Oregon Agricultural Experiment Stations, Veterinary Medicine Account.

Submitted as Technical Paper Number 3928 from the Oregon Agricultural Experiment Station, Corvallis, Oregon.

Presented to the Committee on Diseases of Sheep and Goats at the Seventy-Eighth Annual Meeting of the United States Animal Health Association, October 13, 1974 in Roanoke, Virginia by Dean H. Smith, D.V.M.
granulosum and spinosum. These changes occur in cells at the edge of the advancing point of separation of the hoof from the foot and in superficial cells of persisting layers of stratum spinosum. Necrosis in the strata granulosum and spinosum results in detachment of the corneum.

Papilla are edematous. Leukocytes, especially polymorphonuclear neutrophils, infiltrate the papillae and stratum spinosum.6

Pathogenesis: Beveridge3 and Thomas32 both reported the experimental production of foot rot lesions in sheep using pure cultures of B. nodosus and thus attributed the condition solely to this organism. However, Egerton10 and Roberts24 pointed out that neither of the earlier researchers had excluded F. necrophorum or other bacteria from the experimental environment. They demonstrated that B. nodosus and F. necrophorum were the minimal agents required for reproducing foot rot. They showed that in all cases where foot rot was successfully reproduced, a superficial invasion of F. necrophorum always preceded recognizable growth of B. nodosus in the tissues.

Thomas32 and Egerton10 showed that B. nodosus proliferates in the epidermal layers, primarily the stratum lucidum, and does not invade the dermis. F. necrophorum on the other hand does invade the dermis. Egerton10 also showed that establishment of B. nodosus infection was followed by deeper penetration of F. necrophorum, indicating that B. nodosus enhanced the invasiveness of F. necrophorum. B. nodosus and F. necrophorum are the only organisms shown to penetrate fresh epidermal tissue.10 In early lesions B. nodosus predominated at the point of separation of epidermal tissues where it was often the sole invader. However, there was little associated tissue response and, although B. nodosus is essential to foot rot, its exact role in the destructive process is uncertain. This B. nodosus characteristic of eliciting minimal tissue response, together with the ability to multiply slowly and sustain viability over a long period of time in the presence of limited nutrients, probably accounts partly for the chronicity of foot rot infections. Conversely, invasion by F. necrophorum is usually accompanied by severe inflammation and destruction contributing to the severity of the lesions; however, F. necrophorum remains viable in the tissues for a relatively short time, and without accompanying B. nodosus infection, F. necrophorum lesions are transient.

Neither Egerton10 nor Thomas32 were able to demonstrate tissue damage directly caused by bacterial action. Both workers agreed the basic lesion, separation of the layers of the epidermis or horn, is caused by inflammatory processes. Beveridge3 first demonstrated the production of proteases by B. nodosus and was able to show a correlation between the virulence of a strain and its proteolytic activity. Thomas34 further showed that the B. nodosus proteases had ability to digest epidermal elements but not dermal constituents, thus corresponding with the epidermal location of B. nodosus. It is possible that the proteolytic activity of B. nodosus allows it to invade or penetrate fresh epidermal tissues but does not elicit a significant pathological effect per se.

Multiple other microorganisms have been associated with foot rot. Egerton10 demonstrated that cocco-bacilli and diphtheroids were relatively superficial in location and did not appear to participate directly in the infection. Spirochetes and motile fusiforms penetrated more deeply but grew mainly in tissue already destroyed by the pathogenic process. They did not invade fresh tissue and
appeared to be not essential to the pathogenesis of foot rot.

Roberts\textsuperscript{24} observed that \textit{Corynebacterium pyogenes} greatly enhanced the initial establishment of the fastidious anaerobes \textit{B. nodosus} and \textit{F. necrophorum}. He later demonstrated an intricate synergistic mechanism between \textit{C. pyogenes}, \textit{B. nodosus}, \textit{F. necrophorum} and motile fusiforms common in foot rot lesions.\textsuperscript{24,24} \textit{C. pyogenes} provides a growth factor for \textit{F. necrophorum} as well as probably using oxygen, decreasing Eh, and supplying catalase, thus stimulating \textit{F. necrophorum} growth in the superficial epidermis of the interdigital integument. At the same time, \textit{F. necrophorum} produces a leucocidal toxin which protects \textit{C. pyogenes} as well as itself from phagocytosis. Later, as \textit{F. necrophorum} penetrates deep into the epidermis and into an environment unfavorable for the growth of \textit{C. pyogenes}, the required growth factor is supplied by \textit{B. nodosus}. This probably explains the enhanced invasiveness of \textit{F. necrophorum} in the presence of \textit{B. nodosus} as mentioned earlier. The bacterial interplay is further extended by the motile fusiforms which provide a growth stimulating factor for \textit{B. nodosus}.

Of all the microorganisms involved in the aforementioned synergistic mechanisms causing foot rot in sheep, the only organism not freely available from the environment is \textit{B. nodosus}. The only habitant known for \textit{B. nodosus} is the epidermal matrix of the hoof,\textsuperscript{36} thus the spread of foot rot is contingent on the transmission of this organism by infected sheep.

\textbf{Related Conditions of the Ovine Foot:} Benign Foot Rot (Scald, Nonprogressive Foot Rot): This is essentially an inflammation and necrosis of the interdigital skin that follows infection by \textit{F. necrophorum} and strains of \textit{B. nodosus} of low virulence.\textsuperscript{31} Egerton and Parsonson\textsuperscript{11} showed that the low virulence of these strains of \textit{B. nodosus} was related to a low proteolytic index. Morgan \textit{et al.}\textsuperscript{21} demonstrated that these benign \textit{B. nodosus} infections could persist in flocks for prolonged periods without reverting to the highly virulent characteristics.

Ovine Interdigital Dermatitis (O.I.D.): This is a \textit{F. necrophorum} infection that closely resembles benign foot rot.\textsuperscript{21} It can be differentiated with certainty from early or benign foot rot only by the absence of \textit{B. nodosus} in smears from the lesion examined microscopically. In agreement with the characteristics of \textit{F. necrophorum}, this is primarily a transient infection dependent on environmental conditions, whereas benign foot rot is more chronic and less dependent on environmental factors.

Infectious Bulbar Necrosis (I.B.N., Heel abscess): This is a necrotizing lesion of the digital cushion, particularly of the heel, arising by extension of infection from lesions of O.I.D.\textsuperscript{22} This appears to be a true mixed infection of \textit{B. nodosus} and \textit{C. pyogenes}. There is evidence that this condition is related to changes in blood supply to the digit.

Foot Abscess (Toe abscess): This is separate disease entity associated with \textit{F. necrophorum} and other bacteria.\textsuperscript{31} As with I.B.N., the condition is more common in lambing ewes or ewes heavy with lamb. The abscess is usually located under the sole in the anterior part near the toe. The pathogenesis of the condition has not been described.

\textbf{MICROBIAL NOMENCLATURE AND CHARACTERISTICS}

\textit{Nomenclature:} \textit{Bacteroides nodosus} is the most recently approved name of
the organism formerly referred to as *Fusiformis nodosus*.\textsuperscript{1,2} Ironically, Beveridge\textsuperscript{3} would have originally placed it in this genus except that the validity of *Bacteriodes* was not generally recognized at that time. Secondly, the definition of *Bacteriodes* in Bergey (1934) included “good growth on ordinary culture media” which did not apply to this organism.

*Fusobacterium necrophorum* is the name now assigned to the organism formerly referred to as *Fusiformis necrophorus*, *Sphaerophorus necrophorus*, or *Actinomyces necrophorus*.\textsuperscript{1}

**Bacteriodes nodosus:** The isolation of *B. nodosus* was first reported by Beveridge in 1938 and more fully described by him in 1941.\textsuperscript{3} It is a large gram negative rod having characteristic nodules usually at both ends especially upon primary isolation. It is basically non-reactive biochemically\textsuperscript{1} and is non-motile and non-spore forming. It is an obligate anaerobe and is routinely grown in an atmosphere of 10% CO\textsubscript{2} in H\textsubscript{2} at pH 7.5 and 37°C. Colonies grown on agar plates containing horse blood are non-hemolytic, colorless, and usually have smooth surfaces, but occasionally have a ground-glass appearance. The colonies etch into the agar surface and usually appear to be growing in a pit. Sometimes, however, this depression can only be seen when the colonies are removed. Liquid media have been described as being capable of supporting *B. nodosus* growth.\textsuperscript{22,23}

*B. nodosus* appears to be an obligate parasite of the hoof and even under the most favorable conditions cannot live away from the host for more than two weeks.\textsuperscript{36} It does, however, have the ability to multiply slowly and remain alive for long periods in the presence of limited nutrients.\textsuperscript{24}

There are three recognized antigens of *B. nodosus*. They are the surface or K antigen, the somatic or O antigen, and the proteolytic enzymes found in the supernatant fluid of liquid media.

The K antigens have served as the basis for classifying *B. nodosus* into at least 3 types. It is probable that several K antigens are present in some isolates. This system cannot be used as an index to virulence, because isolates from both benign and virulent cases of foot rot belong to each of the 3 original types.\textsuperscript{17} Work on the ultrastructure of *B. nodosus*\textsuperscript{30,35} has revealed piliated organisms both when grown on agar and during the logarithmic phase of growth in liquid media. These pili, which are antigenic and are most likely the K antigens, are readily dislodged and moderately heat-labile. Vaccination with a purified preparation of pili elicits an agglutinin response identical to that given by injection of the commercial vaccine prepared from formalized whole cultures. The possible role of pili in the pathogenesis of foot rot has not been determined, but they seem to play a role in eliciting protective antibodies. Incomplete phage (bacterial viruses) have been observed in some purified pili preparations and may be responsible for the rapid lysis of the organism at the end of the logarithmic phase of growth. Whether the presence of phage is associated with pathogenicity remains to be determined.

The O antigens on the other hand are heat-stable, apparently have at least one common antigen among the 3 original types identified by K agglutination tests, and are probably responsible for the indirect hemagglutination reaction.

The proteolytic enzyme antigen is the only known characteristic of *B. nodosus* that is associated with virulence. A proteolytic index has been devised...
by measuring the zones of precipitation and clearing surrounding *B. nodosus* strains growing on casein agar. Virulent strains consistently produced a much wider zone than did benign strains, and the application of fully virulent cultures to the inflamed interdigital skin of benign cases caused a rapid conversion to virulent foot rot.\(^{11}\)

A fluorescent antibody stain has been developed for use as a means of diagnosis of *B. nodosus*.\(^{26}\) This stain reacted specifically with organisms from 29 farm samples of cattle and sheep from 7 countries that were confirmed cases of foot rot. From this it seems reasonable to conclude that the preparation contains antibody to cell surface antigens which are present throughout the species. Unfortunately, the features of similarity in surface antigens shown by this test cannot be extended to imply cross-protection *in vivo*.

**VACCINATION AND IMMUNITY**

*Effectiveness of Vaccine:* Field trials using oil- and alum-adjuvanted preparations of *B. nodosus* bacterins in Australia,\(^{14,15}\) New Zealand,\(^{28}\) and England\(^{25}\) generally agree on the beneficial effects of vaccination. Immunity against *B. nodosus* infection was rarely "all or none" but foot rot in vaccinated sheep lasted a shorter time, was less invasive and affected fewer feet in vaccinates than in controls. Significant therapeutic value resulting from vaccination was also noted in trials by Skerman\(^{28}\) and Egerton.\(^{12,15}\) Duration of immunity is not definitely known, but is generally thought to be 6 months or less, thus vaccination at the time of peak exposure is important. Enhanced resistance to infection is provided by a second dose of vaccine.\(^{28}\)

The relationship of the antigenic structure of *B. nodosus* to immunity is not definitely known; however, Egerton et al.\(^{14}\) refer to an outbreak of foot rot in vaccinated sheep that was associated with a strain of *B. nodosus* subsequently shown to be serologically distinct from the two strains in the vaccine. In a field trial of an Australian-produced vaccine in one flock in Oregon, Snyder\(^ {29}\) showed that the vaccine did not protect against foot rot. No serologic characterization of *B. nodosus* strains from the unprotected flock was done. McGowan\(^ {18}\) has also reported that the Australian vaccine did not hold up under U.S. conditions. Again, the reason for these unfavorable results with the vaccine were not determined.

*Mechanism of the Immune Response:* The mechanism of resistance to *B. nodosus* infection has not been definitely determined. Egerton and Roberts\(^ {13}\) demonstrated a correlation between high circulating levels of bactericidal antibody and resistance to infection. They suggested that resistance was due to the destruction of *B. nodosus* by antibody and complement diffusing into the infected epidermis.

The K and O antigens of *B. nodosus* have not been directly correlated with pathogenicity or, conversely, with resistance to infection. Antibodies to both the K and O antigen have been demonstrated in normal sheep. K and O agglutinin titers rose slightly after natural chronic infection, but were increased about 100 times greater than this after vaccination of the sheep with an oil-adjuvanted vaccine. Resistance in sheep vaccinated in this manner was usually associated with O antibody titers of 2000 or greater, however, there was not such a close relationship between high K antibody titers and resistance.\(^ {16}\)
With alum-adjuvanted vaccination, it was found that O titers rose very little after vaccination even in sheep subsequently shown to be highly resistant. K titers, however, increased markedly and sheep with K titers of 10,000 or more were usually those resistant to subsequent experimental infection. Therefore, no definite correlation between agglutination titer and resistance to infection has been established, and the specific immunogenic antigen has not been identified.

Serological identification of sheep which have suffered from foot rot may be determined using a protease test; however, immunoglobulin antiproteases must be distinguished from naturally occurring serum protease inhibitors such as \( \alpha \)-macroglobulin. Whether a macroglobulin is involved in resistance of sheep to \( B. nodosus \) infection has not been determined, but because of its large molecular size, \( \alpha \)-macroglobulin may not be able to diffuse into the epidermis — the site of infection.

The possible role of cell-mediated immunity in resistance to \( B. nodosus \) infection as suggested by Egerton and Roberts and Cooper has not been extensively studied.

**TREATMENT AND CONTROL OF FOOT ROT**

It is possible to control and eliminate foot rot through a rigorous program of hoof trimming and foot bathing with solutions of formalin, arsenicals, copper sulfate or chloromycetin together with isolation or culling of infected sheep. Parenterally administered antibiotics have been shown effective in treating foot rot cases.

The role that \( B. nodosus \) plays in foot rot of cattle is not definitely known; however, it has been recognized in multiple cases of bovine foot rot. In one case the cattle were known to have no previous exposure to sheep. In another case, cattle were incriminated as the source of infection for new uninfected sheep brought onto a farm attempting to eradicate foot rot from the premises and establish a “clean” flock. In this case, transmission of \( B. nodosus \) from a steer to sheep was definitely demonstrated under experimental conditions and the sheep developed virulent foot rot. This transmissibility of \( B. nodosus \) infection from cattle should thus be considered in any sheep foot rot control program.
REFERENCES


REPORT OF THE COMMITTEE OF DISEASES OF SHEEP AND GOATS 1974

Chairman: F. James Schoenfeld, Salt Lake City, Utah.
Co-Chairman: C. C. Beck, Manchester, Mich.


The Committee met as instructed with seven members and seventeen visitors, which included two from Canada. The suggested agenda subjects were discussed by the committee and guests and the proceedings of the committee are now reported.

1. Dr. Richard F. Hall brought the committee up to date concerning Vibrio Vaccine studies. A study is underway in connection with Dr. Bruce Lang, Parasitologist, concerning the development of a “Fluke Vaccine” for sheep and cattle. Other parasitic problems are being studied in connection with Washington State, concerning antibody response in parasitized animals.

2. Dr. Ferron L. Andersen, Parasitologist, gave an illustrated lecture on parasite environment in relations to irrigation. He also presented carcass disposal and sheep management as it relates to Hydatid Disease. The full text of Dr. Andersen's studies will be submitted as part of this report.

3. Dr. M. E. Macheak brought to the committee's attention the report on Predator Control 1971, which was prepared by the advisory committee on predator control of the Bureau of Sport Fisheries and Wildlife to the Council on Environmental Quality and the Department of Interior. The recommendations of this report were read. A discussion concerning the use of toxicants was brought forth. It is recommended that a further study of this report be made.

4. Dr. D. H. Smith gave a summary report on "The review work, Ovine Foot Rot", by Dr. John A. Schmitz, and Mr. Joseph L. Gradin, of the Department of Veterinary Medicine, Oregon State University. The completed report is submitted and included in the committee report.

5. Mr. O. H. Timm, presented a resolution concerning funding for foot rot research. It was approved by the committee to be sent to the Resolutions Committee for action.

6. Dr. A. Klingsport reported on Scrapie in the United States, and also the Field Trial, Mission, Texas. He also reported on Bluetongue in the United States. They are included in the committee report.

SCRAPIE

Scrapie was reported in one flock in De Kalb County, Illinois, during fiscal year 1974. This outbreak occurred in a mixed flock of purebred and grade Hampshire, Rambouillet, and Suffolk sheep. There were two outbreaks reported.
in fiscal year 1973, and three outbreaks each reported in 1971 and 1972. These are the smallest number of outbreaks reported since fiscal year 1954. The number of flocks under surveillance has dropped from 230 in fiscal year 1973 to 148 in 1974.

The De Kalb County, Illinois outbreak was reported to a veterinary practitioner by the owner. The Illinois infected flock is being held under State quarantine pending slaughter arrangements. The source of the De Kalb County, Illinois, outbreak was considered to be another De Kalb County flock which was also considered to be the source of a scrapie outbreak in Parke County, Indiana, in fiscal year 1972. The De Kalb County source flock was dispersed; however, regulatory officials located and slaughtered all bloodline animals sold from the flock and located and placed under surveillance all nonbloodline exposed animals sold from the flock.

A Somerset County, New Jersey, flock infected in fiscal year 1972 was previously reported as not being slaughtered but held under State quarantine. The owner refused to slaughter this flock on the grounds that he did not sell breeding animals and therefore was not spreading disease. However, after 24 months of quarantine it became apparent that scrapie was entrenched in the flock, cases continued to occur, and the owner agreed to slaughter the flock of 45 Suffolk sheep and five goats in June 1974.

### SCRAPIE FIELD TRIAL, MISSION, TEXAS

The scrapie field trial has been underway since November 1964 (116 months). During this period scrapie has been confirmed by histopathological study or by mouse inoculation in 305 animals. These 305 cases have occurred on infected premises No. 3 in the following category of animals either taken to Mission or born on the premises.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Scrapie bloodline exposed sheep</td>
<td>223</td>
</tr>
<tr>
<td>b. Field suspects held for observation</td>
<td>12</td>
</tr>
<tr>
<td>c. Nonbloodline exposed sheep</td>
<td>33</td>
</tr>
<tr>
<td>d. Nonbloodline exposed goats</td>
<td>37</td>
</tr>
</tbody>
</table>

305

The scrapie field trial has demonstrated that bloodline animals exposed on infected and source flock premises will continue to develop scrapie and provide foci of infection for continuing spread of the disease unless slaughtered. The losses in bloodline animals continue to occur in succeeding generation of such animals reared on infected premises.

From October 1969 to July 1974, scrapie has been confirmed by histopathological examination or by mouse inoculation in 33 nonbloodline blue (not previously exposed) sheep and 37 nonbloodline glue goats born or taken to Mission and reared in contact with affected Cheviot, Montadale, and Suffolk sheep. Thirty-six of the affected goats were born at Mission and are of the following breeds or crosses: Angora; Nubian; Angor X Nubian X Toggenburg; Nubian X Toggenburg; Nubian X mixed dairy; and Nubian X Toggenburg X mixed dairy. These goats were affected at ages ranging from 36 to 60 months. One goat was a mixed breed dairy goat purchased from an infected Suffolk flock and affected 13 months after arriving at Mission.
Of the 33 affected blue sheep, 30 were born and reared on the infected premises at Mission and were of the following breeds: Hampshire; Rambouillet; Suffolk; and Targhee ranging in ages from 32 to 51 months. The additional blue affected sheep have the following history: One Hampshire was taken to Mission and placed in exposure at 8 months of age and showed signs of scrapie in June 1972 at 88 months of age, died at 89 months of age and was confirmed scrapie by histopathological examination. One Rambouillet ewe was taken to Mission and placed in exposure at 3 months of age and showed signs in August 1972 at 88 months of age, was destroyed 15 days later and scrapie confirmed by histopathology. One Targhee ewe was taken to Mission and placed in exposure at 6 months of age and showed signs in August 1972 at 88 months of age, was destroyed one month later and scrapie confirmed by histopathology. These findings provide further evidence that scrapie can and does spread laterally when healthy animals are held in contact with animals developing scrapie, further, that pre-natal or birth exposure is not required for the spread of scrapie; however, the incubation period appeared to be lengthened when first exposure occurs at 3 to 8 months of age.

Natural scrapie has now been spread by contact to every breed of previously nonexposed, nonbloodline sheep and goats that have been used in the Mission study. Successful passage of scrapie from affected sheep and goat tissue to sheep, goats, and mice have been demonstrated. These studies are being continued and are used to assist in diagnosis and for checking susceptibility of animals. Various other studies are being continued to determine the route, age, and methods by which scrapie transmission occurs naturally.

**BLUETONGUE**

During fiscal year 1974, bluetongue was confirmed by virus isolation in five cattle herds, three in Colorado and two in Oregon; and in 11 sheep flocks, four in California, one in Idaho, one in New Mexico, three in Oregon, and two in Texas.

In addition 57,473 modified complement-fixation (MCF) tests were run at Veterinary Services Laboratories, Ames, Iowa, and at ten approved laboratories with the following results:

<table>
<thead>
<tr>
<th></th>
<th>Total Tested</th>
<th>Negative</th>
<th>Suspicious</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>47,687</td>
<td>43,076</td>
<td>4,135</td>
<td>476</td>
</tr>
<tr>
<td>Sheep</td>
<td>795</td>
<td>680</td>
<td>84</td>
<td>31</td>
</tr>
<tr>
<td>Goats</td>
<td>1,728</td>
<td>1,658</td>
<td>67</td>
<td>3</td>
</tr>
<tr>
<td>Wildlife</td>
<td>516</td>
<td>339</td>
<td>59</td>
<td>118</td>
</tr>
<tr>
<td>Zoo animals</td>
<td>6,567</td>
<td>6,326</td>
<td>222</td>
<td>19</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>57,293</strong></td>
<td><strong>52,079</strong></td>
<td><strong>4,567</strong></td>
<td><strong>647</strong></td>
</tr>
</tbody>
</table>

Positive MCV samples were collected from the States of Arizona, Arkansas, California, Colorado, Florida, Idaho, Indiana, Kansas, Kentucky, Louisiana, Michigan, Missouri, Montana, Nebraska, Nevada, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, South Dakota, Texas, Utah, Virginia, Washington, Wisconsin, and Wyoming.
7. **Biologics:** Concern was shown for the proposed removal of Epididymitis Vaccine from the market as read from a letter by Dr. G. V. Peacock. A progress report on the status of Ovine mixed Bacterin was presented as herein contained.

**PROGRESS REPORT ON STATUS OF OVINE MIXED BACTERIA**

People interested in the viability of the sheep industry may or may not know the present status of the Ovine Mixed Bacterin. Production of this biological product was discontinued voluntarily by licensed commercial laboratories. This action was taken concurrently with the discontinued production of 17 other biological product codes including seven Mixed Bacterins, seven Named Bacterins, one Antibacterial Serum, one Serum and one Toxoid.

The formula for Mixed Bacterin (Ovine) contained 30% *Pasteurella multocida*, 30% *Corynebacteria* spp., 20% *Salmonella schottmuelleri* and 20% *Staphylococcus albus* and aureus (10% each).

Mixed Bacterins as a biological products class are being replaced by Named Bacterins. The biologics industry has agreed to remove *Staphylococcus* species and *Salmonella schottmuelleri* from all products. However, additional time has been permitted to produce data for individual licensees of some of the aerobic named bacterin products containing *Staphylococcus* species. The industry has further been given until July, 1975, to determine the feasibility of being able to develop a valid potency assay for *Corynebacterium pyogenes*.

Mouse potency test procedures have been developed and proposed as regulations for the serial to serial assay of biological products containing *Pasteurella multocida*. Potency test comparisons between mice and sheep have been established demonstrating the number of organisms per milliliter of bacterin required in a sheep dose to satisfactorily protect sheep against experimental challenge. It is expected that a bacterin containing *P. multocida* with a label recommendation for use in sheep will be on the market soon.

8. Dr. E. E. Kerr and Dr. T. B. Snodgrass of USFDA reported that testing was in progress with Selenium for use in sheep and cattle.

9. The committee again recommends that Veterinary Service of APHIS take steps to outline specific epidemiological procedures and designate persons for diseases of sheep and goats.

10. This report is submitted to the Executive Committee for approval by the Committee of Diseases of Sheep and Goats as appointed by the president of this association.
INTRODUCTION

Since 1966 animal health officials have felt that bovine tuberculosis existed among dairy cattle in northern Illinois. Holstein steers from the area periodically disclosed lesions of tuberculosis at slaughter. Investigations generally led to a market or to a feedlot from which further tracing was impossible.

In April, 1973, an extensive enzootic of tuberculosis was found in a Holstein herd in northern Boone County. The entire herd was slaughtered after 61 reactors disclosed tuberculous lesions on postmortem examination. Contact herds and herds involved in sales and purchases were tuberculin tested, but the source of infection was never established.

In December, 1973, a second dairy herd disclosed eight reactors following a report of tuberculous lesions in a nonreactor at slaughter. The herd was located by tracing the Market Cattle Identification (MCI) backtag which was affixed to the cow. Lesions of tuberculosis were found in five of the reactors. *Mycobacterium bovis* was isolated. This herd was also depopulated. Another tuberculous herd had been removed from this premises in 1928. The source of the current infection was not established.

Both herd owners had purchased animals from a livestock dealer, now deceased, whose records had been destroyed. In addition, the second herd had used rented bulls and had grazed replacement heifers in a community pasture during the summer months.

Concerned herd owners urged the Agricultural Committee of the Boone County Board of Supervisors to request a tuberculin test of all dairy herds in the county. State and Federal animal health officials concurred. A special area test of Boone County dairy herds was conducted during a four week period in late March and early April, 1974, by State and Federal veterinarians.

MATERIALS AND METHODS

All stanchioned cattle and all cows going through milking parlors in Boone County dairy herds were tuberculin tested in the caudal fold using 0.1 ml United States Department of Agriculture Contract Mammalian Tuberculin. A new

---

1. Tuberculosis Epidemiologist, Northern Region, United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Madison, Wisconsin.
4. Veterinary Medical Officer, United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Mattoon, Illinois.
disposable 1.0 ml. tuberculin syringe equipped with 1 26 ga. x 3/8-inch needle was used in each herd. The syringe was filled with 0.1 ml. of tuberculin before injecting each animal tested.

The test was observed in 72 + 4 hours. Responses were recorded in accordance with the standard Animal and Plant Health Inspection Service code. Herds were classified negative (N), or suspect (S). In negative (N) herds, there were no animals reported with P1, X2, or greater responses to tuberculin. Herds which contained animals with P1, X2, or greater responses were classified as suspect(S). In suspect herds, all animals with tuberculin response were retested.

Retests were conducted within ten days of the caudal injection. The comparative-cervical (C-C) test was employed. Biologically standardized trichloroacetic acid precipitated avian and bovine purified protein derivative (PPD) tuberculins of equal potency produced by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services Laboratories, Ames, Iowa, were used.

The avian PPD was made from M. avium Strain D4. Concentration was 1.128 mg/ml. The bovine PPD was made from M. bovis Strain AN5. Its concentration was 0.996 mg/ml. The dose of each PPD was 0.1 ml. Injections were made with 1.0 ml. disposable tuberculin syringes equipped with 26 ga. x 3/8-inch needles.

Before injection the skin fold thickness at each test site was measured to the nearest 0.5 mm. with a modified vernier caliper. At 72 + 6 hours post-injection, skin thicknesses were remeasured. Increases were recorded as the response to each tuberculin. Responses were plotted on a scattergram and animals classified as "Negative for M. bovis," suspect, or reactor according to established criteria.

Reactors were slaughtered in an official establishment operating under United States Department of Agriculture, Animal and Plant Health Inspection Service, Meat and Poultry Inspection supervision. Specimens were collected and submitted to Veterinary Services Laboratories, Ames, Iowa, for histopathologic and bacteriologic examination.

RESULTS

Results of the caudal fold tests are summarized in Table 1. There were 7,114 cattle tested in 171 herds. Only 44.4 percent (76) of the herds and 96.0 percent (6,831) of the cattle were reported as negative. In 55.6 percent of the herds (95), a total of 283 (4.0 percent) of the cattle tested developed tuberculin responses of P1, X2, or greater and were classified as suspects. These 95 herds contained an additional 529 animals with responses less than P1 or X2. All animals with tuberculin response in suspect herds, except 19 in two herds, were retested using the C-C test.

Results of the C-C retests of the caudal fold suspects are summarized in Table 2. There were 5.2 percent (5) of the herds and 1.75 percent (5) of the cattle classified as reactors. Five (5.2 percent) of the herds or 1.75 percent (5) of the cattle were still suspicious. The remaining 89.6 percent of the herds (85) and 96.5 percent of the animals (273) were reclassified as negative.

Figure 1 is a scattergram plotting of the tuberculin responses of the C-C retest of caudal fold suspects. This response pattern closely parallels the data accumulated for all C-C tests conducted nationally during FY 1974. Figure 2 is a
scattered plotting of the responses for all 793 cattle restested by C-C test. There were 6 reactors, 5 caudal suspects and 1 caudal deviator, (0.8 percent); 20 suspects (2.5 percent); and 767 negatives (96.7 percent).

Postmortem examination of the six reactors disclosed five animals with skin lesions. The other reactor revealed no gross lesions of tuberculosis. This animal and one of the skin lesion cases were from one herd.

DISCUSSION

Although the Boone County area test failed to disclose additional *M. bovis* infection, it reconfirmed the high incidence of tuberculin sensitivity previously reported from the north Central states.1,3,4,6 By use of the C-C test, herd status was established within ten days after the initial caudal tuberculin test.

Before the adoption of the C-C test, suspect herds would not have been retested for 60 to 90 days with the caudal test. During the interim, herd status would have remained unknown. If a herd was tuberculous, there would be further opportunity for a spread of the disease. Frequently, only suspect animals were quarantined. There was always the possibility of spreading tuberculosis to other herds through the sale or movement of exposed cattle. The reliability of a caudal retest, even after 60 to 90 days, is not really known. Tuberculous herds have been missed because only the suspects were retested and they were classified negative. As a result, the herd was released from surveillance. Since the skin of the neck is more sensitive than the caudal fold, the C-C test can be conducted before general desensitization occurs. There have been no cases reported in which the C-C test has failed to identify an *M. bovis* infected herd.*

In Boone County, 83 of the 95 herds (87.4 percent) which were suspicious on the standard caudal test were reclassified “Negative for *M. bovis.*” When all 171 herds tested are considered, use of the C-C test resulted in an increase of specificity from 38.3 percent (76) for the caudal test to 93.0 percent (159). There were still 12 herds (7.0 percent) with reactors and/or suspects to the C-C test.

In 5 of the 6 reactors, skin lesions were found on postmortem examination. Veterinarians conducting the test also reported skin lesions in several of the herds with suspects. There have been reports that skin lesions may reduce the specificity of the C-C test.3

It has been observed that sensitization of cattle by tubercle bacilli is maintained, on the average, over a more extended period than that due to skin lesions and other forms of sensitivity.8 If the C-C test had been delayed for 60 to 90 days, one can only speculate as to the results. Since the test was conducted in a cattle population with high risk for bovine tuberculosis, delay in retesting was not considered feasible. In herds where there is no history of exposure to tuberculosis, the C-C test can be delayed for at least 60 days. If the results of the first C-C retest are inconclusive, a second C-C test is conducted 60 to 90 days later.
BIBLIOGRAPHY

TABLE 1
RESULTS OF CAUDAL FOLD TESTS IN BOONE COUNTY

<table>
<thead>
<tr>
<th></th>
<th>HERDS</th>
<th>CATTLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NUMBER</td>
<td>%</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>76</td>
<td>44.4</td>
</tr>
<tr>
<td>SUSPECT</td>
<td>95</td>
<td>55.6</td>
</tr>
<tr>
<td>TOTAL</td>
<td>171</td>
<td>100.0</td>
</tr>
</tbody>
</table>

TABLE 2
RESULTS OF COMPARATIVE-CERVICAL RETESTS OF CAUDAL FOLD SUSPECT HERDS

<table>
<thead>
<tr>
<th></th>
<th>HERDS</th>
<th>CATTLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NUMBER</td>
<td>%</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>85</td>
<td>89.6</td>
</tr>
<tr>
<td>SUSPECT</td>
<td>5</td>
<td>5.2</td>
</tr>
<tr>
<td>REACTOR</td>
<td>5</td>
<td>5.2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>95</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Figure 1--Comparative-Cervical Retest of Caudal Fold Suspects Within 10 Days

(BOONE COUNTY, ILLINOIS)

Increased Skin Thickness in mm. - Avian PPD

Increased Skin Thickness in mm. - Bovine PPD

O - No Gross Lesions
Figure 2--Comparative-Cervical Retest of Caudal Fold Suspects and Deviators Within 10 Days

(BOONE COUNTY, ILLINOIS)
THE STATUS OF THE STATE-FEDERAL TUBERCULOSIS ERADICATION PROGRAM

by
Ralph W. Bennett, D.V.M.*

Since the inception of the Tuberculosis Eradication Program in 1917, able and dedicated men have stood before this assemblage and begged, pleaded, and threatened dire consequences if the vigilance against Mycobacterium bovis was relaxed. This year will be no exception.

We have come a long way down the road over the past 57 years toward the ultimate goal of total eradication of bovine tuberculosis. The goal is in sight, but the view is obscured by the clouds of apathy on the part of the livestock industry and the veterinary profession as a whole.

Perhaps this apathy is best exemplified by the situation in our accredited herds in fiscal year 1974, when three of the 30 infected herds discovered, were accredited herds for a herd infection rate of 44.52 per 100,000 herds compared to a herd infection rate of 1.39 per 100,000 herds for non-accredited herds as shown in figure 1.

This is almost an unbelievable situation when we consider that accredited herds must be tested and found negative annually. One of the three accredited herds found to be infected with M. bovis in fiscal year 1974 was discovered as a result of tracing of a lesioned animal found on routine slaughter. Subsequent testing of this herd by regulatory veterinarians revealed 119 reactors, of which 64 disclosed lesions of tuberculosis on slaughter. The negative annual tests of this herd performed by an accredited veterinarian must be highly suspect. It is probable that this accredited veterinarian, and many other accredited veterinarians, that are engaged in testing accredited herds, have been lulled into a false sense of security by the assumption that accredited herds are somehow exempt from M. bovis infection. This false sense of security must be overcome by increased efforts to upgrade the testing techniques of all veterinarians to insure that accepted procedures of tuberculin injection and reading are followed.

In the first 18 years of the program, from 1917 to 1935, the reactor rate dropped from a high of 4.88 percent in 1918 to .72 percent in 1935. This significant reduction in the reactor rate was accomplished primarily because of the dedication of the veterinarians doing the tuberculin testing in those early days of the program. Today's veterinarian must be no less dedicated to the eradication of bovine tuberculosis through the professional application and reading of the tuberculin test.

Figure 2 shows that no change has been made in the status of the various States insofar as accredited free areas and modified accredited areas are concerned. Some changes have been made, however, in the States which had not discovered M. bovis infection in the past 5 years. Two States, Alabama and

*Chief Staff Veterinarian, Tuberculosis Eradication, Cattle Diseases, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture.
Indiana, fell from this category in fiscal year 1974, and an additional five States: Arizona, Oklahoma, Kansas, Arkansas, and Mississippi, reached this plateau for a net gain of three States in this category.

Thirty lesioned herds were found in the United States in fiscal year 1974 (figure 3). Twenty-eight of these herds were confirmed as *M. bovis* and two herds were suspicious of *M. bovis*. This represents a drop of eight herds from the 38 lesioned herds found in fiscal year 1973. This may be indicative of a drop in *M. bovis* infection in the United States; however, it may be more indicative of a possible trend by the livestock industry and certain elements of the regulatory agencies to complacency as a result of the present low incidence of bovine tuberculosis. Let us hope the former is the case.

It is interesting to note that one of the herds tuberculin tested as a result of tracing of a lesioned regular kill animal disclosed 181 reactors out of 182 cattle tested. The only negative animal was a new-born calf. Of the 181 reactors, 43 revealed lesions indicative of tuberculosis upon slaughter.

The decline over the past 2 years in the number of lesioned herds found as a result of traceback testing continues with only 18 (60%) of the 30 infected herds discovered in fiscal year 1974 being found in this manner (figure 4). Tuberculin testing for all other reasons resulted in the disclosure of twelve infected herds. This is a very poor record for slaughter surveillance for tuberculosis and must be improved drastically to accomplish total eradication of bovine tuberculosis.

There is, of course, a direct correlation between the number of *M. bovis* lesioned animals found on regular kill which are not traced to a herd of origin and the number of lesioned herds found through traceback. Figure 5 shows that the number of *M. bovis* lesioned animals not traced to a herd of origin has steadily risen over the past 2 years, while the number of infected herds found through tracing of these animals has steadily declined over the same period of time, indicating that increased emphasis must be placed on epidemiological methods. It is true that a portion of these animals originate in Mexico, but we must guard against the easy solution of a problem traceback by incriminating imported animals.

Figure 6 shows the location by State of the 30 lesioned herds found in fiscal year 1974 and the 10 herds depopulated this year. It is interesting to note that *M. bovis* infection with the exception of California continues to be concentrated primarily in the eastern one-half of the country.

Depopulation of *M. bovis* infected herds dropped drastically in fiscal year 1974, with only 10 of the 30 affected herds being depopulated (figure 7). Seven States depopulated every known tuberculosis-affected herd. The ten herds depopulated in fiscal year 1974 is the lowest number depopulated since fiscal year 1964 when eight herds were depopulated. The primary reason for the decline in the number of herds depopulated this year is the unrealistic Federal indemnity rate for exposed animals of $100.00 for grade and $200.00 for purebred exposed animals. In addition, most State authorities for the payment of indemnity on exposed animals, is limited to token amounts or no payment at all. Efforts are underway to raise the amount of Federal indemnity for this class of animal; however, the outlook for success at this time is not very promising. Individual States are urged to press for authority to make indemnity payments on exposed animals in those States not now having this authority and to increase the amount...
of payment in those States which are now limited to small payments. It is difficult to convince an owner that he should depopulate exposed animals when he faces the prospect of great financial loss even though, in the long run, he will probably be money ahead by doing so. Since depopulation of tuberculosis-exposed animals is the only way one can be sure every last vestige of *M. bovis* has been eliminated from a herd, the question is how many tuberculous animals remain in those herds not depopulated to serve as a reservoir of infection for all of the clean herds in the country?

Individual animal identification is the key to successful tracing of suspected tuberculous animals to their herds of origin. Figure 8 shows that of the 98 suspicious lesioned regular kill animals bearing individual identification, 76 percent were successfully traced. Conversely, of the 97 suspicious lesioned regular kill animals bearing no individual identification, only 23 percent were successfully traced. The importance of animals being individually identified and Meat and Poultry Inspection personnel collecting such identification cannot be overemphasized.

This matter of individual identification being the key to successful traceback is further emphasized by the fact that 82 percent of the adult lesioned slaughter cattle were successfully traced, whereas only 26 percent of the lesioned feeders were successfully traced (figure 9). The reason for the difference is the fact that a far greater percentage of adult cattle are individually identified.

If individual identification is the key to successful tracebacks, and we believe it is, then increased submissions of suspected tuberculous lesions from regular kill animals by Meat and Poultry Inspection is the handle that will open the door to the early discovery of tuberculosis-affected herds. Unfortunately, the number of suspected tuberculous lesions submitted from regular kill animals has remained substantially constant the past several years as shown in figure 10.

Figure 11 shows that of the 81 slaughter establishments slaughtering 100,000 or more adult cattle annually, 30 submitted no suspected tuberculous lesions at all during the year. Thirty-one submitted up to three lesions each during the year, and 20 submitted not less than 3.5 nor more than 37 lesions each.

Perhaps even more revealing, is the fact that in fiscal year 1974, in 57 federally inspected slaughter establishments, each slaughtering over 20,000 cows annually, 34 plants submitted no granulomas for laboratory examination for evidence of tuberculosis, 44 plants submitted between .2 and 3 granulomas per 20,000 cows slaughtered, and 9 plants submitted between 3.5 and 35 granulomas per 20,000 cows slaughtered (figure 12). The granuloma submission rate must be increased for the slaughter surveillance and traceback program to be successful in eradicating bovine tuberculosis. It is taking altogether too long to get back to infected herds with the present submission rate.

The number of veterinarians trained to perform the comparative-cervical test has increased from 124 in fiscal year 1973 to 339 in fiscal year 1974 (figure 13). Now that there is an adequate number of veterinarians trained to perform the comparative-cervical test, it is strongly suggested that all animals classified as suspect or deviator be retested with a comparative-cervical test.

During the past year, it has become increasingly difficult to find a slaughter establishment that will take tuberculosis reactor animals due to the cooking restrictions on the meat. Some reactors are being slaughtered at rendering plants, on the farms of origin, and at State Diagnostic Laboratories with postmortems
in each case being performed by regulatory veterinarians. Such slaughter
methods preclude any salvage from the reactors and adds considerable expense
to the tuberculosis program because of the inefficiency of performing
postmortems under rendering plant and field conditions. No satisfactory answer
to this problem has, as yet, been found.

In summary, only through continued vigilance of all concerned to detect
bovine tuberculosis whether through regular kill postmortem procedures in
slaughter establishments or through tuberculin testing of animals on farms, and
the eradication of each foci of infection found, will the Bovine Tuberculosis
Eradication Program succeed.
### M. BOVIS INFECTION RATES

**(Fiscal Year 1974)**

<table>
<thead>
<tr>
<th>Herd Status</th>
<th>Number of Herds</th>
<th>Number of M. bovis Herds</th>
<th>Rate Per 100,000 Herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-accredited</td>
<td>1,950,852</td>
<td>27</td>
<td>1.39</td>
</tr>
<tr>
<td>Accredited</td>
<td>6,738</td>
<td>3</td>
<td>44.52</td>
</tr>
</tbody>
</table>

---

### BOVINE TUBERCULOSIS AREA STATUS

**(June 30, 1974)**

- **ACCREDITED FREE AREA**
- **MODIFIED ACCREDITED AREA**
- **NO M. BOVIS REACTORS FOR OVER 5 YEARS**

---

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

Lesion Herds Confirmed and Suspicious
(Fiscal Year 1974)

Detected Herds with Tuberculosis Infection
M. BOVIS LESION CASES ON REGULAR KILL
NOT TRACED TO HERD OF ORIGIN
(Fiscal Year)


PROPORTION OF LESION HERDS DEPOPULATED
(Fiscal Year 1974)
Tuberculosis Eradication

Lesion Herds-FY 1964 Through 1974 and Those Depopulated

Fiscal Year


184 141 111 86 74 70 50 67 52 38 30

Lesion Herds

Number Depopulated

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

195 Suspicious Lesion Cases (Regular Kill)

Animals Identified and Unidentified

(Fiscal Year 1974)

Unsuccessful 77%

Successful 23%

98 with Identification

97 No Identification

U.S. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE
**195 SUSPICIOUS LESION CASES (Regular Kill)**

**BY SLAUGHTER CLASS**

*(Fiscal Year 1974)*

- **FEEDERS**
  - 81 cases
  - 26% successful
  - 74% unsuccessful

- **ADULTS**
  - 114 cases
  - 82% successful
  - 18% unsuccessful

**Tuberculosis Eradication**

**TUBERCULOSIS TRACEBACK CASES**

*(VS 6-35)*

- **TOTAL CASES CLOSED**
  - 1970: 1,025
  - 1971: 922
  - 1972: 879
  - 1973: 1,044
  - 1974: 1,042

- **CASES CONFIRMED OR SUSPICIOUS OF TUBERCULOSIS**
  - 1970: 240
  - 1971: 150
  - 1972: 152
  - 1973: 134
  - 1974: 195

**FISCAL YEAR**

- 1970
- 1971
- 1972
- 1973
- 1974

U.S. DEPARTMENT OF AGRICULTURE

VETERINARY SERVICES

ANIMAL AND PLANT HEALTH INSPECTION SERVICE
Tuberculosis Eradication

**FEDERAL ESTABLISHMENTS SLAUGHTERING OVER 100,000 CATTLE** BY GRANULOMA SUBMISSION RATE

*(Fiscal Year 1974)*

- **20 EST.**
  - 3.5-37 CASES
  - PER 100,000
  - SLAUGHTERED

- **30 EST.**
  - (NONE)

- **31 EST.**
  - 0.3-3.0 CASES
  - PER 100,000
  - SLAUGHTERED

*EXCLUDING CALVES*

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

---

Tuberculosis Eradication

**FEDERAL ESTABLISHMENTS SLAUGHTERING OVER 20,000 COWS** BY GRANULOMA SUBMISSION RATE

*(Fiscal Year 1974)*

- **44 EST.**
  - 0.2-3.0 CASES
  - PER 20,000
  - SLAUGHTERED

- **34 EST.**
  - (NONE)

- **9 EST.**
  - 3.5-35 CASES
  - PER 20,000
  - SLAUGHTERED

U.S. DEPARTMENT OF AGRICULTURE
VETERINARY SERVICES
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARIANS APPROVED* TO CONDUCT COMPARATIVE-CERVICAL TEST
(As of August 1, 1974)

*TOTAL APPROVED - 339
1974 REPORT OF THE COMMITTEE ON TUBERCULOSIS AND JOHNE'S DISEASE

Chairman: P. L. Smith, Sacramento, California
Co-Chairman: A. R. McLaughlin, Madison, Wisconsin


The Committee on Tuberculosis and Johne's Disease met on Tuesday and Wednesday afternoons. The following presentations were made:
1. Research findings on Band 24, Dr. W. L. Mallman
2. Swine TB in South Dakota, Dr. M. D. Mitchell
3. Report on C-C test results for FY 74, Dr. L. D. Konyha
4. Mycobacteria Isolated from Tuberculosis Swine, Dr. C. A. Thoen
5. Pennsylvania's Johne's Disease Program, Dr. J. Dick
6. Current Johne's Disease Research, Dr. A. B. Larsen

Committee discussions covered tuberculosis in cattle and swine as well as Johne's Disease.

I. TUBERCULOSIS IN CATTLE

The committee was informed that there are approximately 350 veterinarians who have been approved to conduct the comparative cervical test. It is recommended that all retests of animals with response to the caudal fold tuberculin test be conducted by approved personnel using the C-C test.

UMR were discussed. The following changes are recommended:

That the title be changed to UMR — Bovine Tuberculosis Eradication.

Part IV — Quarantine Procedures — Paragraph 4 to read: Herds in which NGL reactors only occur and no evidence of M. bovis infection has been disclosed may be released from quarantine after a 60-day negative retest on the entire herd.

Part V — Special Retests of High-Risk Herds, Paragraph 3 (Retests of Newly Assembled Herds on Depopulated Premises) add the following sentence: If the premises are vacated for one year, these requirements may be waived.

Part X — Modified Accredited Status, Paragraph 2 (Trace Testing) Subsection a. to read: All cattle in herds of origin or cattle associated with those showing evidence of tuberculosis at slaughter must be quarantined and tested.

A state official proposed that UMR be changed to waive retests in herds tested because of straight kill lesion animals with suggestive histopathology and
no isolation of *M. bovis*, if reactors disclosed were NGL on postmortem examination. The committee feels further documentation is needed before such a change can be recommended.

The subcommittee appointed at the 1973 meeting reported on their findings concerning the utilization of paramedical personnel for surveillance tuberculin testing. The subcommittee was instructed to continue its investigation by submitting its report to all appropriate state and federal animal health officials for comments. It is anticipated that full committee will act on the subcommittee's recommendation at the 1975 meeting.

II. TUBERCULOSIS

The subcommittee appointed in 1973 presented a proposed UMR-TB eradication in swine. It was accepted by the committee and is forwarded for adoption by the Executive Committee.

III. RESOLUTIONS

Three resolutions were submitted for the Resolution Committee's consideration concerning funding of a swine tuberculosis program; increase of indemnities for tuberculin test reactors in the bovine eradication program; and development of intrastate and interstate resolutions for the control of the movement of zoo and exhibition animals.

(PROPOSED)
UNIFORM METHODS AND RULES —
TUBERCULOSIS ERADICATION IN SWINE

Uniform Methods and Rules for the Establishment and Maintenance of a Tuberculosis Eradication Program in Swine

TABLE OF CONTENTS
MINIMUM STANDARDS

PART I ........................................... DEFINITIONS
PART II ........................................... IDENTIFICATION OF SWINE
PART III .. REPORTING OF GROSS LESIONS OF TUBERCULOSIS
PART IV ................................. PROGRAM PROCEDURES

Part I: DEFINITIONS

1. "Swine Tuberculosis" — a disease in swine caused primarily by organisms of the *Mycobacterium avium* complex, and occasionally by *M. bovis* or *M. tuberculosis*.

2. "Swine" — swine refers to domestic porcine of all ages.

3. "Tuberculin" — a product that is approved by and produced under specifications of the USDA for injection into animals for the purpose of detecting tuberculosis.

4. "Official tuberculin test" — a test for tuberculosis applied and reported by approved personnel in accordance with these Uniform Methods and Rules.

5. "No Gross Lesion (NGL) Animal" — an animal in which a lesion(s)
indicative of tuberculosis is not found during slaughter inspection.

6. "Passed for Cooking (PFC) Animal" — an animal in which the character and extent of the lesions to tuberculosis found during slaughter inspection indicate a localized condition not severe enough to warrant carcass condemnation, provided the affected organ or other part is condemned.

7. "Condemned Animal" — an animal in which the lesions of tuberculosis are generalized; or the lesions are extensive in tissues of either the thoracic or abdominal cavity; or the lesions are multiple, acute and actively progressive; or when the character or extent of the lesions otherwise is not indicative of a localized condition.

8. "Direct to Slaughter" — direct to slaughter shall mean the shipment of swine from the premises of origin directly to a slaughter establishment without diversion through assembly points.

9. "Permit" — an official document issued by a Veterinary Services or State representative or accredited veterinarian at the point of origin of a shipment of swine to be moved direct to slaughter which shows that the animals are either tuberculosis reactors or tuberculosis exposed, the identification of each animal, the name of the owner of such animals, the slaughter establishment to which the animals are to be moved, and that cleaning and disinfection and slaughter certification is required.

10. "Subject to Inspection" — carcass disposition based upon gross post mortem findings.

Part II: IDENTIFICATION OF SWINE

1. The State should have and enforce dealer control laws or regulations that require dealers to maintain the identification of swine and records of transactions for each animal purchased or sold.

2. Swine moved in channels of trade should be identified and recorded as to origin and destination at the first concentration point (dealer, livestock market, buying station, slaughter establishment, etc.). Swine marketed for slaughter should be identified by the nationally coded slap tattoo system or other approved system.

Part III: REPORTING OF GROSS LESIONS OF TUBERCULOSIS

A close working relationship with State and Federal Meat Inspection personnel is an essential part of the swine tuberculosis program. Tissue specimens should be submitted routinely only from swine with gross lesions of tuberculosis involving the thoracic cavity. These submissions should be reported on the form provided for such submissions of tuberculous lesions from nonreactors.

Routine passed for cooking (PFC) and condemned carcasses will be reported on a separate form in those States that have a swine tuberculosis program.

Part IV: PROGRAM PROCEDURES

A. Swine herds infected with Mycobacterium bovis or Mycobacterium tuberculosis.

When herds are found to be infected with M. bovis or M. tuberculosis (or where infection with these organisms is strongly suspected through disclosure of
generalized lesions of the thoracic cavity upon slaughter), the following measure shall be taken:

1. Quarantine all animals on the premises.
2. Conduct epidemiologic studies to determine the source(s) of infection and possible spread. Such studies will include testing of exposed herds of swine as instructed in VS Memorandum 552.16, June 16, 1961; and cattle as instructed in VS Memorandum 552.15, December 22, 1972; or other animals as indicated.
3. Reactor cattle and herd retests in cattle will be handled as recommended in UMR Tuberculosis ERadication in cattle.
4. Reactor swine will be tagged and shipped direct to slaughter under permit subject to inspection. Carcass disposition and indemnity payments when allowed, will be based upon postmortem findings. Where test results indicate herd infection is widespread complete depopulation procedures should be strongly recommended.
5. Premises where tuberculous swine are disclosed shall be thoroughly cleaned and disinfected with a disinfectant permitted by Veterinary Services, APHIS, USDA, and in a manner satisfactory to cooperating State and Federal officials.
6. Swine herd retests will be conducted as necessary to bring about quarantine release, with a minimum of two negative tests at not less than 60-day intervals.
7. Indemnity payments to owners of swine may be made in accordance with State or Federal regulations when funds are available.

B. Swine Herds Infected with Mycobacterium avium Complex.

When gross lesions of tuberculosis, other than generalized lesions of the thoracic cavity (A), are reported by meat inspection, the following measures shall be taken:

1. Trace reported lesioned swine to herd of origin.
2. If less than 5% of the carcasses in a lot exhibit gross lesions of tuberculosis and this is the first case in a 6-month period from this producer, send a letter informing him of the lesion(s) found and of the possible causes and means of eliminating the causes of infection.
3. If 5% or more of the carcasses in a lot exhibit gross lesions of tuberculosis, or this is a second instance of lesions in less than 5% of a lot within a 6-month period for one producer, conduct an epidemiologic study, including management practices and necropsies, to determine the possible source(s) of infection.
   a. Make recommendations in writing to the owner for elimination of the disease, based upon the epidemiologic findings.
   b. Herd should be placed under quarantine when allowed by existing State or Federal regulations. Identify and depopulate remaining animals direct to slaughter under permit, as they reach market weight. Carcass disposition and indemnity payments when allowed (7) will be based on postmortem findings.
4. Tuberculin testing in herds from which PFC or condemned carcasses have originated shall be done as instructed in VS Memorandum 552.16, June 16, 1961, only for the purpose of retention of valuable breeding stock at the owner's request. All sows, prospective replacement gilts and boars shall be tested. Reactors shall be tagged and go direct to slaughter under permit subject to
inspection. Carcass dispositions and indemnity payments when allowed (7) will be based on postmortem findings. All animals in the herd not tested shall be sent to slaughter as in (3b). Testing and culling of reactors shall continue in such herds until no reactors are found on two successive tests not less than 60 days apart.

5. Premises where tuberculous swine are disclosed and sent to slaughter under (3b) or (4) shall be thoroughly cleaned and disinfected with a disinfectant permitted by Veterinary Services, APHIS USDA, in a manner satisfactory to cooperating State and Federal officials.

6. Quarantines applied under (3b) shall be released only after the herd is either completely depopulated or passes the required negative tests under (4) and after cleaning and disinfection has been completed and all other recommendations in (3a) have been complied with.

7. Indemnity payments to owners of swine may be made in accordance with State or Federal regulations when funds are available upon completion of recommendations under (3a) above.
THE PAPOVAVIRUS GROUP

S. McConnell

Preface:
The following is a summary of the data presented to the Animal Virus Committee by Dr. Kenneth Takemoto, Laboratory of Viral Diseases, NIAID—NIH, Bethesda, Maryland and current literature.

Introduction:
The family Papoviridae consists of two genera; Papillomavirus and Polyomavirus. These small DNA viruses differ somewhat in size and in the molecular weight of their nucleic acid, but share a number of other properties. The history, properties of the virion and a number of other parameters were reviewed recently by Eddy (1969) and Walker et al (1973) and will not be detailed in this report.

The current knowledge on polyomaviruses, recently isolated from man, will be reviewed.

Description of the Group:
Family: Papoviridae
Genus: Papillomavirus
Genus: Polyomavirus

The similarities and differences between the two genera are listed in tabular form:

<table>
<thead>
<tr>
<th>Genus: Papillomavirus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Size of virion</td>
<td>55 nm</td>
</tr>
<tr>
<td>2. Morphology</td>
<td>icosahedral capsid with 72 capsomers, non-enveloped, multiply in the nucleus.</td>
</tr>
<tr>
<td>3. Group Members</td>
<td>rabbit papillomavirus, canine papillomavirus, bovine papillomavirus, human papillomavirus.</td>
</tr>
<tr>
<td>4. Other probable members</td>
<td>Viruses causing papillomata of other species of animals.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genus: Polyomavirus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Size of virion</td>
<td>45 nm</td>
</tr>
<tr>
<td>2. Morphology</td>
<td>icosahedral capsid with 72 capsomeres, non-enveloped, multiply in nucleus.</td>
</tr>
<tr>
<td>3. Group members</td>
<td>polyomavirus M-1; K virus, rabbit vacuolating virus, simian vacuolating virus (SV40), human polyomaviruses (3 serotypes).</td>
</tr>
</tbody>
</table>

Biological Features of the Group
1. Papillomavirus
   No readily available system available for in vitro cultivation. Virus studies limited to in vivo systems.
2. Polyomavirus
These viruses cause inapparent infections in most hosts. Many possess an oncogenic potential. The viruses can be studied by in vitro methods. These viruses may be lytic or they may produce an abortive infection. In the latter case the virus replicative cycle is incomplete, resulting in altered or transformed cells. Transformed cells contain a virus specified "T-antigen" which can be demonstrated in the cell nucleus using specific fluorescent antibody staining.

Recent Isolates of Polyomavirus from Humans

The following table summarizes the most recently isolated and identified polyomavirus and lists source of virus, isolation system used and the investigators reporting the isolation.

Table 1. New Polyomaviruses Isolated from Humans

<table>
<thead>
<tr>
<th>Polyomavirus Strain</th>
<th>Isolated From</th>
<th>Cells used for Isolation</th>
<th>Investigators</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. C. Brain*</td>
<td>Human fetal brain cells</td>
<td>Padget et al</td>
<td></td>
</tr>
<tr>
<td>B. K. Urine†</td>
<td>VERO</td>
<td>Gardner et al</td>
<td></td>
</tr>
<tr>
<td>DAR and EK Brain*</td>
<td>AGMK</td>
<td>Weiner et al</td>
<td></td>
</tr>
</tbody>
</table>

*Isolated from patients with progressive multifocal leukoencephalopathy.
†Isolated from the urine of a patient under immunosuppressive therapy.

VERO and AGMK are monkey kidney cell lines.

Both the JC and BK virus strains possess hemagglutinin and show a weak antigenic relationship to SV 40. They differ in host cell specificities in that the JC virus does not replicate in monkey cells whereas the BK virus strain does.

The DAR and EK strains to not possess a hemagglutinin, do replicate in monkey cells and are strongly related antigenically to SV 40.

These viruses apparently infect humans early in childhood and may cause mild or subclinical infections. Transmission appear to be vertical and serologic evidence suggests that antibody appears at about 2 years of age and by 15 years approximately 70% of individuals tested have specific antibody.

The evidence further suggests that these viruses may remain in a suppressed or latent state until the host's defenses are suppressed or lowered by disease and/or stress and then initiate clinical disease in the infected individual.

Immunosuppressed patients can be screened for the BK virus using electronmicroscopy techniques. There is a continuous shedding of the virus in the urine, such that 12cc's of urine pelleted directly only an EM grid provides an excellent screening test. Often, antibody is present and causes clumping of virus, enhancing the results obtained.
REFERENCES

USAHA 1974  
UNITED STATES ANIMAL HEALTH ASSOCIATION  
COMMITTEE — ANIMAL VIRUS CHARACTERIZATION  

Chairman: S. McConnell  
Co-Chairman: C. J. York  

The Committee on Animal Virus Characterization has actively participated in a number of activities during the past year. The members serve this organization through their interplay with the many committees of the USAHA, State agencies, National and International organizations.

The Committee members consult with the American Type Culture Collection on accessioning of viruses of importance to the USAHA especially those agents of current diagnostic importance. Mr. David Stevens, Curator of the V & R Committee of the ATCC has generously provided his services as an ex-officio member and meets with the Committee yearly. Committee members individually participate in the activities and programs of the National Institute of Health and the WHO/FAO Board of Comparative Virology as these programs relate to evaluation, collection, characterization and storage of reference reagents. These ancillary activities are essential to this organization’s program as they relate to diseases of food animals, companion animals and zoonosis of importance in regulatory medicine.

The Committee sponsored a program on the Papovavirus group as well as current taxonomic proposals for the arboviruses which will be considered at the next meeting of the ICNV. The Arboviruses which constitute a broad category of viruses grouped together on the basis of insect transmission are now more definitively characterized by morphological, serological, and biochemical relationships and are distributed in four families of viruses: i.e. Rhabdoviridae (rabies, vesicular stomatits, bovine ephemeral fever). Reoviridae (reovirus, bluetongue); Bunyaviridae (rift valley fever; and Togaviridae (Venezuelan equine encephalomyelitis, louping ill). Under this current system, hog cholera, bovine virus diarrhea and equine arteritis viruses can be properly classified in the family Togaviridae with which they share common morphological and biochemical characterizations but have not been shown to be insect transmitted. This nomenclature to be adopted by the ICNV was discussed and will be included as part of this report.

The papovaviruses are small DNA oncogenic viruses whose importance to animal and human health are becoming increasingly more recognized as their frequency of recovery increases, particularly in immunosuppressed or immunodeficient animals. The recent isolation of papoviruses in urine and central nervous tissue in human subjects and their previously reported isolation from...
many animal species indicates that Veterinarians need to pay more attention to
the potential dangers of this virus group. Current methods of isolation,
identification and characterization were discussed and will be appended to this
report.

In closing, the members wish to express their great appreciation to Dr. Robert Crandall for his untiring efforts in consolidating and verifying the
voluminous number of viruses that were considered for inclusion in the animal
reference virus recommendations which will be published in the American
Journal of Veterinary Research as a contribution of the Animal Virus
Characterization Committee.
ARBOVIRUSES — ECOLOGIC AND ANTIGENIC GROUPING

The arboviruses (arthropod-borne viruses) were defined originally as viruses maintained in nature by a biological transmission cycle between susceptible vertebrate hosts and hematophagous (blood sucking) arthropods. True biological transmission requires infection and propagation of the virus in the arthropod as well as in the vertebrate host, with virus present in the blood of the host during some stage of the infection. This manner of ecologic categorization has been of great practical use to the epidemiologic virologist, the central person involved in epidemic-epizootic disease control. As the epidemiologist remains the primary user of accrued information in this field, the term arbovirus still has considerable merit, and will continue to be used ecologically. However, in the interests of precision and informativeness, it will no longer be used taxonomically.

Classification of viruses meeting the ecologic definition has been based upon antigenic relationships in a scheme elaborated primarily by Dr. Jordi Casals and his colleagues at Yale University. As the shift to classification based upon virion physical properties evolved, and it was realized that the ecologic definition predicted little regarding physical properties, there was some anxious anticipation that the viruses and serogroups would be dispersed into the many taxa of a universal classification scheme. After all, arbovirologists are currently dealing with about 400 serologically distinct viruses which fall into more than 35 serogroups with over 70 ungrouped viruses left over. What has happened, however, as physicochemical data has been accumulated and collated, is that a very workable number of virus groups has emerged, and in most instances these groups have earned separate taxonomic status. Historic groupings, such as Group A, Group B, etc., have maintained their identity, if not their names. It is satisfying that the current status of arbovirus taxonomy acknowledges the foresight of Dr. Casals in initially making groupings on serologic bases.

PHYSICOCHEMICAL BASES FOR CLASSIFICATION

In any universal classification scheme, individual virus characteristics are carefully weighed to construct a hierarchy which "we would like to believe but
are unable to prove." Characteristics of nucleic acid and protein constituency may eventually predominate as classification criteria, but in practice most virion physical properties now are derived from electron microscopic examination. At this one practical level of resolution, virion morphology may be the most stable product of the viral genome. The diversity of virus morphology and morphogenesis allows distinction of many groups. In arbovirology, electron microscopy is particularly useful because it can be carried out on large numbers of viruses, and it can be used when viruses are propagated in varying substrates ranging from suckling mouse brain or liver to various cell cultures. It is particularly manageable when virus titers reach $10^6$ infectious units/gram or more, and such titers are common among arboviruses in host systems of choice. Electron microscopy has limitations as an independent basis for taxonomic placement, but it integrates well in characterization schemes. In practical arbovirology this means that morphologic-morphogenetic characteristics have a major place in defining groups, whereas serologic cross-reactivities are needed for identifying all the members of groups. The key to this scheme is that no one has ever found two arboviruses which are serologically related, but morphologically-morphogenetically distinguishable. Luckily, a *Rickettsia prowazeki*—*Proteus OX 19* analogue has not been found. At the present time large numbers of electron microscopic and serologic data bits are being collated by arbovirologists for characterization as well as taxonomic purposes. Because arbovirologists have been working in some different organizational structures than have virologists who handle other virus groups, a brief description is appropriate to bring recent developments into perspective.

Since 1966, the International Committee for the Taxonomy of Viruses (ICTV) (formerly the International Committee on Nomenclature of Viruses—ICNV), has assumed a dominant position in all viral classification matters. Its Arbovirus Study Group, under the chairmanship of Dr. James Porterfield, National Institute of Medical Research, London, has been concerned with most of the vector-borne viruses of animals, and fewer viruses have been relegated to other Study Groups. The American Committee on Arthropod-Borne Viruses (ACAV) has been the other focal point for data accumulation and distribution. This committee, which now has international representation, is responsible for the *Catalogue of Arthropod-Borne Viruses of the World* (working and published versions), the Arthropod-Borne Virus Information Exchange, and the Arbovirus Abstract Service. The ACAV has two standing subcommittees of particular interest here: the Subcommittee on Evaluation of Arthropod-Borne Status (SEAS) and the Subcommittee on the Interrelationships of Catalogued Arboviruses (SIRACA). The former subcommittee is concerned with the validity of biological transmission data, and the latter subcommittee has assumed responsibility for collating data on physiochemical and serologic characterization. Under the chairmanship of Dr. Jordi Casals, SIRACA has continually updated grouping data and evaluated subgroup relationships, and it is now contributing to the integration of minor serogroups and antigenically ungrouped viruses into the universal taxonomy scheme. This function often requires experimental work by members as well as review of known data. Possibly because of these activities of the ICTV Arbovirus Group and the ACAV, there is little overlapping effort by other agencies; neither the
WHO/FAO Comparative Virology Programme nor the Western Hemisphere Animal Virus Characterization Committee have entered the area of arbovirology, to my knowledge. One other institutionalized activity should be included here, that of hazard classification of etiologic agents. Hazard classification of etiologic agents of human disease is based on relative risks of introduction of exotic agents, risks of severe new epidemic diseases, risks of escape of viruses from laboratories, and risks to laboratory workers. The position of the U.S. Public Health Service on these matters is primarily implemented by limitation of import and transfer permits and by facilities reviews made as part of applications for financial support (see Classification of Etiologic Agents on the Basis of Hazard\(^2\)). In contrast, hazard classification of etiologic agents of animal disease is primarily concerned with quarantine, eradication and immunization programs, and is strongly supported by law and U.S. Department of Agriculture administrative policy. Because so many viruses infect both man and domestic and/or wild animals, cooperation between regulatory agencies must extend to hazard classification.

Nearly all arboviruses which have been adequately or presumptively classified fall into one of six genera, which in turn fall into four of the RNA virus families. This is shown in Fig. 1, which is a modification of the scheme revised annually by Dr. Joseph Melnick.\(^7\) Figure 1 goes beyond the genus and family designations currently accepted by the ICTV by including some genus and family names which are still under discussion. A similar informal scheme was used recently by Fenner.\(^4\) Descriptions and comments on the taxonomic status of each of the six genera containing arboviruses follow. Comprehensive lists of all the viruses in these genera will be published soon (Catalog of Arthropod-Borne Viruses of the World, 2nd edition, in preparation).

**Togaviridae — Alphavirus**

The taxonomy of alphaviruses, formerly called Group A arboviruses, has progressed well because the member viruses grow so well in cell culture. The presently recognized 20 viruses of this genus are spherical, 50-70 nm in diameter, with an icosahedral capsid (\(T = 3\)), and a unit membrane envelope with filamentous projections. They are heat, acid, lipid solvent, and detergent labile. Their RNA is single stranded and linear, has a molecular weight of \(4 \times 10^6\) daltons, and is infectious. Virions have 3 polypeptides; 2 are envelope glycoproteins with molecular weights of about 53,000, and one is the capsid structural unit with a molecular weight of 30,000. Nucleocapsid proteins have group specificity (shared by alphaviruses), but both subgroup and serotype specificities are contained in the glycoproteins.\(^8\) Details of physiochemical properties have been compared by Horzinek.\(^9\)\(^10\) Synthesis of viral constituents takes place in cytoplasm, and viral RNA synthesis is associated with membranous structures. Morphogenesis is distinctive; nucleocapsid formation often occurs upon host cell membranes and is often exaggerated. Envelopment occurs by budding of nucleocapsids through host membranes.\(^11\)\(^12\)

Alphaviruses are diverse in their association with disease in man and domestic animals. Some viruses are virtually avirulent in nature (Sindbis virus) and some are extremely lethal (Eastern equine encephalomyelitis virus in man and horses). At least 4 disease patterns are known: 1) encephalomyelitis
(Venezuelan equine encephalomyelitis in horses), 2) myositis-arthritis (chikungunya in man), 3) lymphoreticular disease (Venezuelan equine encephalomyelitis virus infection in hamsters), and 4) undifferentiated febrile illness (Semliki Forest virus in man). All alphaviruses are mosquito-borne, but because particular viruses have limited geographic distribution, the disease patterns vary in different parts of the world.

By restricting membership in the Alphavirus genus to the 20 viruses which originally constituted a serogroup, the new taxonomy remains valuable from the epidemiologic viewpoint. The genus definition, which in fact is a list of the common virion properties, remains extremely precise. This precision is maintained by relegating those non-vector-borne viruses with rather similar physicochemical properties (e.g., rubella and lactic dehydrogenase virus) to a separate genus (Rubivirus) of the Togaviridae family.

Togaviridae — Flavivirus

The flaviviruses (flavi = yellow; for the prototype yellow fever virus), formerly called Group B arboviruses, include 52 distinct members. Like the alphaviruses, they 1) are spherical, 2) have a unit membrane envelope with filamentous projections, 3) contain single stranded linear RNA (4×10^6 daltons) which is infectious, and 4) are heat, acid, solvent, and detergent labile. They differ from alphaviruses in size (40-50 nm diameter), in having ill-defined internal structure, being trypsin sensitive, and having different virion polypeptide constituency. Virions have three polypeptides; two of them are in the envelope (53,000-58,000 dalton glycoprotein — site of major serologic cross-reactivity; 9,000 dalton non-glycoprotein), and one is in the core (13,000-16,000 dalton "nucleocapsid" structural unit). Viral synthesis takes place in cytoplasm, and morphogenesis occurs by budding, primarily upon endoplasmic reticulum membranes without prior formation of distinct core particles; this morphogenesis and coincident proliferation of intracellular membranes is quite distinctive.

Flaviviruses are the etiologic agents of a wide range of diseases of man and domestic animals. Dengue, yellow fever, and several encephalitides (e.g., St. Louis and Japanese encephalitis, louping ill) are caused by member viruses with diverse organ tropisms and capacities to cause fatal host damage. Particular viruses are mosquito-borne, others are tick-borne, and a few have maintained serologic ties and yet no longer require an arthropod cycle and are spread by direct contact (Modoc, Rio Bravo viruses).

Present physicochemical data suggest that, like the alphaviruses, the flaviviruses constitute a very precisely defined genus. Key virion parameters vary minimally among those viruses which have been studied, and the complex serological intragenus relationships continue to have epidemiologic value. As in the case of the alphaviruses, similar non-vector-borne viruses, which include hog cholera, bovine virus diarrhea (mucosal disease), and equine arteritis viruses (vector relationship of the latter is questioned), are placed into a separate genus (Pestivirus) of the Togaviridae family.

Reoviridae — Orbivirus

The Orbivirus genus is a new grouping of 32 arthropod-borne viruses which have physicochemical characteristics rather like reoviruses. Virions are 65-80 nm
in diameter and have a double shell; the inner shell is an icosahedral capsid with 32 very distinct hollow capsomers.\(^{15}\) Unlike reoviruses they are very pH 3 labile and only relatively resistant to lipid solvents or detergents (titer loss $10^1$ to $10^2 \log 10$ units under standard conditions).\(^{17}\) Their RNA is double stranded and divided into 10 segments (total molecular weight $12 \times 10^6$ daltons). Virions have seven polypeptides ranging from 32,000-155,000 daltons. Viral synthesis takes place in cytoplasm and morphogenesis is associated with intracytoplasmic granular or filamentous inclusion bodies and tubular structures. Ultrastructural features are distinctive enough for identification of genus members if negative contrast preparations of free virus particles are included.

Orbiviruses have diverse ecologic niches also. Bluetongue, epizootic hemorrhagic disease of deer (EHD), and Colorado tick fever viruses are representative pathogens, but many member viruses have not been associated with disease. Vectors include mosquitoes, ticks, *Culicoides* and phlebotomine flies.

The construction of the *Orbivirus* genus served to maintain the precise definition of the *Reovirus* genus, as well as to bring together 16 independent serogroups (some with one member) from the arbovirus collection. Construction of the Reoviridae family emphasizes the fundamental position of nucleic acid structure in the universal taxonomy. However, except for these considerations, this classification has little practical value for epidemiologic use because the pathogenic member viruses must still be treated individually.

Rhabdoviridae — *Rhabdovirus*

The Rhabdoviridae family contains viruses with enough physical and biological differences to make definition complex. At the genus level, only the 23 viruses that can infect animals are included, and definition is further biased in favor of vesicular stomatitis (VSV) and rabies viruses.\(^{19}\) These rhabdoviruses are bullet shaped, about 70 x 180 nm in size (range 140-380 x 65-100 nm). They are made up of a precisely formed and cylindrically coiled nucleocapsid with surrounding unit membrane envelope covered by projections. They are heat, acid, lipid solvent and detergent labile. Their RNA is single stranded, linear, and complimentary to the transcribing strand (molecular weight $4 \times 10^6$ daltons). VSV virions have 5 polypeptide species: L, 160,000 daltons; G, 65,000 daltons, glycopeptide of surface projections, site of serotype specificity, and neutralization target; N, 54,000 daltons, nucleocapsid polypeptide with serogroup specificity; NS, 42,000 daltons, phosphoprotein of unknown function; and M, 27,000 daltons, membrane-associated polypeptide. Synthesis of virion constituents takes place in cytoplasm (nucleus required for rabies synthesis), and morphogenesis entails nucleocapsid coiling into nascent budding sites upon host cell membranes. Virion morphology and morphogenesis are so distinct that electron microscopy can substitute for the lack of serologic cross-reactivity among most animal rhabdoviruses.

Although many rhabdoviruses of animals are not known to cause disease in nature, others cause diseases as diverse as ephemeral fever of cattle and rabies of most mammals. Many of the viruses are neurotropic in experimental animals, but are epitheliotropic as well in nature.\(^{20}\)

Taxonomically, construction of the *Rhabdovirus* genus and Rhabdoviridae
family has been an obvious step. Variance in details of physical properties of virions has been quantitated, so that questions of membership need not be argued for every new virus with an elongated form. For example, Marburg virus, although having a rhabdovirus-like shape, should be excluded from the group on the basis of exceptional substructure. Placement in the Rhabdovirus genus has removed several viruses from the “ungrouped arbovirus” category, but because of the exceptional diversity of member viruses, practical value to the infectious disease virologist is yet to be decided.

Bunyaviridae — *Bunyavirus*

The *Bunyavirus* genus contains the 86 viruses formerly constituting the Bunyamwera serologic supergroup, the latter being formed to acknowledge distant serologic cross-reactions among several arbovirus serogroups. Morphologic characterization first indicated the validity of maintaining the taxonomic status of the supergroup, and now other physicochemical data confirm this. Bunyaviruses are spherical or oval, 90-100 nm in diameter, and consist of a unit membrane envelope with fuzzy projections surrounding a rather unstructured interior from which a helical nucleocapsid may be extracted. Virions are acid, lipid solvent and detergent labile. Their RNA is single stranded and linear and probably is divided into 3 segments. Virions have 3 major polypeptides (83,000 and 30,000-45,000 dalton glycopeptides of envelope; 20,000-25,000 dalton “nucleocapsid” polypeptide). Constituent synthesis takes place in cytoplasm, and morphogenesis occurs without prior core formation via budding primarily into vesicles of the Golgi complex. This morphogenesis is distinctive so that thin-section electron microscopy is worthwhile. Negative contrast microscopy is difficult except on concentrated partially purified virus preparations.

Although many member viruses are neurotropic, many others are hepatotropic in experimental animals and man (e.g., serogroup C). All viruses are mosquito-borne, and all have rather limited geographic distribution.

The taxonomy of bunyaviruses is under consideration by the ICTV and its Arbovirus Study Group at this time. It has been agreed that the viruses of the Bunyamwera serologic supergroup should constitute a new genus, but family definition is unsettled. It may be presumptuous to judge practical value at this time, other than to acknowledge that this new genus brings a large number of “left-over” viruses into the universal taxonomy scheme.

Bunyaviridae — Unnamed Presumptive Genus

In the course of ultrastructural studies of ungrouped and minor serogroup arboviruses, 45 viruses (including sero-relatives) were found to have similar thin-section morphology-morphogenesis but no serologic relation to the *Bunyavirus* genus. We tentatively placed these viruses into a separate, unnamed genus of the Bunyaviridae family. The size and shape of these viruses are similar to those of members of the *Bunyavirus* genus. They have a similar 2.5-nm-wide helical nucleocapsid and unit membrane envelope, but in some cases an ordered arrangement of surface projections has been resolved, rather than the fuzzy surface seen on viruses of the *Bunyavirus* genus. The single stranded RNA is segmented. Virion polypeptides are similar in that a 25,000 dalton polypeptide is associated with the RNA and a 65,000-75,000 dalton polypeptide is part of the envelope, but no intermediate size protein is found. Morphogenesis is
indistinguishable from that of viruses of the *Bunyavirus* genus.

Several viruses in this grouping are significant pathogens (e.g., Rift Valley fever, Nairobi sheep disease, and Congo-Crimean hemorrhagic fever viruses). Both neurotropic and hepatotropic viruses are included, and there are diverse vector associations, including phlebotomine fly, tick and mosquito-borne viruses.

Because of the minimal nature of the evidence tying these viruses together, the ICTV Arbovirus Study Group regard them as "other possible members" of the *Bunyavirus* taxon. Whether they are grouped in this way or as a second genus of the Bunyaviridae family, the merit of bringing these viruses together is best judged from the order brought to the Arbovirus Catalogue. The largest number of previously ungrouped and minor serogroup arboviruses examined have turned out to fit in this taxon.

**Other Arboviruses**

Although more than 67 arboviruses have not been classified, the primary reason has been a lack of experimental effort rather than apparent shortcomings of the universal taxonomy scheme. Most of those viruses which were considered arboviruses, but which do not fit into the described genera, have been properly placed elsewhere: 1) Cotia virus, Poxviridae; 2) Nodamura virus, Picornaviridae; 3) African swine fever virus, Iridoviridae; and 4) Nariva virus, Paramyxoviridae. Arenaviruses, which at one time were thought to be vector-borne, have been shown to be spread in nature by contact, and have been shown to stand as a separate taxonomic set. Few arboviruses have proven to be "unclassifiable," but current work on Quaranfil, Johnston Atoll, Hughes and Nyamanini viruses suggests continuing taxonomic difficulties. Because of all this recent progress, the main impact of arbovirus taxonomy in the future will most likely be related to the emergency of new exotic diseases and etiologic agents. New arboviruses are still described frequently, and new diseases require a sophisticated public health or veterinary research effort. An ability to respond to new problems meaningfully will continue to justify taxonomic programs and maintain enthusiasm for the subject.
ARBOVIRUSES

REFERENCES


79th ANNUAL MEETING
November 2-7, 1975
PORTLAND HILTON HOTEL
Portland, Oregon

80th ANNUAL MEETING
November 6-12, 1976
AMERICANA HOTEL
at BalHarbour
Miami Beach, Florida

81st ANNUAL MEETING
October 16-21, 1977
RADISSON HOTEL, DOWNTOWN
Minneapolis, Minnesota