PROCEEDINGS

SEVENTY-FIFTH

ANNUAL MEETING

UNITED STATES ANIMAL HEALTH ASSOCIATION

and

FOURTEENTH ANNUAL CONFERENCE OF
AMERICAN ASSOCIATION OF VETERINARY LABORATORY
DIAGNOSTICIANS

SKIRVIN HOTEL
Oklahoma City, Oklahoma

October 24, 25, 26, 27, 28, 29, 1971
PROCEEDINGS

SEVENTY-FIFTH
ANNUAL MEETING

of the

UNITED STATES
ANIMAL HEALTH
ASSOCIATION

Skirvin Hotel
Oklahoma City, Oklahoma
October 24, 25, 26, 27, 28, 29, 1971
CONTENTS

Report of the Committee on Animal Virus Characterization — S. McConnell, 
et al. ................................................................. 1
International Aspects of Animal Disease Control — E. E. Saulmon .......... 10
Report of the 1971 Committee on Import-Export — John F. Quinn, et al. ... 14
Spread of Scrapie Among Sheep and Goats: Scrapie Field Trial, Mission, 
Texas — A. L. Klingsporn and J. L. Hourrigan ................................ 16
Report of the Committee of Infectious Diseases of Sheep and Goats — J. L. 
Hourrigan, et al. .................................................................. 25
1971 Report of the Committee on Evaluation and Development of 
State-Federal Programs — J. L. O’Harra, et al. ............................. 28
Report of the Committee on Parasitic Diseases and Parasiticides — J. H. 
Brashear, et al. ..................................................................... 35
Report of the Committee on Professional Education and Extension — R. C. 
Hammond, et al. .................................................................. 47
The Albion Mastitis Project — A. R. Smith ........................................ 53
Report of the Mastitis Committee — J. S. McDonald, et al. ................. 62
Getting One Good Idea from a Meeting Isn’t Good Enough Anymore — 
William F. McCulloch ................................................................ 66

CATTLE

Infectious Diseases
Clinical Aspects of Bluetongue in Oregon Cattle — G. E. Reynolds ...... 74
Parainfluenza-3 Virus in Intercurrent Infections Causing Bovine Respiratory 
Disease — M. L. Frey and R. A. Ball ........................................ 80
Immunogenicity in Calves of a Bovine Viral Diarrhea Vaccine Inactivated 
with Beta-propiolactone — G. Lambert, A. L. Fernelius, and R. L. Smith . 84
Report of the Committee on Infectious Diseases of Cattle — John F. 
Hudelson .............................................................................. 90

Anaplasmosis
Promising Therapeutic Agents for the Elimination of Anaplasma Marginale in 
the Carrier Animal — K. L. Kuttler ........................................... 92
Excerpts from a Report on the Evaluation of an Attenuated Anaplasma 
Marginale Vaccine — F. C. Neal and G. T. Edds ............................ 99
<table>
<thead>
<tr>
<th>Brucellosis</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplemental Negative Cream BRT in Wisconsin — G. C. Janney</td>
<td>105</td>
</tr>
<tr>
<td>Proposal for a Swine Herd Validation Program - Via Market Swine Testing — P. B. Doby</td>
<td>111</td>
</tr>
<tr>
<td>Progress of the State-Federal Brucellosis Eradication Program — H. C. King</td>
<td>113</td>
</tr>
<tr>
<td>Current Public Health Problems of Swine Brucellosis — S. L. Hendricks</td>
<td>123</td>
</tr>
<tr>
<td>Evaluation of the Responses to the Questionnaire on the Cooperative State-Federal Brucellosis Eradication Program</td>
<td>137</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leptospirosis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>An In Vitro Growth Inhibition Test for Leptospiral Neutralization — D. N. Tripathy, L. E. Hanson, and W. A. Krumrey</td>
<td>138</td>
</tr>
<tr>
<td>Leptospirosis in Cattle and Swine in Minnesota — J. W. Glosser, et al.</td>
<td>144</td>
</tr>
<tr>
<td>Chemotherapy of Renal Leptospirosis: <em>Leptospira grippotyphosa</em> and <em>Leptospira hardjo</em> in Hamsters and Swine — O H. V. Stalheim</td>
<td>155</td>
</tr>
<tr>
<td>Report of the Committee on Leptospirosis — L. E. Hanson, et al.</td>
<td>160</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FOREIGN ANIMAL DISEASES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Overview of the 1971 Texas Venezuelan Equine Encephalomyelitis Epizootic — R. O. Spertzel</td>
<td>162</td>
</tr>
<tr>
<td>African Swine Fever — E. C. Sharman</td>
<td>176</td>
</tr>
<tr>
<td>Computer Retrieval of Animal Disease Research Records — P. Sutmoller and R. Trautman</td>
<td>180</td>
</tr>
<tr>
<td>Foot-and-Mouth Disease in Sheep and Goats: Early Virus Growth in the Pharynx and Udder — J. W. McVicar and P. Sutmoller</td>
<td>194</td>
</tr>
<tr>
<td>Experimental Multiple Infection of Animals with Foot-and-Mouth Disease Viruses — G. E. Cottral and P. Gailiunas</td>
<td>441</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DISEASES OF HORSES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation of the Immunodiffusion Test for the Diagnosis of Equine Infectious Anemia — E. Roth, et al.</td>
<td>222</td>
</tr>
<tr>
<td>Immunodiffusion Detection of Antibodies to Equine Infectious Anemia Virus Infection Associated Antigen — D. O. Morgan</td>
<td>232</td>
</tr>
<tr>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Equine Infectious Anemia: A Survey of the Equine Population of Cape May County, New Jersey — M. J. Kemen</td>
<td>241</td>
</tr>
<tr>
<td>A Prospectus on Equine Infectious Anemia with Guidelines — 1971</td>
<td>249</td>
</tr>
<tr>
<td>Immunodiffusion Test for Equine Infectious Anemia — L. Coggins and V. Patten</td>
<td>254</td>
</tr>
<tr>
<td>A Prospectus and Guidelines on African Horse Sickness — 1971</td>
<td>265</td>
</tr>
</tbody>
</table>

**PHARMACEUTICALS**

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voluntary Certification of Animal Drugs: “Where Do We Stand?” — F. H. Holt</td>
<td>270</td>
</tr>
<tr>
<td>The Livestock Drug Industry in the 1970’s — D. A. Phillipson</td>
<td>275</td>
</tr>
<tr>
<td>Safety Evaluation of An Amicarbalide Isethionate Formulation in Ponies — V. K. Weidle and G. T. Edds</td>
<td>281</td>
</tr>
<tr>
<td>Report of the Pharmaceuticals Committee — S. F. Scheidy</td>
<td>296</td>
</tr>
</tbody>
</table>

**DISEASES OF POULTRY**

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Newcastle Situation in the United States — E. E. Grass</td>
<td>298</td>
</tr>
<tr>
<td>Transmissible Diseases of Poultry Committee — H. E. Goldstein, <em>et al.</em></td>
<td>309</td>
</tr>
</tbody>
</table>

**PUBLIC HEALTH**

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental Health Aspects and Animal Diseases Associated with Animal Wastes — J. H. Steele</td>
<td>315</td>
</tr>
<tr>
<td>Public Health, Radiological Fallout and Toxicology — R. L. Parker, <em>et al.</em></td>
<td>321</td>
</tr>
</tbody>
</table>

**SWINE**

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characterization of Protective Antibodies to Transmissible Gastroenteritis Virus in Serum and Milk of Sows Exposed to the Virus — M. H. Abou-Youssef and M. Ristic</td>
<td>329</td>
</tr>
<tr>
<td>Committee on Transmissible Diseases of Swine — D. P. Gustafson, <em>et al.</em></td>
<td>342</td>
</tr>
<tr>
<td>Report of the Sub-Committee on Transmissible Gastroenteritis of Swine — E. H. Bohl, <em>et al.</em></td>
<td>345</td>
</tr>
<tr>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td><strong>HOG CHOLERA</strong></td>
<td></td>
</tr>
<tr>
<td>Processing Food Waste into a Feed Ingredient — T. W. Powell</td>
<td>348</td>
</tr>
<tr>
<td>The Status of the State-Federal Hog Cholera Eradication Program — D. R. Stauffer</td>
<td>350</td>
</tr>
<tr>
<td><strong>SALMONELLOSIS</strong></td>
<td></td>
</tr>
<tr>
<td>The Results of Feeding Salmonella-Contaminated Meat Meal to Growing Finishing Swine — W. F. Nape and C. Murphy</td>
<td>365</td>
</tr>
<tr>
<td><strong>TUBERCULOSIS</strong></td>
<td></td>
</tr>
<tr>
<td>Should Tuberculosis Be Eradicated from all Species — W. L. Mailmann</td>
<td>410</td>
</tr>
<tr>
<td>The Status of the State-Federal Tuberculosis Eradication Program — A. F. Ranney and J. D. Roswurm</td>
<td>418</td>
</tr>
</tbody>
</table>
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vii
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G. L. Crenshaw, Davis, California

R. L. Pyles, Jefferson City, Missouri
H. R. Smith, Cincinnati, Ohio
Committee on Parasitic Diseases and Parasiticides — 1972 (Continued)

J. H. Hopson, Albuquerque, N. Mexico
H. F. Groves, Columbus, Ohio
H. B. McGrath, Kansas City, Missouri
A. C. Newman, Opelika, Alabama
R. D. Radleff, College Station, Texas
I. H. Roberts, Albuquerque, New Mexico
R. H. Singer, Bryan, Texas
D. E. Zinter, Beltsville, Maryland
John Poole, Hyattsville, Maryland
W. C. Tobin, Denver, Colorado

Committee on Pharmaceuticals — 1972

Dr. Roland Gessert, Chairman, New York, New York
Dr. Fred Kingma, Co-Chairman, Washington, D.C.

D. J. Anderson, Ft. Worth, Texas
A. Freeman, Chicago, Illinois
C. C. Beck, Ann Arbor, Michigan
H. E. Gouge, St. Joseph, Missouri
K. Mayer, Chicago, Illinois
G. T. Edds, Gainesville, Florida
George Mitchell, St. Louis, Missouri
S. F. Scheidy, Bryn Mawr, Pennsylvania

Committee on Professional Relations — 1972

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Dr. N. B. Haynes, Co-Chairman, Ithaca, New York

C. C. Beck, Ann Arbor, Michigan
W. C. Burnet, Kansas City, Missouri
G. L. Crenshaw, Davis, California
C. Dobbins, Athens, Georgia
W. L. Henning, University Park, Pennsylvania
R. I. Hostetler, Pullman, Washington
Moses Simmons, Denton, Texas
T. P. Siburt, Blacksburg, Virginia
K. Weinland, Lafayette, Indiana
Robert Swope, University Park, Pennsylvania

Committee on Public Health, Radiological Fallout and Toxicology — 1972

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

Louis Locke, Patuxent, Maryland
William B. Buck, Ames, Iowa
John C. Jefferies, Hyattsville, Maryland
John Spaulding, Washington, D.C.
Hillman A. Nelson, Ames, Iowa
Raymond Fagan, Richmond, Virginia
Calvin W. Schwabe, Davis, California
J. H. Stewart, Washington, D.C.
Ted Rea, APO, New York
E. C. Sharman, Washington, D.C.
E. E. Wedman, Corvallis, Oregon
W. E. Jennings, Santa Rosa Beach, Florida
J. F. Stara, Cincinnati, Ohio
J. H. Steele, Houston, Texas

Committee on Public Relations and Local Arrangements — 1972

Dr. C. L. Campbell, Chairman, Tallahassee, Florida

W. L. Bendix, Richmond, Virginia
Norman Powers, Lake Luzerne, New York
Wilson Powell, Tallahassee, Florida
Howard Obenchain, Washington, D.C.
R. S. Pyles, Wichita, Kansas
Committee on Transmissible Diseases of Poultry — 1972

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

Raleigh Allen, Washington, D.C.  C. W. Wilder, Augusta, Maine
F. G. Buzzell, Augusta, Maine  B. S. Pomeroy, St. Paul, Minnesota
L. C. Grumbles, College Station Texas  W. Schoefield, St. Louis, Missouri
J. E. Hanley, Dade City, Florida  J. B. Thomas, Columbia, South Carolina
R. Hogue, Lafayette, Indiana  J. B. Roberts, Roland, Oklahoma
A. E. Janawicz, Montpelier, Vermont  T. B. Ryan, Raleigh, North Carolina
T. L. Landers, Chamblee, Georgia  S. A. Moore, Washington, D.C.
W. C. Patterson, Jr., Athens, Georgia  H. W. Towers, Dover, Delaware

Porter Halbert, San Augustine, Texas

Committee on Rabies — 1972

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

Marvin Goff, Ames, Iowa  Mel Abelseth, Albany, New York
A. L. Brown, Lincoln, Nebraska  W. G. Winkler, Atlanta, Georgia
T. D. Njaka, Charleston, West Virginia

Committee on Salmonella — 1972

Dr. A. A. Erdmann, Chairman, Madison, Wisconsin
Dr. W. E. Lyle, Co-Chairman, Madison, Wisconsin

C. E. Boyd, Columbia, South Carolina  N. W. Rokey, Mesa, Arizona
W. C. Ferrall, Hartford, Connecticut  R. L. Parker, Atlanta, Georgia
J. R. Hay, Chicago, Illinois  E. E. Grass, Sacramento, California
John Walker, Hyattsville, Maryland  J. S. Culbertson, Washington, D.C.
Charles Hasserman, Des Plaines, Illinois  E. V. Jeszenka, Harrisburg, Pennsylvania
J. G. Miller, Tifton, Georgia  William Dubbert, Washington, D.C.

Committee on Diseases of Sheep and Goats — 1972

Dr. J. L. Hourrigan, Chairman, Hyattsville, Maryland
Dr. G. L. Crenshaw, Co-Chairman, Davis, California

C. C. Beck, Ann Arbor, Michigan  Ward Van Horn, Buffalo, South Dakota
Donald W. Baker, Albuquerque, New Mexico  W. W. Hawkins, Bozeman, Montana
W. A. Hickman, Pierre, South Dakota  James Schoenfeld, Salt Lake City, Utah
Blaine McGowan, Davis, California  A. L. Klingsporn, Hyattsville, Maryland
R. E. Simmons, Boise, Idaho  J. H. Womack, Citrus Heights, California
T. B. Snodgrass, Dallas, Texas  L. R. Barnes, Indianapolis, Indiana
OFFICERS AND COMMITTEES

Committee on Diseases of Sheep and Goats — 1972 (Continued)

O. H. Timm, Dixon, California
H. E. Metcalf, Denver, Colorado
W. W. Clark, Mission, Texas
T. A. Kincaid, Jr., La Vernia, Texas

Committee on State-Federal Relations — 1972

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

W. L. Bendix, Richmond, Virginia
D. Spangler, Chicago, Illinois
T. A. Ladson, College Park, Maryland
O. H. Timm, Dixon, California
J. C. Shook, Mechanicsburg, Pennsylvania
J. H. Brashear, Oklahoma City, Oklahoma
H. W. Towers, Dover, Delaware
H. E. Goldstein, Columbus, Ohio

Committee on Transmissible Diseases of Swine — 1972

Dr. D. P. Gustafson, Chairman, Lafayette, Indiana
Dr. L. R. Barnes, Co-Chairman, Indianapolis, Indiana

R. A. Bankowski, Davis, California
E. M. Dwyer, Boston, Massachusetts
E. H. Bohl, Wooster, Ohio
James E. Fox, Ashland, Ohio
James B. Nance, Alamo, Tennessee
Miodrag Ristic, Urbana, Illinois
John Villari, Sewell, New Jersey
H. W. Dunne, University Park, Pennsylvania
S. S. Nicholson, Baton Rouge, Louisiana
E. A. Butler, Des Moines, Iowa
John R. Ragan, Nashville, Tennessee
Don Kruger, Olympia, Washington
Gary Combs, Hyattsville, Maryland
Don Brothers, Paducah, Texas
W. A. Stewart, Ames, Iowa
E. O. Haelterman, Lafayette, Indiana

Committee on Tuberculosis and Paratuberculosis — 1972

Dr. J. G. Flint, Chiarman, St. Paul, Minnesota
Dr. R. M. Scott, Co-Chairman, Montpelier, Vermont

V. H. Berry, Washington, D.C.
E. L. Brower, Trenton, New Jersey
C. E. Boyd, Columbia, South Carolina
B. Eller, Denver, Colorado
O. J. Halverson, Salem, Oregon
E. L. Hendee, East Lansing, Michigan
A. F. Kaufmann, Atlanta, Georgia
A. B. Larsen, Ames, Iowa
W. L. Mallman, East Lansing, Michigan
A. F. Ranney, Hyattsville, Maryland
A. P. Schneider, Boise, Idaho
R. J. Schroeder, Downey, California
H. Q. Sibley, Austin, Texas
P. L. Smith, Sacramento, California
R. J. Stadler, Hartford, Connecticut
Charles Thoen, Rochester, Minnesota
A. E. Lewis, Ottawa Ontario, Canada
W. C. TOBIN
President-Elect

OLIN H. TIMM
First Vice President

JOHN C. SHOOK
President

J. F. ANDREWS
Second Vice President

W. L. BENDIX
Secretary-Treasurer
<table>
<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
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<tbody>
<tr>
<td>26. Dec. 6-8, 1922</td>
<td>Chicago, Ill.</td>
<td>*Dr. T. E. Munce, Harrisburg, Pa.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
</tr>
<tr>
<td>27. Dec. 5-7, 1923</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. J. Butler, Helena, Mont.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
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<tr>
<td>Date Range</td>
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<tr>
<td>30. Dec. 3-1, 1926</td>
<td>Chicago, Ill.</td>
<td>*Dr. John R. Mohler, Wash., D.C.</td>
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<tr>
<td>33. Dec. 4-6, 1929</td>
<td>Chicago, Ill.</td>
<td>*Dr. Chas. G. Lamb, Denver, Colo.</td>
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<tr>
<td>34. Dec. 3-5, 1930</td>
<td>Chicago, Ill.</td>
<td>*Dr. A. E. Wight, Wash., D.C.</td>
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<tr>
<td>36. Nov. 30-Dec. 1-2, 1932</td>
<td>Chicago, Ill.</td>
<td>*Dr. Peter Malcolm, Des Moines, Iowa</td>
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<tr>
<td>37. Dec. 6-8, 1933</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. T. Faulder, Albany, N.Y.</td>
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<tr>
<td>43. Dec. 6-8, 1939</td>
<td>Chicago, Ill.</td>
<td>*Dr. J. L. Axby, Indianapolis, Ind.</td>
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<tr>
<td>44. Dec. 4-6, 1940</td>
<td>Chicago, Ill.</td>
<td>*Dr. H. D. Port, Cheyenne, Wyo.</td>
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<tr>
<td>45. Dec. 3-5, 1941</td>
<td>Chicago, Ill.</td>
<td>*Dr. E. A. Crossman, Boston, Mass</td>
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<tr>
<td>47. Dec. 4-6, 1943</td>
<td>Chicago, Ill.</td>
<td>Dr. W. H. Hendricks, Salt Lake City, Utah</td>
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<td>49. Dec. 5-7, 1945</td>
<td>Chicago, Ill.</td>
<td>Dr. C. U. Duckworth, Sacramento, Calif.</td>
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<tr>
<td>50. Dec. 4-6, 1946</td>
<td>Chicago, Ill.</td>
<td>*Dr. William Moore, Raleigh, N.C.</td>
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<tr>
<td>53. Oct. 12-14, 1949</td>
<td>Columbus, Ohio</td>
<td>*Dr. T. O. Brandenburg, Bismarck, N.D.</td>
<td></td>
</tr>
<tr>
<td>57. Sept. 23-25, 1953</td>
<td>Atlantic City, N.J.</td>
<td>*Dr. T. Childs, Ottawa, Canada</td>
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</tr>
<tr>
<td>60. Nov. 28-30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. A. L. Brueckner, Baltimore, Md.</td>
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### RECORD OF PREVIOUS MEETINGS – Continued

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<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
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<tbody>
<tr>
<td>62. Nov. 4-6, 1958</td>
<td>Miami Beach, Fla</td>
<td>Dr. John G. Milligan, Montgomery, Ala.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>65. Oct. 3-Nov. 1-3, 1961</td>
<td>Minneapolis, Minn.</td>
<td>Dr. A. P. Schneider, Boise, Idaho</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>67. Oct. 15-18, 1963</td>
<td>Albuquerque, N.M.</td>
<td>Dr. T. J. Grennan, Jr., Providence, R.I.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>70. Oct. 10-14, 1966</td>
<td>Buffalo, N.Y.</td>
<td>Dr. C. L. Campbell, Tallahassee, Fla.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
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*Deceased
†Reprinted in 54th Annual Report
††Reprinted in the 66th Annual Report

+This was the last meeting of the Interstate Association of Livestock Sanitary Boards
INVOCATION
HARRY E. GOLDSTEIN, D.V.M.

Bless us, heavenly Father, forgive our erring ways, Grant us strength to serve Thee put purpose in our days . . . Give us understanding enough to make us kind — So that we may judge all people with our heart and not our mind . . .

And teach us to be patient in everything we do, Content to trust Your wisdom and to follow after You . . . And help us when we falter and hear us when we pray And receive us in Thy Kingdom to dwell with Thee this day.

And direct us in all our doings with Thy humbleness that we may glorify Thy Holy name in transacting the business of the Seventy-fifth Convention of the United States Animal Health Association.
MEMORIAL SERVICE

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

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

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

LAWRENCE E. GREEN — (University of Pennsylvania 1915) — died April 13, 1971
WILBUR F. GUARD — (Ohio State University 1912) — died July 15, 1971
MERLE Riemenschneider — (Colorado State University 1935) — died April 26, 1971

We respectfully request all present to arise and remain standing to participate in a silent prayer for the peaceful repose of the souls of those deceased members.

SILENT PRAYER

Thank you for your respectful participation.

As we bid farewell to these colleagues who have been called on before, let us be thankful that these men have had the opportunity to so ably contribute to their respective fields, and that we have had the privilege of knowing and working with them. Let us be thankful for the ideals that in a country where men can think, act and work on their own initiative. It is this type of “ideology” exemplified by our departed friends that needs to be sustained by us who represent the living. The finest tribute we can pay these men is to continue with dedication on the same high plane set by their examples.
MESSAGE OF THE PRESIDENT-ELECT

by J. C. Shook, V.D.M.
United States Animal Health Association

It is with mixed emotions and — perhaps, considerable trepidation — that I accept this opportunity to address our organization as its president-elect. The office seems to bear a strange and ill-fated omen for us in the Northeast.

Just a year and a half ago, you will recall, President-Elect Dr. Jean Smith, of Connecticut, died during his term in office.

Since our meeting in Philadelphia a year ago, a lot of political water has gone over the dam in Pennsylvania, and I was placed in the uneviable position of having to live entirely on non-existent salary of the president-elect of the United States Animal Health Association.

I am sure that it had nothing whatever to do in bringing on President Nixon’s economic freeze.

Good fortune, however, has smiled in my direction and I am sincerely happy for the opportunity to continue in regulatory work.

I want all of you to know that I am deeply grateful for the opportunity to serve this organization which has meant so much to regulatory veterinary medicine, and to me personally over the years.

We easterners look upon the west as open spaces, lots of livestock, expansive palis and mountains, and a friendly philosophy found nowhere else in the country. Our stay here in Oklahoma City has been most enjoyable, and we look forward to the remainder of the week here.

On behalf of the Association, I want to express appreciation and gratitude to Dr. Jay Brashear and his committee for these arrangements. I know the time and effort they have put into making them. Thanks again to Jay and Betty Ann and our Ella for a job well done.

Since its inception, this organization has paved the way for regulatory veterinary medicine to control, prevent, and eradicate many diseases of our livestock and poultry populations. This has led to increased production through lower losses and better efficiency.

We have formed a close liaison with diagnosticians and, in addition, have taken important steps — such as meat and milk hygiene — to better serve consumer interests.

On the other hand, our organization — if it has had some faults — has been, perhaps, too narrow in its perspective.

Let me emphasize that this is not meant to be derogatory in any sense, but all of us — especially veterinarians — have a tendency to be self-sufficient and see things in their own way. If we are honest with ourselves, we must recognize the fact that we have sometimes allowed ourselves to get into a well defined channel where the sailing is smooth and easy.

Efforts have been made regularly to inject new vitality into our programs. In the years which I have been associated with the organization, the president each year has taken new strides to broaden our approach. New committees have been formed;
old ones have been reorganized, and our executive office has improved with a resulting brightening of the association's image.

There has been progress, but we have not gone far enough and, I feel, we have been too modest. There are still too many people asking who and what is the U.S.A.H.A. These are people—not the general public—but people who should know and who should be seeking membership and a working interest in this organization.

With rapid changes taking place in our agricultural economy, and new emphasis on environmental control and improvement, with new discoveries in technology of disease diagnosis, control and eradication, and the threat of foreign animal diseases being introduced into our country, we need to assert ourselves and let our presence be heard.

We must not seek a dictatorial or arbitrary position regarding our programs, but we—in an organization which represents both the regulatory segment of veterinary medicine, industry, and the technologically related groups—need to strengthen our leadership role.

One of the important steps that must be taken is to keep our members better informed about the activities of our association, particularly those activities in which the executive secretary is involved. For this purpose, I am suggesting establishing some sort of information exchange that will be informative without stressing that office unduly. This must work both ways. Committee chairmen and the members themselves must keep the officers and directors informed of potential and real problems promptly so that information can be disseminated and appropriate measures taken.

Positive steps also must be taken to eradicate diseases and to carefully evaluate vaccination programs to determine whether any could or should be abandoned. In this respect, the U.S.A.H.A. must assume a firm leadership role instead of waiting to follow industry's initiative.

This can be approached on a purely economic basis. It does not require a master mind in mathematics to demonstrate that it is cheaper to prevent or eradicate a disease than it is to control it and live with it. There have been a number of examples in which regulatory agencies have failed to take necessary preventive measures with a resultant introduction of a disease into a new area and a costly and time consuming effort to bring it under control.

One of the things we need to consider is a more active role for the Federal-State Relations Committee. It has long been the practice of this committee to meet once a year and discuss the gamut of regulatory problems. Sometimes we have failed to follow up as strongly as we could have. Having served on this committee for a number of years, I sometimes had the feeling that we relaxed just a little too much following our meetings. To me, this borders on a condition that I call wheel spinning.

This may sound a bit harsh, but I assure you it is not meant to be unjust criticism. These discussions are necessary, and it is important that we recognize the problems. It is my opinion that we could better serve industry if we follow up on these points several times a year. It would back up our efforts with action and add muscle to our programs. This would strengthen our position the following year—and when future budget hearings are held. It has been my experience that legislators—both state and federal—need to be kept apprised regularly of the needs of
agencies and industries or they lose sight of the relative importance of these groups. It is often more effective from an organization like ours than from people within the agency.

In this connection, I am not suggesting that the entire Federal-State Relations Committee necessarily be called upon to meet frequently. I am suggesting that we should consider some method of implementing the objectives set forth by this committee ... possibly by the follow up action on the part of certain committee members or association officers.

We are in an era of rapid change and we dare not lose sight of these trends and their impact on this organization and those whom it represents. All of us have experienced the expansion of average herd size in number of producing animals. Now we are hearing predictions — and have actually seen a beginning tendency — toward vertical integration in livestock and dairy production. Already dairy herds in excess of 1,000 milking animals — unheard of a decade ago — are becoming commonplace in some sections of the country. In other sections, they have begun developing single corporate organizations that carry on — all at one location — a complete plan that includes raising animals through a breeding program, a fattening regimen, a slaughter facility, and a product marketing service.

With these changes have come new and bigger problems. Typical is that of pollution of air, water and the environment. Particularly important to us are the pollution problems caused by odors and run-off from large livestock operations.

These problems became critical with increased competition for land for urbanization ... competition brought on by our expanding population. This problem will be greatly intensified in the future and we must be prepared to meet it and master it.

All of us are painfully aware also of the competition for tax dollars and the acute need for these dollars to carry on our vital regulatory functions. In order to meet this current challenge, I believe we must adjust programs to increase efficiency and effectiveness. There are a number of ways to accomplish this.

A close look is necessary at the possible interchange of personnel, especially those with certain added skills in various fields. The value of working together in this way — of closer cooperation between state and federal agencies — is immeasurable. This has been demonstrated in recent disease outbreaks in certain areas of our country.

Sophisticated techniques of diagnosing disease have been developed, and are being developed rapidly. But these techniques in many cases involved expensive equipment and facilities along with highly trained professional staffs. Many agencies are finding it almost prohibitive to keep up with the latest methods in order to provide industry and the profession with the ultimate in services. It is my firm belief that the answer to this need must be found in a mutual cooperation — a closer working relationship — among all regulatory agencies, both state and federal, to avoid costly duplication of effort. Laboratory equipment and professional staffs with special capabilities should be kept busy in their particular fields. Certainly they should not be relegated to routine procedures and activities because their work load is not sufficient to occupy a major portion of their time. I am convinced that if agencies work together on a regional basis, along with their federal counterparts, a considerable saving could result and more comprehensive professional services could be provided. We must remove the fences along our state
borders and work more closely together.

The result, I believe, would not only be saving in dollars, but also the prevention of overlapping of activities and an even more important saving in professional man hours. If this type of integration is not forthcoming, I fear we will soon be facing a critical shortage of scientists and that the competition of these individuals will be even more acute among our agricultural interests. For this reason, we must carefully guard and conserve manpower expenditures to adequately continue to meet our challenges.

In addition to changes and progress that I have mentioned, there will come undoubtedly, other new developments such as man-made replacements for some products of our livestock industry. Some of these products already are being marketed.

It is imperative that we not take this lightly and that we keep pace with all such developments. We must be careful that we do not lose our competitive position due to apathy on the part of industry and regulatory agencies.

Research unquestionably will continue to give us new tools with which to work. We must make the best possible use of these new developments if we are to grow stronger and more progressive in the future.

Before closing, I want to call your attention to an overlooked weakness in the field of veterinary medicine. It is regulatory veterinary education that in my opinion has been neglected in the past.

I believe this organization, the United States Animal Health Association, should take an objective look at the teaching programs of our veterinary colleges and carefully evaluate what is being done and the goals that are in sight. This organization then must be prepared to work with our veterinary educational institutions and allied veterinary organizations in establishing programs which will assure a sufficient number of qualified regulatory veterinarians for the future.

The appreciation for regulatory veterinary medicine must be part of our educational system and the educational programs must be upgraded. When this is accomplished, then those who are in administrative positions will need to reorganize classification systems and adjust salary schedules appropriately.

I have proposed very little that is really new, although I have touched on a number of pressing problems that currently confront us. I pledge myself and, hopefully, our entire organization to making a greater effort to solve these problems. We know what we want for the future, but we can only attain it by working together in seeking to achieve our goals.

The way is clear if we cooperate in every way possible.

We can expect to face emergencies in the future, such as those that we encountered during the past years. We must strive, however, to prevent emergencies from becoming catastrophies that could cause irreparable damage to our industry and our economy.

Now, one last note about our organization. The U.S.A.H.A. has had few internal problems such as those that often plague other organizations with this many members.

It is my opinion that we have escaped many of these difficulties because our membership is composed of hard-working, sincere and dedicated people, and because we have had competent leadership.

We have, however, developed a condition common to many large organizations
MESSAGE OF THE PRESIDENT-ELECT

and now find ourselves facing serious fiscal difficulties. The board of directors are aware of the situation and have studied it carefully. We pledge our efforts to solving this problem and regaining solvency for the association.

We have been talking in generalities here but hopefully we have set a direction for us. The details of implementation and the final outcome will rest with the committees and the membership. Let’s make 1972 a good year. Thank you.

Before I leave the rostrum, It is my privilege to make a presentation to our President.

In the years I have been associated with this organization I have developed a sincere admiration for the officers who assumed leadership roles. Many times we do not really get to know these people intimately however, and our admiration lacks depth.

Having worked with Dr. Mitchell during the past year, I feel I learned to know him much better and my appreciation for his straight forward approach to the affairs of the organization and regulatory veterinary medicine has grown tremendously.

He may have had to look in the mirror and assert “I am the President”, but he didn’t have any trouble convincing me he was the man for the job.

Doctor Mitchell, it is a real honor for me to present you with this certificate and this tie tac on behalf of the association. I trust you will display them proudly and that this organization can look forward to many more years of your association with us.
MESSAGE OF THE PRESIDENT
OKLAHOMA CITY, OKLAHOMA
TUESDAY, OCTOBER 26, 1971

M. D. Mitchell, D.V.M.
President

Governor Hall, members of the Association, Ladies and Gentlemen, and Dr. Shook, thank you for this momento and certificate. Words cannot express my appreciation of this presentation. I can assure you that I will wear this with the greatest amount of pride and hope it will never bring the USAHA any embarrassment. This certificate I will take home and hang in my office in Pierre in order that anyone who visits me will be able to view it.

The past year has been a pleasant one for me and I would only hope that during my term as your president some good has come on behalf of the USAHA. Most of you people who know me, know that I like to enjoy what I am doing and if I can't, then I want no part of it. At the same time, I am human to the point that I would hope that the past year would not go down as a year lost or a year of back sliding.

If you would recall, last year I did make a few specific remarks as to what I would like to see accomplished. I did ask for more and faster progress in our efforts to finish the job of eradicating Bovine Tuberculosis. While great strides did not come about, some progress was made. The Hog Cholera Eradication Program has been a huge success in my opinion, and I believe everyone would agree that the past year has been very encouraging to say the least. I am pleased to note that there has been some positive action taken to move the Screw Worm Buffer Zone further South. Perhaps even more would have been accomplished had not some unforeseen events taken place.

The outbreak of VEE among the horses in the State of Texas was probably one of the biggest new markers of the year and many man years of labor and a lot of money was spent on this project, which may have been used in other areas had the disease stayed down south of the border.

This past year has made me aware of some other things also. For example, before I was installed as president one or two people told me that I would be surprised as to how many would step forth and offer their services in order to help me conduct the business and render the service this Association is dedicated to do. There were many who did just that, some I had no idea would give any thought or have any interest in helping me, came forth and did even more than could be expected. Others, whom I sort-of expected would pitch in, did just that. All in all, I want to express my thanks to each and every one of you for your support. It has been a great year, you are the ones who made it possible. No one person could possibly do it alone.

During the year I became aware, probably more than anyone else except possibly the Secretary, that our expenditures have climbed upward just as every other cast has risen. Now we should take a serious look at our finances and start to get our house in shape rather than to wait until we are, in fact, in trouble.

We have a busy week ahead of us, so rather than me wasting your time by
standing up here, I think we better go on into the business at hand.

Again, I want to thank each and every one of you for the part you play in making the USAHA the fine organization it is. I know my successor will get the same good support. I am looking forward to a fruitful and enjoyable week.

Thank you for listening.
RESPONSE TO ADDRESS OF WELCOME

Dr. C. L. Campbell
Tallahassee, Florida

Your Excellency, Governor Hall, Mr. President, Members of the United States Animal Health Association, and guests.

In the now nineteen years in which I have been a member of this Association, I have twice had the privilege of responding to the Address of Welcome extended to us by an esteemed citizen of the host state. This, however, is my first chance to visit this fine Southwestern State and so as not to appear too unknowledgeable, I felt that I should best spend some time in researching material so as to glean information concerning the stature of Oklahoma. I know, of course, of the paramount rank of Oklahoma as a breeding cattle state and that oil and natural gas gushed freely from its many wells. I was amazed, however, to discover that there are some 80,000 of these oil wells and a tenth that many natural gas wells which flow in all but five of your 77 counties. In fact, I would presume that Oklahoma City is the only capital in the United States where oil derricks may be found on the Capitol lawn, and while not necessarily esthetic, there have none the less proved lucrative to the extent of swelling your state coffers by some $8 million in royalties.

Also of great interest in my delving into Oklahoma lore was the fact that this Association met in Guthrie, Oklahoma, a city just a few miles north of here, in 1905, two years prior to the territory’s being granted statehood. At that time Guthrie was the capital of the territory, a condition which was altered five years later when Oklahoma City assumed that prominence.

Oklahoma is a young state as compared to my native State of Florida. I believe that you were the 46th state to be admitted to the Union. You have a progressive young governor and a dynamic young State Veterinarian. With such a combination, Oklahoma can only be expected to be one of the foremost agricultural states in our nation.

My research developed many historical facts concerning Oklahoma, but it was not until my arrival here that I experienced the warmth and friendliness of your state’s citizens, and apparent inherent characteristic of the natives of the Southwest.

While my observations since arriving can testify to the congeniality of your people, I do recall however some remarks made by your very able State Veterinarian, Dr. Brashear, who was responding to the Address of Welcome in Philadelphia last year. He referred to the Oklahoma Territory and the five civilized Indian Tribes, and stated that as an Oklahoman, he often compared those places he visited with his native state. In drawing an analogy with Pennsylvania’s founders who but a short time prior thereto had been unwanted British citizens, he noted that Oklahoma too had been civilized by Indians and outlaws unwanted in other parts of the United States.

Apparently, Governor Hall, from what I have read during the past few weeks, your Sooners are still not too popular in some regions of our country, like SMU, Pitt, USC, Texas, Colorado and just this past week-end Kansas State. And judging from the overpowering scores, it occurs to me that these Sooners will continue alienating themselves to the extent of becoming the Nation’s No. 1 antagonists.

In all sincerity, Governor Hall, on behalf of the United States Animal Health...
Association, I would like to say that we are indeed happy for the privilege of meeting in this land of youth and promise, and to express our thanks for your very gracious greeting. I assure you that the warmth of your welcome is exceeded only by our desire to enjoy your hospitality.
The Secretary is most happy to greet all of the members and guests of this, the Seventy-Fifth Annual Meeting of the United States Animal Health Association, here in Oklahoma City, Oklahoma. The hotel informs us that reservations have been very encouraging and that we should have a fine meeting with, hopefully, record attendance. The Secretary certainly hopes that you have all found accommodations that are both satisfactory and pleasant. The local committee has been working extremely hard to provide the members with some pleasant entertainment during the perhaps all-too-brief periods when we will not be working. We hope that you will all take full advantage of these outings, not only because Doctor Brashear and his group have worked so hard to set them up, but because they certainly will be most enjoyable. The program for the ladies this year has been planned with especial care. We hope to have a particularly large attendance for our distaff side. I hope you will all join me in wishing the ladies a most pleasant week here and urge them to participate fully in those things that have been provided for their entertainment.

You will notice in your program that we have again attracted what might be considered satellite meetings of national groups, both industry and government, who find it profitable to meet with us at our annual meeting. This year, in addition to the American Association of Veterinary Laboratory Diagnosticians, I believe there are nine such additional groups. They are most welcome. We are delighted to have them meet with us and, after their own meetings, participate with us in the United States Animal Health Association’s deliberations. It is hoped that both the committee programs and the general program this year meet with your approval and that you all profit thereby. You will notice that we have made some slight change in the format, particularly with respect to the Report of the Committee on Nominations and the election of officers. These two functions have been scheduled for Thursday at noon, rather than at the close of business on Friday. We hope in this way to have a much better representation of the membership in the audience. There has been some discussion, and, as a matter of fact, we have had a request to give serious consideration to trying to arrange our departure at the close of business on Thursday. The Secretary and the program committee have tried to work this out, but to date we have been unable to accommodate all the speakers and committees that wish program space without running the meeting over into Friday. However, this is your meeting, and it is hoped that any of you who have thoughts concerning this matter will express them to the Secretary, along with any suggestions you may have for improving our meeting to make it not only more interesting but more informative.

About the only major problem that arose during the recently ended fiscal year was the appearance in the United States of Venezuelan equine encephalomyelitis. The United States Animal Health Association, as you probably are aware, had
called attention to the possibility of this disease appearing, in the Proceedings of both last year and the previous year. At the time it did appear and vaccination began, there was a great deal of misunderstanding about the vaccine and about its use and safety, which resulted in a rash of emergency rules and regulations restricting the movement of horses around the country. As a result of this what might be called general confusion, President Mitchell called a meeting of our Executive Committee in Chicago on August 17, inviting also members of the biological manufactureres, United States government military experts, and others, so that we might have the facts and, if possible, end the confusion. This meeting was extremely well attended, and the minutes thereof will appear as an addendum to the minutes of this year's Executive Committee meeting. A great deal of the confusion and misunderstanding about Venezuelan equine encephalomyelitis and the vaccine was cleared up, and, as a result, most, if not all, state barriers were dropped and we have gone ahead with the task of vaccinating most of the horses in the United States. It is hoped that by so doing and perhaps by encouraging the continued use of the commercial product now available, any severe outbreak of Venezuelan equine encephalomyelitis will be prevented, as well as any potential threat to our human population.

The most urgent part of this report will deal with the Association's finances. You will note that when we ended our 1970 fiscal period, as of September 30 of that year, our auditor had established our net worth as $32,997.72. We finished up the fiscal year immediately past on September 30, 1971, with our auditor establishing our net worth at $21,791.89. We are the victims of inflation and, at this point in time, it does not seem to be improving. The administration is endeavoring to hold inflation, once it is brought under control, to somewhere around 2 or 3 percent annually; but even that is still inflation. Every attempt was made during this past fiscal year to hold expenses at a minimum and, at the same time, fulfill the responsibility of the Association not only to its membership but to the nation's animal agriculture and related fields. Our cash balance at the beginning of the fiscal year, October 1, 1970, was $3,907.74. During the year, this balance was increased by cash receipts in the amount of $41,119.47, which made a total cash amount available for the year of $45,026.74. Our expenditures for the year totaled $40,606.80, leaving us a cash balance as of September 30, 1971, of $4,419.94.

Disregarding the cash balance left over from the two fiscal years and simply totaling receipts and expenditures for the immediately past year, our excess of revenue amounted to $512.67. Under the United States Revenue Code, we are officially a nonprofit organization, and a mere five hundred dollars of revenue over expenditures for the period would appear to substantiate it. However, our problem is that it was necessary during the year to liquidate one of the two 4% United States Treasury Bonds that were part of our capital assets. The bond was sold at a discount based on the New York market, with the approval of the Board of Directors when they met on July 21, 1971, in connection with the American Veterinary Medical Association convention in Detroit. Had it not been for the sale of this bond, we would have been several thousand dollars in the red. All of this is in the Treasurer's report, as certified by our accountant. Also attached to this report, which will appear in the Proceedings, is a breakdown of our expenditures over the past three fiscal years, with an estimate of our minimum budget for the
1972 year. Our expenditures for fiscal 1969 were $28,460.13 and for fiscal 1970, $32,270.19. Expenditures for fiscal 1971 were $39,441.44, as shown on the cost-analysis statement (the difference between this figure and the one in the financial statement is that the former was developed as of August 18, 1971, and the latter as of September 30, 1971). We have estimated our budget for the 1972 fiscal year at $43,160.00. You will note that the cost of our annual meeting jumped from $1,528.21 in fiscal year 1969 to $3,272.54 in fiscal 1971. Our largest item, of course, is printing, and here our cost increase was amazing. Our total printing bill in 1969 was $11,363.36; in 1971, as of August 18, it was $18,934.52. Under a new arrangement with the American Association of Veterinary Laboratory Diagnosticians, as you probably know, the Executive Committee increased the amount supplied them for expenses from $200 to $600 annually.

An additional source of revenue must be found. The $100 annual dues paid by official and allied-organization members, in the opinion of the Treasurer, would be difficult to increase at this time. We have raised the registration fee from $10 to $20, and, in the case of non-members, to $30, which does include a copy of the Proceedings. This has helped considerably, but it is still far short of our current needs. With roughly 866 individual members, a $5 increase in dues would produce some $4,000 added revenue, and an additional $10 would of course produce in excess of $8,500 increased revenue. We are going to try to hold expenditures down as much as possible; but even so, doubling individual dues would keep us right on the verge of deficit operations, which is not good business and leaves nothing for emergencies. We were fortunate that the special meeting in Chicago cost the Association nothing, because we had a luncheon at O'Hare Airport and priced it to take up the cost of the meeting room.

Each member of the Executive Committee will receive at this meeting a complete financial report signed by our auditor. I have not prepared enough for all the members, but any member wishing a full financial statement prior to the time it appears in this year's Proceedings may get one at once by simply dropping a postcard to the Secretary and asking for one.

The fact remains, as stated in the beginning, we have got to find at this meeting an additional source of revenue if this Association is to remain solvent and active.
UNITED STATES ANIMAL HEALTH ASSOCIATION
1444 East Main Street
Richmond, Virginia 23219

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR PERIOD
OCTOBER 1, 1970 THROUGH SEPTEMBER 30, 1971

DECREASED BY EXPENDITURES:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual Meeting</td>
<td>$3,392.99</td>
</tr>
<tr>
<td>Printing</td>
<td>$19,268.31</td>
</tr>
<tr>
<td>Office Supplies</td>
<td>$847.82</td>
</tr>
<tr>
<td>Salary</td>
<td>$11,087.50</td>
</tr>
<tr>
<td>Social Security Tax</td>
<td>$566.50</td>
</tr>
<tr>
<td>Communication</td>
<td>$2,252.70</td>
</tr>
<tr>
<td>Travel: Dr. M. D. Mitchell</td>
<td>$250.68</td>
</tr>
<tr>
<td>Dr. J. C. Shook</td>
<td>$76.46</td>
</tr>
<tr>
<td>Dr. W. C. Tobin</td>
<td>$520.42</td>
</tr>
<tr>
<td>O. H. Timm</td>
<td>$575.20</td>
</tr>
<tr>
<td>Dr. David L. Smith</td>
<td>$87.36</td>
</tr>
<tr>
<td>Dr. J. L. O'Harra</td>
<td>$358.00</td>
</tr>
<tr>
<td>Ella R. Blanton</td>
<td>$100.00</td>
</tr>
<tr>
<td>Other Meetings</td>
<td>$228.03</td>
</tr>
<tr>
<td>American Association of Veterinary Livestock Diagnosticians</td>
<td>$600.00</td>
</tr>
<tr>
<td>Miscellaneous Expense</td>
<td>$363.02</td>
</tr>
<tr>
<td>Bank Service Charge</td>
<td>$31.81</td>
</tr>
</tbody>
</table>

Total Decreased by Expenditures: $40,606.80

CASH BALANCE — SEPTEMBER 30, 1971:

- Cash on hand-September 30, 1971 $277.00
- Southern Bank and Trust Company
  Richmond, Virginia
    Checking Account $4.99
    Savings Account $4,006.50
    Local Arrangements Account $129.45
- Trevose Savings and Loan Association
  Morrisville, Pennsylvania $1.00
- Sandia Savings and Loan Association
  Albuquerque, New Mexico $1.00

Total Cash Balance: $4,419.94
REPORT OF THE SECRETARY-TREASURER

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

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR PERIOD
OCTOBER 1, 1970 THROUGH SEPTEMBER 30, 1971

CASH BALANCE – OCTOBER 1, 1970:

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

**INCREASED BY CASH RECEIPTS:**

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual Dues</td>
<td>$8,660.00</td>
</tr>
<tr>
<td>Official Dues</td>
<td>5,200.00</td>
</tr>
<tr>
<td>Proceedings</td>
<td>2,599.00</td>
</tr>
<tr>
<td>Reprints</td>
<td>4,215.97</td>
</tr>
<tr>
<td>Registration Fees</td>
<td>10,245.00</td>
</tr>
<tr>
<td>Foreign Animal Books</td>
<td>735.00</td>
</tr>
<tr>
<td>Interest Income</td>
<td>1,964.50</td>
</tr>
<tr>
<td>Sale of one U.S. Treasury Bond</td>
<td>7,500.00</td>
</tr>
</tbody>
</table>

**TOTAL BEGINNING BALANCE AND RECEIPTS**

$45,026.74
UNITED STATES ANIMAL HEALTH ASSOCIATION
1444 East Main Street
Richmond, Virginia 23219

SUMMARY OF OPERATIONS
FOR PERIOD OCTOBER 1, 1970 THROUGH SEPTEMBER 30, 1971

REVENUE:

Total Cash Receipts $41,119.47
Less — Expenditures 40,606.80

Excess of Revenue over expenditures $ 512.67

NET WORTH — SEPTEMBER 30, 1971

Cash on hand 277.00
Accounts Receivable 3,240.36

Balance:
Southern Bank and Trust Company, Richmond, Virginia
Checking Account 4.99
Savings Account 4,006.50
Local Arrangements Account 129.45

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 3,100.00
U.S. Treasury Bond 10,000.00
Furniture and Fixtures 906.59

NET WORTH — SEPTEMBER 30, 1971 $21,791.89
ANALYSIS OF CHANGE IN NET WORTH:

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Worth, September 30, 1971</td>
<td>$32,997.72</td>
</tr>
<tr>
<td>Decreased by:</td>
<td></td>
</tr>
<tr>
<td>Sale of one Treasury Bond</td>
<td>$10,000.00</td>
</tr>
<tr>
<td>Accounts Receivable</td>
<td>2,653.72</td>
</tr>
<tr>
<td>Furniture and Fixtures</td>
<td>26.17</td>
</tr>
<tr>
<td>Sub Total</td>
<td>12,679.89</td>
</tr>
<tr>
<td>Increased by:</td>
<td></td>
</tr>
<tr>
<td>Inventory - Supplies and Proceedings</td>
<td>$961.39</td>
</tr>
<tr>
<td>Excess of Revenue over Expenditures</td>
<td>512.67</td>
</tr>
<tr>
<td>Net Worth, September 30, 1971</td>
<td>$21,791.89</td>
</tr>
</tbody>
</table>

Henry H. Budd
Accountant
**UNITED STATES ANIMAL HEALTH ASSOCIATION**

**COMPARATIVE COST ANALYSIS STATEMENT**

<table>
<thead>
<tr>
<th>Item</th>
<th>Fiscal Year 1969</th>
<th>Fiscal Year 1970</th>
<th>Fiscal Year 1971</th>
<th>(estimated) Fiscal Year 1972</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual Meetings</td>
<td>$ 1,528.21</td>
<td>$ 2,773.89</td>
<td>$ 3,272.54</td>
<td>$ 4,300.00</td>
</tr>
<tr>
<td>Printing</td>
<td>11,363.36</td>
<td>12,272.59</td>
<td>18,934.52</td>
<td>19,000.00</td>
</tr>
<tr>
<td>Postage &amp; Communication</td>
<td>1,573.08</td>
<td>2,127.02</td>
<td>2,417.55</td>
<td>2,800.00</td>
</tr>
<tr>
<td>Office Supplies</td>
<td>427.36</td>
<td>666.64</td>
<td>719.55</td>
<td>900.00</td>
</tr>
<tr>
<td>Salary</td>
<td>10,500.00</td>
<td>10,800.00</td>
<td>11,100.00</td>
<td>11,100.00</td>
</tr>
<tr>
<td>Social Security Tax</td>
<td>378.43</td>
<td>655.55</td>
<td>373.10*</td>
<td>600.00</td>
</tr>
<tr>
<td>Travel</td>
<td>2,257.75</td>
<td>2,400.64</td>
<td>1,722.12</td>
<td>2,300.00</td>
</tr>
<tr>
<td>Auditor</td>
<td>200.00</td>
<td>200.00</td>
<td>200.00</td>
<td>225.00</td>
</tr>
<tr>
<td>AAVLD</td>
<td>200.00</td>
<td>200.00</td>
<td>600.00</td>
<td>600.00</td>
</tr>
<tr>
<td>Office Equipment</td>
<td>14.52</td>
<td>139.88</td>
<td>71.24</td>
<td>1,300.00**</td>
</tr>
<tr>
<td>Bank Service Charge</td>
<td>17.42</td>
<td>33.98</td>
<td>30.82</td>
<td>35.00</td>
</tr>
<tr>
<td></td>
<td>$28,460.18</td>
<td>$32,270.19</td>
<td>$39,441.44***</td>
<td>$43,160.00****</td>
</tr>
</tbody>
</table>

*Employers cost of Social Security Tax for last quarter of fiscal year is to be paid.

**Office equipment will have to be bought when we move from present quarters. We have been using free the State’s desk and chairs.

***Miscellaneous charges still to be included in this amount.

****Estimated cost of rent per month when we move from present quarters, minimum, $150.00, plus utilities. Minimum cost per year, $1800.00 to be added to the estimated figure above.

Income                                         | $30,937.67        | $28,038.96        | $31,000.00        | $31,000.00                   |
REPORT OF THE COMMITTEE ON NOMINATIONS, RESOLUTIONS AND INTERNAL AFFAIRS

The Committee met the afternoon of October 27 and 28. Members present were: John Safford, Grant Kaley, Clarence Campbell, John Quinn and John O'Harra, acting chairman.

The Committee requests the Executive Secretary to prepare appropriate resolutions or expressions of appreciation to all who have contributed to the hospitality and the success of this meeting held in Oklahoma City and transmit same.

Your Committee took action on resolutions received as follows:
A. Northeastern Animal Health Association resolution concerning vaccination of horses for VEE. The resolution was recognized in the Committee report on Infectious Diseases of Horses.
B. Northeastern Animal Health Association resolution pertaining to special and limited licenses – referred to the Committee on Biologics.
C. Public Health and Radiological Fallout Committee resolution proposing name change of the Committee – referred to the Board of Directors for action.
D. Anaplasmosis Committee resolution on anaplasmosis free herds. The resolution appears as a part of the committee report which has been approved and is a matter of record.
E. Committee on Parasitic Diseases and Parasiticides resolution on demonstration of heel fly control – referred back to the Committee suggesting the resolution be directed to functional organizations.
F. Committee on Meat and Poultry Hygiene resolution requesting legislation for quarantine authority – referred back to committee for clarification.
G. A resolution received from A.A.V.L.D. – on diagnostic reference assistance – referred to the Committee on Biologics.

The following resolutions were approved and are recommended for adoption by the members of the United States Animal Health Association:

Resolution No. 1

ORIGIN OF RESOLUTION: United States Animal Health Association Committee on Infectious Diseases of Horses
DATE OF ORIGIN: October 25, 1971
NASDA COMMITTEE:
DATE RECEIVED BY NASDA COMMITTEE:
SUBJECT OF RESOLUTION: VEE Control for 1972

WHEREAS, it is evident that VEE continues to appear among unvaccinated horses in Texas; and
WHEREAS, epidemiologists and all others who have closely observed the
disease during the past few years anticipate that VEE will appear in unpredictable areas of the United States during the Spring and Summer of 1972; and

WHEREAS, it is recognized that the most effective means of preventing the spread of VEE is to have an immune horse population; consequently, all horses possible should be vaccinated prior to the 1972 mosquito season; and

WHEREAS, it is recognized that at this time the horse is the only sentinel animal effective for determining where the virus exists and that sick horses will be the first detectable indication of an outbreak; and

WHEREAS, it is recognized that several classes of susceptible horses exist and will continue to exist in areas where vaccination has been practiced, namely: (1) new horses moving into the area, (2) foals born subsequent to the vaccination program, (3) foals vaccinated too young to develop lasting immunity, (4) vaccinated horses that failed to develop immunity, and (5) horses that were overlooked and never vaccinated; and

WHEREAS, it is recognized that the lack of a system for the individual indentification of horses is a most serious obstacle to administering VEE as well as other disease programs;

NOW, THEREFORE, BE IT RESOLVED that the following protective actions be taken as soon as possible by all parties concerned:

1. USDA, effective January 1, 1972, require that all horses, mules, and donkeys moving interstate be accompanied by a certificate of vaccination for VEE signed by an approved veterinarian during 1971 or 1972.

2. USDA, as soon as possible, determine the interval at which vaccinated horses should be re-vaccinated.

3. USDA and all states establish all VEE cases and suspect cases as a required reportable disease.

4. USDA and the states re-evaluate, expand and improve the system to expedite disease reporting.

5. USDA establish or assure an adequate VEE laboratory diagnostic capability and maintain work priorities to insure that diagnoses are promptly made and reported on all suspect cases.

6. USDA take steps to insure that an adequate reserve stockpile of vaccine is maintained and be in a position to again implement state by state compulsory vaccination programs should a situation develop where such action should be taken.

7. USDA expedite and supplement where necessary the "Continuing Studies Related to Efficacy and Safety of the Vaccine in Equines" currently agreed upon between USDA and the licensed producer of commercial VEE vaccine.

8. The states of Texas, Louisiana, Arkansas, California, Arizona, Oklahoma and New Mexico, effective January 1, 1972, require that all horses, mules and donkeys being transported within the respective states be accompanied by a certificate of VEE vaccination signed by an approved veterinarian during 1971 or 1972.

9. The management of all race tracks, horse shows, rodeos and other activities where horses are concentrated require that all entries have a certificate of VEE vaccination signed by an approved veterinarian during 1971 or 1972.

10. VSDA publicize to the horse owner and the veterinary profession the advisability of vaccinating as many horses for VEE as possible.

11. Veterinary practitioners be apprised of the foregoing and make every
REPORT OF THE COMMITTEE

effort to promptly report all suspect cases so that any new outbreak will be quickly
diagnosed and recognized.

12. USDA give priority to the development of a practical individual animal
identification system.

ACTION TAKEN BY STANDING COMMITTEE

ACTION TAKEN BY RESOLUTIONS COMMITTEE

ACTION TAKEN BY NASDA

Resolution No. 2

ORIGIN OF RESOLUTION: U. S. Animal Health Association
Import-Export Committee

DATE OF ORIGIN: October 26, 1971

NASDA COMMITTEE

DATE RECEIVED BY NASDA COMMITTEE:

SUBJECT OF RESOLUTION: Mandatory Agricultural Clearance of International
Carriers

RESOLVED, That the United States Animal Health Association in formal
session at its seventy-fifth Annual Meeting held October 24-29, 1971, at Oklahoma
City, Oklahoma, strongly urges that legislation be enacted to provide mandatory
agricultural clearance of carriers as a condition of entry into the United States; and
RESOLVED FURTHER, That copies of this Resolution be forwarded to the
National Association of State Departments of Agriculture, to the Secretary of the

ACTION TAKEN BY STANDING COMMITTEE

ACTION TAKEN BY RESOLUTIONS COMMITTEE

ACTION TAKEN BY NASDA

BACKGROUND INFORMATION
REGARDING THE DEVELOPMENT OF THIS RESOLUTION

The Bureau of Census, Immigration and Naturalization Service and the United
States Public Health Service each have enabling legislation requiring clearance of
carriers of passengers and freight on ships and planes entering the United States
from foreign countries. Currently, the United States Department of Agriculture
lacks such authority for animals and animal products, and it is vitally needed to
provide for adequate inspection of such agricultural products to assist in preventing
the introduction of exotic animal diseases into this country.

Resolution No. 3

WHEREAS, there has recently been a tendency to de-emphasize the role of
veterinary public health and food hygiene in some veterinary schools of the United
States, and,
WHEREAS, without adequate instruction in these areas graduating veterinarians will be less capable of meeting the broad needs of the public,

BE IT RESOLVED that the Committee on Public Health, Radiological Fallout and Toxicology recommends that the faculties of all veterinary schools give adequate coverage and adequate perspective to the application of the role of the graduate veterinarian in the terrestrial and aquatic food chain as well as other aspects of the environment essential to the health and welfare of mankind.

Resolution No. 4

ORIGIN OF RESOLUTION: Anaplasmosis Committee — USAHA Organization
DATE OF ORIGIN: October 26, 1971
NASDA COMMITTEE
DATE RECEIVED BY NASDA COMMITTEE
SUBJECT OF RESOLUTION: Living Attenuated Anaplasmosis Vaccine

Be it resolved: the USAHA urges the Veterinary Biologics Division, USDA to deny licensing any living, attenuated anaplasmosis vaccine until such time as possible transmittal factors, adverse vaccine reactions, interference with diagnostic procedures to complicate interstate and international movement of cattle and other problems of concern to the industry and regulatory officials be satisfactorily resolved.

STATEMENT OF JUSTIFICATION

Anaplasmosis is a serious disease of cattle particularly in certain endemic areas in the United States. Regulatory officials in some states and countries now require import cattle to meet free anaplasmosis qualifications. Living attenuated vaccines can cause prolonged vaccine titres interfering with the test for this disease therefore complicating and denying movements of cattle. The industry also must be assured any future licensing of a new living vaccine will not be capable of transmitting the disease.

ACTION TAKEN BY STANDING COMMITTEE
ACTION TAKEN BY RESOLUTIONS COMMITTEE
ACTION TAKEN BY NASDA

Resolution No. 5

ORIGIN OF RESOLUTION: Committee on Infectious Diseases of Cattle — USAHA Organization
DATE OF ORIGIN: October 26, 1971
NASDA COMMITTEE
DATE RECEIVED BY NASDA COMMITTEE
SUBJECT OF RESOLUTION

Whereas, a condition commonly known as "Sudden Death Syndrome" exists in feedlots throughout the United States with sudden deaths occurring in heavier cattle not manifesting any previous illness; and
Whereas, the etiology of the "Sudden Death Syndrome" is obscure; and no consistent preventive methods are known; and
Whereas, it is conservatively estimated that the "Sudden Death Syndrome" losses to the feedlot industry are in excess of $15,000,000 annually;
Now therefore be it resolved that the United States Animal Health Association recommend that funds be appropriated for research on the cause(s) and prevention of The Sudden Death syndrome in feedlot cattle.

Committee on Infectious Diseases of Cattle

This "Sudden Death Syndrome" is of great economic importance throughout the United States and especially in the large feedlot areas of the midwest and Western areas. This syndrome is associated with heavy feedlot cattle that are found dead without clinical signs. The major necropsy lesion is that of peritracheal hemmorhage and edema. Rapid decomposition is usually observed. Many signs of clostridial infections are often noted; however, at this time it has not been determined whether the primary cause of death is physiological or infectious.

Resolution No. 6

ORIGIN OF RESOLUTION: Committee on Leptospirosis of the USAHA Organization
DATE OF ORIGIN: October 27, 1971
NASDA COMMITTEE: Committee on Leptospirosis
DATE RECEIVED BY NASDA COMMITTEE: October 27, 1971
SUBJECT OF RESOLUTION: Licensed Plate Antigens for Leptospirosis Testing

BE IT RESOLVED THAT, only Veterinary Biologics Division licensed antibodies be used to conduct microscopic plate tests for the diagnosis of leptospirosis in cattle and swine and that known positive and negative sera be included in the tests.
BE IT FURTHER RESOLVED THAT, the Executive Committee of the USAHA transmit this recommendation to regulatory officials, to the AAVLD and to the directors of veterinary diagnostic laboratories.
WHEREAS, licensed (USDA) plate antigens are now available for the diagnosis of leptospirosis due to *pomona, grippotyphosa, hardjo, icterohaemorrhagiae* and *canicola* serotypes:
BE IT RESOLVED THAT, only Veterinary Biologics Division licensed antigens be used in the microscopic plate tests for the diagnosis of leptospirosis in cattle and swine and that known positive and negative sera be included in the testing.

ACTION TAKEN BY STANDING COMMITTEE: Approved October 28, 1971
ACTION TAKEN BY RESOLUTIONS COMMITTEE
ACTION TAKEN BY NASDA
Resolution No. 7

ORIGIN OF RESOLUTION: Committee on Leptospirosis of the USAHA Organization

DATE OF ORIGIN: October 27, 1971

NASDA COMMITTEE: On Leptospirosis

DATE RECEIVED BY NASDA COMMITTEE: October 27, 1971

SUBJECT OF RESOLUTION:

THEREFORE, BE IT RESOLVED THAT, the Executive Committee of the USAHA encourage consultations and meetings between producers of veterinary biologics, representatives of the Veterinary Biologics Division, Plant and Animal Health Services, USDA, and representatives of the Leptospirosis Committee of the USAHA to discuss, formulate and recommend workable procedures to develop new leptospiral immunizing agents.

WHEREAS, bacterins containing *grippotyphosa* and *hardjo* serotypes would be useful in controlling these infections, and

WHEREAS, as considerable information and technology is available to expedite their development and licensure:

ACTION TAKEN BY STANDING COMMITTEE: Approved October 28, 1971

ACTION TAKEN BY RESOLUTIONS COMMITTEE:

ACTION TAKEN BY NASDA:

Resolution No. 8

ORIGIN OR RESOLUTION: Mastitis Committee — Organization

DATE OF ORIGIN: Wednesday, October 27, 1971

NASDA COMMITTEE

DATE RECEIVED BY NASDA COMMITTEE

SUBJECT OF RESOLUTION: Streptococcus Agalactiae Eradication from Dairy Cows

The following resolutions were unanimously adopted.

Be it resolved that the U.S.A.H.A. recommends:

(1) Adoption, by each state, of a program for the control and eradication of *Streptococcus agalactiae* infection in dairy cattle, and

(2) Each state prohibit importation of pre-parturient and lactating dairy cattle unless such cattle shall be shown to be free from *Streptococcus agalactiae* infection.

JUSTIFICATION:

As *Streptococcus agalactiae* is an eradicable, infectious and contagious disease that causes serious economic loss to the dairy industry, the Committee strongly feels that infected animals should not be moved intrastate or interstate.

ACTION TAKEN BY STANDING COMMITTEE

ACTION TAKEN BY RESOLUTIONS COMMITTEE

ACTION TAKEN BY NASDA
Resolution No. 9

ORIGIN OF RESOLUTION: Committee on Foreign Animal Diseases
DATE OF ORIGIN: October 27, 1971
NASDA COMMITTEE
DATE RECEIVED BY NASDA COMMITTEE
SUBJECT OF RESOLUTION: Authority to control Movement of Livestock In The U.S. Prior to Confirmation of Disease

NOW THEREFORE BE IT RESOLVED, THAT the USDA in cooperation with the appropriate states, take action to obtain the Legislative authority for state and federal livestock officials to institute quarantine and control procedures within the United States when an exotic disease is recognized as an imminent threat to its livestock and before the disease actually gains entry into this country.

BE IT FURTHER RESOLVED, THAT copies of this Resolution be forwarded to the National Association of State Departments of Agriculture and to the Secretary of the United States Department of Agriculture.

JUSTIFICATION

VEE erupted explosively in June 1971 in south Texas as predicted. Control procedures including spraying and vaccination were massive and expensive. Because equine traffic control procedures could not be implemented until a laboratory confirmed diagnosis was made, the movement of horses out of Cameron and Hidalgo counties of Texas in late June and early July contributed greatly to the dissemination of the VEE virus in the 1971 Texas outbreak. Additionally, in spite of our awareness of the steady northward progression of the disease through Tamalipas State, Mexico, we could not implement one of the most effective procedures to control the spread of disease. Fortunately an effective vaccine was available for contingency use.

But what of other "exotic" diseases for which no vaccine exists? How can we possibly expect to control a rapidly spreading disease without the authority to institute control procedures prior to the arrival of the disease? It is increasingly apparent that the present speed and mode of livestock transportation increases the threat of introduction and spread of a new disease(s). Therefore a re-evaluation of our concepts of disease prevention and control is required which will provide authority for state and federal livestock disease control officials to act before the fact rather than after the disaster.

Therefore, the Committee on Foreign Animal Diseases submits the following resolution for consideration by the U.S. Animal Health Association and recommends its adoption:

Whereas animal disease control procedures cannot be imposed within this country until the disease which threatens the health of U.S. livestock is confirmed in this country by laboratory diagnosis and

Whereas lack of authority to implement disease control procedures results in unnecessary and undesirable delays in preventing the spread of exotic diseases and
Whereas authority to prohibit livestock movement in the areas immediately threatened may be the primary control measure to contain a disease within a limited area in this country until other control procedures can be implemented:

**ACTION TAKEN BY STANDING COMMITTEE:**

**ACTION TAKEN BY RESOLUTIONS COMMITTEE:**

**ACTION TAKEN BY NASDA:**

Your Committee submits the following nominees for offices of the United States Animal Health Association:

- **President** ...................................... Dr. J. C. Shook
- **President-Elect** ................................ Dr. W. C. Tobin
- **First Vice-President** .............................. Mr. O. H. Timm
- **Second Vice-President** ............................ Dr. J. F. Andrews
- **Treasurer** .................................... Dr. W. L. Bendix

Your Committee submits the following nominees for regional industry representatives on the Executive Committee of the United States Animal Health Association:

- **Northeast** ............................... Dr. E. S. Bryant of Maine
- **West** ............................... Mr. Bob Laramore of Wyoming
  Dr. William Henning of Pennsylvania
  Mr. Archie Wilson of Montana
- **Central** ................................ Mr. J. R. Bishop of Indiana
  Mr. Ward VanHorn of South Dakota
- **South** ................................ Mr. Joe Finley of Texas
  Mr. Jim Nance of Tennessee

This committee places the aforementioned in nomination for the respective offices of the U.S.A.H.A.
The Animal Virus Characterization Committee has met during the past year with the majority of the membership participating actively. The deliberations of the Committee have centered around (1) the direction and scope of our activities in the future; (2) the relationship of this Committee to various agencies i.e. Agricultural Research Service, National Institutes of Health, American Veterinary Medical Association, World Health Organization (W.H.O.) and others; (3) the role of virus characterization in virus diagnostic and research programs; (4) the development and standardization of reference reagents, and; (5) ways and means of collecting, storing and disseminating virus characterization data.

The Animal Reference Virus Catalogue was published in the American Journal of Veterinary Research (1970) 31: 1915-1928. Still, much data is available on the many virus strains contained in the catalogue. This data is not in a format suitable for publication by many of our current scientific journals. Additionally, where it is acceptable the expense for publication is beyond the means of the Committee. As mentioned in our previous report, specific information is often lacking for many virus groups and international accord is often missing. The format although essential for a complete virus record, is complicated and complex. In spite of this, your committee has made tentative arrangements to have some of this material published beginning in 1972.

The Committee has informally petitioned the Executive Board for the WHO/FAO Program on Comparative Virology to support our Committee as a WHO Reference Center for Data Collection and Evaluation. The response from WHO/FAO has been less than enthusiastic. Presently, the majority of the Committee members are currently serving on one or more of the Working Teams collaborating in the WHO/FAO Program on Comparative Virology. As mentioned in our last report, a center is maintained at the laboratory of one of the Committee members to continually receive, collate and disseminate new characterization data on reference viruses as it becomes available.

The Committee has recommended to the Secretary of Agriculture that a program be funded to produce, standardize, store and distribute reference reagents to assist in research, diagnosis and control of infectious diseases of animals. The recent epizootic of Venezuelan Equine Encephalomyelitis in Texas adequately emphasizes the need for this type activity. In response to this letter liaison has been established and discussions are underway.
REPORT OF THE COMMITTEE

In view of the recent VEE epizootic and the explosive incidence of African Swine Fever in Cuba, it is felt that the collection and dissemination of virus characterization data is an essential means of providing input into an effective animal health program.
REPORT OF THE COMMITTEE ON
LIVESTOCK COMMERCE 1971

Chairman: Glenn B. Rea, Salem, Oregon
L. N. Buttler, Jr., Phoenix, Ariz.; J. H. Brashear, Oklahoma City, Okla.;
F. S. Lee, Brownlee, Nebr.; F. W. Hanson, Springfield, Va.; W. W. Bird,
Nashville, Tenn.; J. E. Hudelson, Topeka, Kans.; J. F. Andrews, Atlanta, Ga.;
C. T. Sanders, Kansas City, Mo.; A. P. Schneider, Boise, Ida.; R. Schnell,
Dickenson, N. Dak.; F. J. Schoenfeld, Salt Lake City, Utah; D. L. Smith,
Indianapolis, Ind.; Ingvard Svarre, Sidney, Mont.; F. W. Peterson, Omaha,
Nebr.

Mr. Chairman:

Your Committee for Livestock Commerce met for the second time to consider
the obligations given to it by the Executive Committee.

We were pleased to have nearly 100% attendance of the Committee plus a
number of visitors who also presented problems and suggestions which were of
assistance to our deliberations.

The committee reviewed communications received during the year from sev-
eral interested groups concerning improvement in the means and procedures nec-
essary for the efficient movement of livestock in interstate trade.

After considerable discussion of Health-Certificates and their present use it was
recommended by the Committee that: (1) the several states who are not presently
using the form as approved by the United States Animal Health Association in 1964
be exhorted to do so; and that (2) in so doing the certification so made relate to the
health of the animal or animals at the specific time the document is issued; (3) that
the inspection and examination performed by the accredited veterinarians issuing
the certificate be in detail and used only for the specific requirements of the state of
destination. In other words the committee recommends that it be a medical docu-
ment instead of one to simply facilitate the movement of livestock.

The Committee further recommends that the Certification to which the
Veterinarian affixes his signature be that which was recommended in 1970 after the
review and approval by the A.V.M.A. which is as follows:

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

________________________________________
Veterinarian

________________________________________
Date
A copy of this certificate with the new wording will be forwarded to all States with a copy of this report if adopted by the Executive Committee.

Further consideration was given as to the means by which livestock might be approved for interstate movement. At least five states represented at our meeting have found that the increased use of a Permit System greatly enhanced the efficiency of the procedure. It was recommended that states when possible adopt a system whereby the shipper by contacting the state of destination could thereby be informed of the necessary requirements involved or in force at the very time of his intended shipment. This then would allow the state of destination to state specifically what requirements by way of tests or inoculations were necessary to satisfy the laws and regulations of the receiving state. Representatives of both Veterinary Practitioner and Livestock groups were most pointed in requesting this provision.

Since many of these permits are requested at odd hours the committee has devised a uniform Certificate for Interstate Movement of Livestock which all states would provide their livestock health officials and accredited veterinarians. This form constitutes a uniform document by which the permit number might be transported from the state of origin to the Chief Livestock Officer in state of destination as well as a document for Interstate Shipment if permits are not granted and one less rigid than a medical health certificate. This then would make unnecessary the use of official health certificates merely for purposes of facilitating the interstate movement of livestock. It would mean that those states not already doing so would have to make public, phone numbers of certain officials authorized to grant such permits. A copy of this recommended certificate is attached. (1) This is the first or initial model — Expect it will undergo considerable change before final form is adopted.

A survey of the several states during the past year revealed that 24 are responsible for the health and welfare of zoo animals. The American Association of Zoo Veterinarians have requested that this organization approve a separate certificate of their own device for the purpose of the movement of zoo animals between zoo facilities. Since the number of zoos in the United States is comparatively small, your committee recommends the approval of the certificate proposed by this organization. A copy of this certificate will be forwarded to the Chief Livestock Official of the state of destination by the originating zoo veterinarian. A sample is attached. (2)

The committee was presented with the problem of rapid and accurate notification of regulation changes to the several states by a given state, pertinent to the interstate movement of livestock. After considerable discussion your committee accepted and recommends for approval of this body a format also attached herewith. (3) It is recommended that the Disease Control Service Office of the Animal Health Division provide the several states with Blank copies of this format in a different color (white) for this use. That each state be responsible for forwarding this format with its changes to each of the other states and or territories. The receiving states will be responsible for the duplication and distribution to all of their accredited veterinarians and livestock officials.

The question of uniform rules and regulations as often proposed and the possibility of their general adoption was examined. As a result of years of effort on the part of regulatory officials present, it was agreed that this after all was a problem for the livestock industry working together in the separate states in order to influence the legislation needed.
It is recommended that this problem be presented to NASDA and the livestock industry for their consideration and recommendation as to its solution. We further recommend that the National Assembly of Chief Livestock Officials be requested to offer their consultation and advise to this end.

Mr. Chairman, that concludes the report of the committee for livestock commerce.
CERTIFICATE AUTHORIZING INTERSTATE MOVEMENT OF LIVESTOCK

Date Issued ____________________ Time ____________________

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

1. Kind of Livestock ____________________ Number of Head ____________________

Additional Identification if Applicable ____________________

Tests or Vaccinations if Required by State of Destination ____________________

2. Consigned From:
   Name of Shipper ____________________ Address ____________________
   Origin of Livestock if Different from Address ____________________

   Signature of Shipper ____________________

3. Consigned To:
   Name ____________________ Address ____________________
   Destination of Livestock if Other than Address ____________________

4. Purpose, Feeding ____________________ Slaughter ____________________ Resale ____________________

5. Permit Number ____________________ (if Required by State of Destination)
   Issued by ____________________

   Signature of Issuing Authorized Officer ____________________ Address ____________________

This Certificate is valid for 1 shipment only, not to exceed 96 hours from time of issue and is void at destination.

AMERICAN ASSOCIATION of ZOO VETERINARIANS
APPROVED HEALTH CERTIFICATE

<table>
<thead>
<tr>
<th>ANIMAL ID. TATTOO</th>
<th>NAME</th>
<th>PHYSICAL DESCRIPTION</th>
<th>HISTORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAND</td>
<td>SCIENTIFIC</td>
<td>AGE</td>
<td>SEX</td>
</tr>
</tbody>
</table>

OWNER CONSIGNEE
ADDRESS ADDRESS
PHONE PHONE

PLACE ISSUED Moved by LAND AIR SEA

This is to certify that the above described animals and found them free from evidence of contagious disease and exposure threats and I further certify that animals have been withheld from this shipment.

Signature ____________________ Date ____________________

Owner's Address ____________________ AAVV Member/Associate

White Copy - Accompany Shipment
Blue Copy - State Office
Yellow Copy - Shipper
Amendments to "Health Requirements and Regulations Governing the Interstate and International Movement of Livestock and Poultry"

Key:
- New Material
- Deleted Material

Date

**GOATS**

Goats for dairy and breeding purposes may enter Shangrila provided they are accompanied by an Official Health Certificate and they have passed a negative test-to-brucellosis and tuberculosis within the past thirty (30) days they qualify with one of the following:

1. **Brucellosis**
   - (a) Originate in a certified brucellosis-free herd or area.
   - (b) Have passed a negative BBA (card) test for brucellosis within thirty (30) days if over six (6) months of age.

2. **Tuberculosis**
   - (a) Originate in a modified tuberculosis accredited area.
   - (b) Have passed a negative tuberculin test within the past thirty (30) days.

NOTE: The above is an example of how changes in interstate regulations might be prepared by a state for dissemination to the other 49 states. Only that portion of regulations that are changed should be forwarded to the other states. These changes should be prepared on a page with the dimensions of this sheet. The receiving state could then duplicate this sheet and forward it on to all practicing veterinarians.

(Shangrila)

Sect. 1, ARS 91-17-5 (amendments)
REPORT OF THE
ANIMAL WELFARE COMMITTEE

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

Model State Law on Animal Welfare
It has been brought to the attention of your Committee that in every state where this bill has been introduced, special interest groups have sought and obtained changes in the language which have destroyed the balance which made the original version acceptable to both the research community and the responsible elements in the humane movement. In state after state these modifications have had the effect of reintroducing all of the controversial elements that your Committee had so painstakingly eliminated during the course of its two years of deliberation on the proposal. It is our opinion that if the law is to have any chance of adoption and effective implementation, the language and effect of the proposal must remain substantially unchanged.

Endangered Species
We once more emphasize that a number of wild species, both foreign and domestic, are nearing the point of complete extinction. The continued sale or slaughter of such animals must be discouraged by every possible means and at every possible opportunity.
Capture of certain species should be permitted only for the purpose of establishing colonies for propagation.

Zoos and Menageries
A model law designed to regulate the public exhibition of wild animals has been prepared by your Committee. However, it now appears that the Federal Animal Welfare Act of 1970, which becomes effective on December 24, 1971, will accomplish many of the same objectives. The Committee has therefore tabled its model law until such time as experience with the federal law indicates more precisely those areas in which the support of state legislation is needed. At that time the model law can be revised to deal only with those areas of concern which can best be handled by state or local controls.

Pre-Slaughter Handling of Livestock
The handling of livestock prior to slaughter has been a perplexing and controversial issue that has defied rational solution.
Your Committee is pleased to report that a two pronged approach to the problem, utilizing research and education, has been undertaken by the Council for Livestock Protection. The Council was incorporated under the laws of the District of Columbia in August 1971. Its first objective will be to develop a method or methods of slaughter which will be ritualistically acceptable while obviating the need to shackle and hoist living animals.

The Council will have cooperation of leading members of the Jewish faith in financing the development of more humane preslaughter practices.
INTERNATIONAL ASPECTS OF ANIMAL DISEASE CONTROL

E. E. Saulmon *

It has been said that the history of international trade and commerce, is in a measure, the history of civilization itself. A proper balance of commerce with other countries is essential for continued growth and economic well-being of the United States or any other progressive nation. International trade in livestock and poultry, and their products and byproducts, is of growing importance throughout the world. The pressures for change resulting from international economic competition, speedy transportation, and worldwide consumer demand, are significantly affecting animal disease control problems for this country. Our counterparts in other countries are in the same situation.

It is evident that any exchange of animals or animal products carries with it the risk of disease introduction. Given the need for such trade, the disease aspect of the trade problem becomes one of balancing national benefits against disease risk. As our needs change, our evaluation of acceptable risk changes. As our transportation improves, our actual risk changes accordingly, and we can only hope that our defenses improve in at least parallel fashion.

The history of worldwide animal disease is one spread largely along trade routes. Traffic on trade routes now routinely moves at hundreds of miles per hour rather than a few miles per day. Fortunately, our ability to determine health status is vastly improved, although this ability still lags behind in increased exposure potential that results from a much larger volume of imports reaching us so much more rapidly.

For purposes of this discussion, this large general subject area can be broken down into import trade and export trade. Within each of these, the relationship of disease control to total national need can be considered with comments on what is being done and what may be needed.

Disease risk from imported animals or products is of immediate concern to all informed veterinarians as well as the livestock and poultry industries. It seems evident, however, that the balance of disease risk against total need for imports is changing rather rapidly for this country.

While we have never adopted the ultimate in disease prevention — shutting off all contact with other countries — we have for many years maintained through prohibitory legislation very rigid sanctions against domestic livestock and meats from much of the world. Enforcement of these, probably assisted by some good luck, has kept us free of foot-and-mouth disease for over 40 years. Because of these sanctions, we have the side-benefits of protection against many other exotic diseases.

There has been pressure against this protective wall, however. One recent ex-

* Deputy Administrator, Veterinary Services, ARS, USDA
ample may be of interest. An exception was made in the prohibitory legislation that permits entry of wild ruminants and swine from infected areas. This is an uncomfortable situation from a disease introduction standpoint, yet the public demand for such specimens was — and is — such that the exception was made.

Many restrictions surround permitted zoological specimens, and we try to keep them upgraded. The Plum Island Animal Disease Laboratory now carries out serological tests for FMD on all wild ruminants entering quarantine. The technology involved was not available in earlier years. Although several hundred such imports have been screened without significant incident, test results for a recent shipment of Roan antelope from Africa indicated that most of the lot had at some time in their lives harbored FMD virus. While no virus was demonstrated, the implications of the serological results were such that the animals were refused entry. How many such animals entered the country in earlier years can only be a matter of speculation. What this incident does illustrate is that public demand for this type of importation—which is a legitimate demand—also produces a disease risk which we must attack with each new tool that research makes available.

The same sort of risk versus demand situation is evident now with regard to new germ plasm for the livestock industry. This demand in the last few years has resulted in a number of importations involving cattle semen from countries from which donor animals are prohibited. Here also the restrictions surrounding importations are lengthy and very expensive for the importer, but the demand is such that the importations are being made. The precautions are considered as excessive by some, but the element of risk cannot be entirely removed even with the laborious procedures established.

Congress, last year, enacted legislation which permits the establishment of an offshore quarantine facility for the purpose of allowing importation of domestic animals from FMD infected countries. Such legislation obviously results from the demand for new blood. When this station is finally in operation, the protective procedures involved will be complex, lengthy, and expensive, but they cannot offer the absolute protection of continued prohibition.

It is also increasingly evident that prohibitions or protective procedures at our own borders are not all we need to think about. As the world shrinks, what affects our neighbors is of larger concern to us. The recent appearance of African Swine Fever in Cuba—and the movement of VEE up through several countries and into the United States—illustrates that no country can pin its hopes on remaining isolated behind its own defence. USDA has requested, and the Congress is now actively considering, expanded legislative authority to cooperate with other countries in this part of the world to combat any disease threatening our livestock and poultry industries. The successful Mexico-United States Foot-and-Mouth Disease Program was an illustration of such cooperation. Because of this effort, we remain one of the largest block of countries in the world—from Panama North through Canada—free of FMD.

However, within a few years, the Pan-American Highway will be completed and link these countries with FMD infected countries in South America. So there is strong effort now, involving ourselves and these other countries to the South, to plan and implement defenses against the disease exposure that will come with the opening of the last link in the highway.
This defense, in which we must actively participate, will be far removed from our actual borders. It must involve a cooperative effort. We cannot dictate either the plan or the action. Whether or not this will be more or less difficult than are the cooperative disease control programs within this country remains to be seen—but at least we have almost 90 years of practical domestic experience to draw upon.

All these changes, in spite of efforts to strengthen our defenses against disease introduction, bring an increasing risk of such introduction. No one of us can remain isolated from this risk. It seems probable that within a few years major airports throughout the country may all be international ports of entry. Containerized shipping is likely to bring animal products directly to interior points. Our ability to rapidly detect any disease introduction whenever and wherever it may occur is of urgent concern. Emergency response capability is a matter of practical necessity, not a text-book exercise.

Coupled with this is the need to work more closely with other countries, and certainly our neighboring countries, to help them keep out or stamp out exotic diseases.

The disease aspect of our export trade is also of growing importance. This has not recently received the depth of attention by our industry—or profession—as has the disease risk from import trade. Historically, however, it was of great concern and needs to receive more attention now.

The historical importance dates back to the establishment of the Bureau of Animal Industry and the First State-Federal Disease Eradication Program. As you will recall, this was in the 1880's against Contagious Bovine Pleuropneumonia. The founding of the BAI and the CBPP program were not due to domestic impact of the disease but to the fact that lucrative foreign markets were shut off because we had the disease. So our forbears corrected the situation—with such determination that they finished before the causative agent of the disease had been isolated.

While we have kept out some diseases, and eliminated others, that were or would be a barrier to export trade, our current situation is one of rising problems associated with export trade in animals and animal products. This is due to the increased number of such shipments and the improved situation in many countries of the health status of their own livestock.

Consequently, the health requirements of other countries for United States livestock are becoming more complicated and comprehensive. While our own laws and regulations require health examination of all exported livestock, and negative status with regard to Bovine Tuberculosis and Brucellosis, these no longer are adequate to cover most export shipments. Within recent years most of the countries that accept our livestock—and a number do not—have continued to add specific requirements covering a variety of diseases.

This is proper and to be expected—we have done the same for livestock we receive—but there are unfortunately still a few individuals here who seem to have forgotten the marketing maxim that the customer is always right and appear to feel that stringent health requirements by other countries are an unacceptable interference with what they would prefer to do.

Our export problems—other than this lack of understanding by some of the international animal health rules of the game—are generally in two areas. One is the difficulty of achieving the necessary understanding with the other country on exactly
what they want. The other is the continuing presence here of certain disease conditions that the other country may find totally unacceptable.

The first is never ending, as the international animal health situation is changing so rapidly that each shipment seems unique. The complexity arises from not only the communication difficulties that often need to be overcome before we really understand what is wanted, but also from the logistics problem of getting this detailed information out to exporters, livestock owners, veterinarians, international transport people, and laboratories—making sure that the right tests are done at the right time, the animals accumulated in proper export facilities, the plane or ship properly fitted, and that the animals remain healthy until loaded for departure.

There are further problems in understanding in that those involved in many other countries do not comprehend the sheer size of this country, our State-Federal system and Division of Animal Health Authority, and the involvement of the veterinary practitioner in what in many countries is strictly a function of government.

All this does not produce an impossible situation—contrary to what some of our livestock people feel when they are first involved in the export business. It does demonstrate, however, that other countries put the same premium on preventing disease introduction that we do, and that any veterinarian or livestock owner involved in such shipments is not engaged in a simple transaction.

The other main bar to our exports is the presence here of diseases others do not want. A quick sampling of some diseases we have that result in prohibited entry by one or more countries includes Bluetongue, IBR, Hog Cholera, Trichinosis, Scrapie, and VEE. Only to the extent we can convince these countries that we have eliminated such conditions, or have procedures that will insure shipment only of animals free of such conditions, can we expect these sanctions to be removed.

So it remains a matter of balance for all countries. What are their total National needs for imports against acceptable disease risk? How far are they willing to go in disease control and eradication at home to maintain or open up international markets for their animals and products?

Beyond this is our growing need to work with other countries to achieve disease control and eradication rather than to depend on outright interdiction or prohibitions. This concept would appear to be necessary and appropriate for continued advancement of animal health worldwide—while at the same time working toward an environment where health requirement barriers can be eased. This is the concept we have continued for several generations in our State-Federal work here.

I would suggest that this association will find itself increasingly concerned with this subject in years to come. Our risks are increasing, as is our need for new knowledge and new outlooks to meet these risks.

As the disease problems are increasing because of better communication and improved international exchange, our challenge is not to hold to the past, but to meet our changing responsibilities.
REPORT OF THE 1971 COMMITTEE ON IMPORT – EXPORT

Chairman: John F. Quinn

The Import-Export Committee of the United States Animal Health Association met in Room 1011 of the Skirvin Hotel in Oklahoma City October 25, 1971. On roll call by Wilson Powell, Secretary, the following members were present: John F. Quinn, Chairman, C. L. Campbell, Co-Chairman, E. L. Browet, Ronald B. Caffey, M. L. Crandall, James R. Hay, M. G. Hynes, John R. Langridge, C. H. Pals, Dean Price, and John C. Shook.

The Committee considered the following Resolution, which was approved and ordered to the Committee on Nominations and Resolutions:

RESOLUTION
MANDATORY AGRICULTURAL CLEARANCE
OF INTERNATIONAL CARRIERS

WHEREAS, the Bureau of Customs, Immigration and Naturalization Service and the United States Public Health Service are United States border clearance agencies, each of which has enabling legislation requiring clearance of carriers of passengers and freight; and

WHEREAS, United States Department of Agriculture lacks such authority;

NOW THEREFORE BE IT RESOLVED, That the United States Animal Health Association in formal session at its Seventy-Fifth Annual Meeting held October 24-29, 1971, at Oklahoma City, Oklahoma, strongly urges that legislation be enacted to provide mandatory agricultural clearance of carriers as a condition of entry into the United States; and

BE IT FURTHER RESOLVED, That copies of this Resolution be forwarded to the United States Department of Health, Education and Welfare, and to the National Association of State Departments of Agriculture.

It has consistently been of concern to the Committee the inspection of imported animal products being brought into the United States. In regard to this concern, the Committee wishes to commend the Agricultural Quarantine Inspection Division for strengthening the procedures on surveillance of animal products being imported into this country. The Committee also commends Consumer and Marketing Service Division of the United States Department of Agriculture for placing more emphasis on surveillance of foreign animal products being produced for importation into the United States.

The Committee calls attention to the inadequacies of the present system of disposal of garbage from foreign ships intering United States waters. With the increased trafficking, this constitutes an ever present hazard to the livestock industry of this nation.

The Committee wishes to bring to the attention of the American Inspection Quarantine Division in view of the current ecological problems with which we are
faced, that at the many stations manned along the borders and coastlines of the United States there most assuredly will be problems involved in the disposal of solid wastes at these stations. A good example of this is a complaint already received by the Animal Health Division of USDA from the City of Port Huron, Michigan. Their complaint involved not only solid waste from animals at the inspection station at Port Huron entering the city sewage disposal system, but also included complaints of odors from nearby residents. This problem is ever increasing in today's society and certainly must be considered in re-evaluation of the Division's programs in their maintenance of these essential quarantine stations.

For some years, the inability of the Division for one reason or another to seal meat lockers on vessels on the East and Gulf coasts of this nation has been a constant problem, and this Committee was quite gratified to receive a report that this problem has now been resolved.

At the meeting of the Committee in 1970, a member of the Plant and Animal Quarantine Division was delegated to provide information on possible sources of revenue to finance increased surveillance on the inspection of animals and animal products coming into the United States. His findings disclosed to the Committee that already existent were tariffs in the form of taxes, and an extension of these tariffs to provide funding for additional inspectional activities was unpalatable to involved governmental agencies administering existing programs. Consequently, the Committee will further explore other possibilities of obtaining revenues which can be used expressly to increase the surveillance over animals and animal products being imported into this country.
SPREAD OF SCRAPIE AMONG SHEEP AND GOATS
SCRAPIE FIELD TRIAL, MISSION, TEXAS

* A. L. Klingsporn, DVM, J. L. Hourrigan, DVM

GENERAL

Scrapie, a naturally occurring disease of sheep and less frequently of goats, is caused by a transmissible, filterable, and self-replicating agent considered by most research workers to be a virus with unusual characteristics. The disease is characterized by progressive degeneration of the central nervous system which causes the animal to rub and scratch, become debilitated and incoordinated. Most affected animals die at from 30 to 54 months of age after manifesting clinical signs of the disease for one to 6 months.

Scrapie has been recognized in Europe for over 200 years. It has been reported in many countries of Europe, in South America in Colombia, in Africa in Kenya and the Republic of South Africa, and in India, Australia, New Zealand, Canada, and the United States. Figure 1. The disease has not been officially reported in Russia; however, indications are that scrapie occurs there.

Scrapie was first reported in Canada in 1938, and in the United States in Michigan in 1947. As of October 1971, the disease has been diagnosed in this country in 199 flocks in 30 States; and in the United States scrapie has affected 294 sheep of the Cheviot, Hampshire, Montadale, and Suffolk breeds. Figure 2.

The Cooperative State-Federal Scrapie Eradication Program began in California in 1952 as an emergency measure. That program provided for histological confirmation of the disease in animals with clinical signs of scrapie, quarantine and slaughter of all sheep and goats in the infected flocks, and tracing and slaughter of all exposed animals moved from the flocks and their immediate progeny. Programs carried out in other States provided for slaughter of infected flocks, but varied as to handling of exposed animals sold from the infected flocks. The program has been amended as study of field outbreaks and review of research findings have dictated.

The latest modifications were made in 1965 when the program was expanded to include slaughter of all bloodline animals. Bloodline animals are defined as the affected animal, all its descendants, sire and dam, and all full or half brothers or sisters. When the disease is limited to well-defined bloodlines and adequate quarantine measures can be maintained, animals to be slaughtered may be limited to bloodline animals. Nonbloodline animals in the infected or source flocks can be held under quarantine for 24 months with monthly inspections. Inspections of these flocks are maintained for an additional 18 months after the quarantine period. This procedure appears to have been successful in three small flocks in Maryland,
New York, and Ohio where the affected animals were recently introduced into the flock. These flocks were placed under quarantine, one in February 1968, one in October 1967, and one October 1966, and no further scrapie has been reported in them. However, in three large flocks in Illinois, two infected flocks and one source flock, where affected animals were born in the flocks additional scrapie occurred within a year in either the bloodline animals taken to Mission or in the nonbloodline animals quarantined on the premises. When investigation reveals that scrapie involves more than one bloodline in the flock or quarantine measures cannot be adequately maintained the entire flock is slaughtered.

We believe that the present program has prevented scrapie from becoming widespread in Cheviot, Hampshire, Montadale, and Suffolk sheep and prevented its spread into other breeds. It is believed that the present program if diligently carried out can effect the eradication of scrapie from the United States. It is fully appreciated that this task will not be easy.

SCRAPIE FIELD TRIAL, MISSION TEXAS

The data obtained from study of field outbreaks, review of the research literature, and reports from other countries have long indicated that scrapie can be a significant disease, can cause substantial losses, and can spread by contact. Unfortunately the natural tendency to suppress reports concerning the occurrence and losses from the disease in some countries, the limited research on the natural disease, and that the eradication program as followed in this country disposed of many animals likely to develop and spread the disease limited the accumulation of data to confirm or deny information about the true nature of the disease, its spread, and losses caused by it.

To provide information in these areas it was proposed in 1964 that a field trial be developed to study natural scrapie and the natural spread of the disease. This proposal was discussed with research workers, State and Federal regulatory officials and members of the sheep and goat industries. They approved the plan and the field trial was initiated at Moore Air Force Base, Mission, Texas, the site of the sterile screwworm production plant.

The field trial study was designed to hold and breed sheep and goats under close observation for extended periods to learn which animals would develop scrapie. As of October 1971, 2224 sheep and 155 goats have been used in this study. In order to study the possible familial relationship of scrapie we have followed the schedule of breeding particular rams to particular ewes and rebreeding these rams and ewes in succeeding years to produce as many full sibs and half sibs as possible. Such full and half sibs make ideal animals for study.

BLOODLINE SHEEP

The bloodline sheep in the Mission flock are of the Cheviot, Hampshire, Montadale, and Suffolk breeds. They were purchased from infected flocks or source flocks in California, Indiana, Illinois, Maryland, Missouri, New York, and Texas. The animals represent 21 bloodlines in which scrapie has been diagnosed in the United States, and were purchased and added to the Mission flock as field outbreaks
occurred. The first bloodline animals were taken to Mission in November 1964 with the most recent additions in December 1970.

During the period from December 1964 to October 1971 scrapie has been diagnosed in 120 bloodline animals in the Mission flock. Not all bloodline or families within given bloodlines appear to be equally susceptible to scrapie at least as far as their developing clinical signs of scrapie is concerned. This is shown by the scrapie data for animals from 10 infected or source flocks taken to Mission for observation in Table 1.

Table 1 — Total Incidence of Scrapie in Lots of Sheep from Infected and Source Flocks Taken to Mission, Texas

<table>
<thead>
<tr>
<th>Flock of Origin</th>
<th>Breed</th>
<th>Number of Sheep in Lot</th>
<th>Months of Study</th>
<th>Percentage of Scrapie Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected Cheviot</td>
<td>40</td>
<td>41</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Infected Suffolk</td>
<td>7</td>
<td>14</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Source Suffolk</td>
<td>101</td>
<td>46</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Infected Suffolk</td>
<td>39</td>
<td>62</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Infected Suffolk</td>
<td>33</td>
<td>54</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Infected Suffolk</td>
<td>5</td>
<td>16</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Infected Suffolk</td>
<td>8</td>
<td>7</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Infected Suffolk</td>
<td>37</td>
<td>38</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Infected Suffolk</td>
<td>47</td>
<td>47</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Source Suffolk</td>
<td>18</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

It must be pointed out that these data represent animals from rather closed lots of sheep selected because of their bloodline relationship to other scrapie affected sheep. The animals taken to Mission from these flocks ranged from only 5 animals in the smallest infected flock to 101 from the largest source flock. The time intervals of the study involve that period from the time the first affected animal in the flock developed signs of clinical scrapie to the time at which the last confirmed case died. This time period was chosen because in a given group of bloodline animals scrapie begins to occur as the animals reach about 27 months of age, peaks out at 35 to 45 months and drops off after 50 months of age and in some lots of sheep appears to stop. Further only animals reaching 24 months of age, a practical minimum age for scrapie manifestation, were considered at risk for the purpose of computing morbidity rates. Even considering the biases of these data it is obvious that losses in scrapie bloodlines of infected and source flocks can reach significant proportions if these animals are allowed to remain alive.

However, even in these scrapie-affected bloodlines there are certain animals which appear to be refractory, or at least do not manifest the clinical signs of scrapie. There are persons who have advocated that it is possible to select these older presumably refractory animals for breeding purposes and develop bloodlines resistant to scrapie. If this theory is valid it could present the opportunity to preserve val-
1971 REPORT OF THE COMMITTEE ON EVALUATION AND DEVELOPMENT OF STATE-FEDERAL PROGRAMS

Chairman: John L. O’Harra, Reno, Nev.
Co-Chairman: John G. Milligan, Montgomery, Ala.

Ex-Officio Members:

USAHA Regional Representatives:

Federal:
E. E. Saulmon, Hyattsville, Md. (ANH); Donald Miller, Hyattsville, Md. (ANH); E. A. Schilf, Hyattsville, Md. (ANH, Cattle Disease); M. J. Tillery, Hyattsville, Md. (ANH, Swine Disease); J. L. Hourrigan, Hyattsville, Md. (ANH, Misc. Disease); C. D. Van Houweling, FDA, Washington, D. C.

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

During the past year a comprehensive review of the Cooperative State-Federal Brucellosis Eradication Program was carried out under the direction of this committee. A detailed questionnaire on program activities was filled out by State and Federal animal health officials in each state. The committee received a tabulation of responses to this questionnaire, and was highly pleased with the wealth of information contained in this report.

Pertinent information revealed through an early evaluation of this report was made available to the Brucellosis Committee of this Association prior to their meeting this year so that they would have this information during their deliberation. A brief summary of the report will be included with the brucellosis report and will be published in the proceedings. The complete report will be furnished to the State Veterinarian and to the Federal Veterinarian in Charge in each state. The report will also be available to interested persons through the Animal Health Division headquarters in Hyattsville, Maryland.

The results of this survey of the Brucellosis Eradication Program pointed up the need for greater adherence to the Uniform Methods and Rules. Many states were deficient in one or more areas. In other cases, there were flagrant violations of these guidelines.
producers of biologics in the United States have expressed interest in the vaccine. The evaluation of the efficacy of the vaccine and its practical use in this country will require additional research and field trial efforts.

Dr. Crenshaw reported observations that, although treatment with antibiotics was useful in early cases of foot rot, under controlled field conditions procedures including trimming and foot baths were found to be more effective.

RAM EPIDIDYMITIS

Dr. Crenshaw reported on a 10-year study of ram epididymitis control in four California flocks. The program included early vaccination of ram lambs followed by re-vaccination annually, and palpation and removal of rams with abnormal testicles.

The practical success of the program, as measured by fewer rams removed by palpation and culling has resulted in a reduction of rams culled from 25% to 3 or 4%. Practical control has been achieved, however, seriological tests will probably be necessary in order to eliminate the disease from a flock.
The three outbreaks occurred one each in Oakland County, Michigan; Beckham County, Oklahoma; and Hamilton County, Texas. One of the outbreaks was reported by the flock owner, one was reported by a State university, and one was reported when tracing bloodline sheep.

All bloodline and nonbloodline exposed sheep in the three infected flocks and their source flocks have been slaughtered with the exception of the five bloodline sheep from the Texas infected flock which were taken to Mission, Texas, for use in the scrapie field trial.

All bloodline sheep sold from the Oklahoma infected and source flocks have been traced and slaughtered. Nonbloodline exposed sheep sold from these flocks have been placed under 42 months surveillance. Bloodlines could not be identified in the Michigan Cheviot outbreak or the Texas Suffolk outbreak; therefore, all exposed sales from these infected flocks and their source flocks are being traced and slaughtered.

A few minutes ago, we heard Dr. A. L. Klingsporn’s paper “Spread of Scrapie among Sheep and Goats at the Scrapie Field Trial, Mission, Texas”, which described measured losses from scrapie and contact spread of the disease to several breeds of sheep and goats.

HAIRY SHAKER DISEASE OF LAMBS

Dr. Crenshaw reported on Hairy Shaker Disease, similar to the condition known as “Border Disease”. The disease was first recognized in New Zealand in 1957, later reported in Scotland in 1959, and has been observed in California flocks for several years. It has also been observed in sheep flocks in other states.

Affected lambs are born with a hairy fleece and some also show a characteristic nervous tremor consisting of rapid rhythmic clonic spasms. When affected lambs are out of white faced ewes and by blackfaced rams pigmented or discolored areas may be seen in the wool covering of poll, neck, shoulders or flanks. A ewe’s first lambs are more likely to be affected, with her dropping unaffected lambs in succeeding years. In affected flocks, 2 of 3% of lambs may be affected. Affected lambs have a poor growth and survival rate with most dying in 30-90 days. Histopathological changes are described in the central nervous system. An agent causing the disease has not been identified; however, the condition can be artificially produced in lambs by inoculating nerve tissue and spleen from affected lambs into the embryonating fetus and by inoculating these tissues into pregnant ewes by the subcutaneous, intraperitoneal, or intravenous routes.

SHEEP FOOT ROT

Dr. McGowan reported that at Davis, California, a practical method had been developed for culturing Fusiformus nodosus organisms in the laboratory and that they had developed a satisfactory method for artificially infecting sheep with the organism. These two procedures will greatly aid development and testing of foot rot vaccine. He also discussed the experiences of Australian workers in regard to the foot rot vaccine being produced and tested experimentally in Australia. Certain
REPORT OF THE COMMITTEE
OF INFECTIOUS DISEASES OF SHEEP AND GOATS

Charman: J. L. Hounrigan, Hyattsville, Maryland
Co-Chairman: G. L. Crenshaw, Davis, California

BLUETONGUE

Your committee wishes to call your attention to the paper “Clinical Aspects of Bluetongue in Oregon Cattle” presented to this assembly yesterday by Dr. Guy Reynolds.

We also discussed Bluetongue (BT) at some length particularly in reference to reports of epizootic hemorrhagic disease (EHD) reported in white-tailed deer this summer and fall in several southeastern states. There has been considerable discussion in scientific circles as to the similarity of BT and EHD viruses. It is well known that both viruses can produce an apparently identical clinical and pathological response in white-tailed deer. A virus has been isolated from white-tailed deer involved in the current disease outbreak in the southeast. At the Veterinary Research Division’s Animal Disease Laboratory at Denver, Colorado, this isolate produced clinical signs seen in bluetongue in sheep and in cattle, performed like BT virus in embryonating chicken eggs and was positive to the florescent antibody test using BT antigen. Active BT research work is continuing at the laboratory.

During fiscal year 1971, bluetongue was confirmed by virus isolations in nine sheep flocks in Arizona, Colorado, Idaho, Nevada, and New Mexico; and in three cattle herds in Colorado, Oregon, and Washington. Additional samples are pending virus isolation studies from cattle in several states.

Bluetongue is a costly disease in the United States and due to its multiple strains and variable viremia in affected animals continues to create problems in increasing frequency in cattle and is developing strains which appear to be more virulent in this species.

SCRAPIE

Scrapie was reported in three flocks in three States during fiscal year 1971. This is the smallest number of scrapie outbreaks disclosed in the United States since fiscal year 1954. The three outbreaks involved two Suffolk flocks and one Cheviot flock. The number of flocks under surveillance has dropped slightly to 306 from 312 last year.
### Mission Field Trial

#### Scrapie Bloodline - Rambouillet Sheep

<table>
<thead>
<tr>
<th>Year</th>
<th>Number</th>
<th>Dead Sheep</th>
<th>&gt;30 Months</th>
<th>&lt;30 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965*</td>
<td>20</td>
<td>9</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1967</td>
<td>24</td>
<td>5**</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1968</td>
<td>17</td>
<td>7**</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1969</td>
<td>32</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>1970</td>
<td>14</td>
<td>0</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>1971</td>
<td>30</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>137</td>
<td>21</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

* Purchased  
** Six of 12 Sheep, Scrapie-Confirmed

- LIVE SHEEP  
- DEAD SHEEP

### Mission Field Trial

#### Scrapie Bloodline - Targhee Sheep

<table>
<thead>
<tr>
<th>Year</th>
<th>Number</th>
<th>Dead Sheep</th>
<th>&gt;30 Months</th>
<th>&lt;30 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965*</td>
<td>19</td>
<td>11</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>1967</td>
<td>18</td>
<td>6</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>1968</td>
<td>22</td>
<td>7**</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>1969</td>
<td>23</td>
<td>3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>1970</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1971</td>
<td>32</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
<td>27</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

* Purchased  
** Five of 7 Sheep, Scrapie-Confirmed

- LIVE SHEEP  
- DEAD SHEEP

---

Figure 5

---

Figure 6
### MISSION FIELD TRIAL

#### NUBIAN GOATS AT MISSION

<table>
<thead>
<tr>
<th>BIRTH DATE</th>
<th>LIVE GOATS</th>
<th>DEAD GOATS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;30 MONTHS</td>
<td>&lt;30 MONTHS</td>
</tr>
<tr>
<td>Year</td>
<td>Number</td>
<td>Number</td>
</tr>
<tr>
<td>1965*</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1966</td>
<td>1</td>
<td>5**</td>
</tr>
<tr>
<td>1967</td>
<td>7</td>
<td>9**</td>
</tr>
<tr>
<td>1968</td>
<td>5</td>
<td>8**</td>
</tr>
<tr>
<td>1969</td>
<td>12</td>
<td>1</td>
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<tr>
<td>1970</td>
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<td>0</td>
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<tr>
<td>1971</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>25</td>
</tr>
</tbody>
</table>

* Purchased
** Ten of 22 Goats, Scrapie-Confirmed

- LIVE GOATS
- DEAD GOATS
- Scrapie Confirmed
- Not Scrapie

---

### MISSION FIELD TRIAL

#### HAMPSHIRE SHEEP AT MISSION

<table>
<thead>
<tr>
<th>BIRTH DATE</th>
<th>LIVE SHEEP</th>
<th>DEAD SHEEP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;30 MONTHS</td>
<td>&lt;30 MONTHS</td>
</tr>
<tr>
<td>Year</td>
<td>Number</td>
<td>Number</td>
</tr>
<tr>
<td>1965*</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>1967</td>
<td>7</td>
<td>4**</td>
</tr>
<tr>
<td>1968</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>1969</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>1970</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>1971</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>21</td>
</tr>
</tbody>
</table>

* Purchased
** Two of 4 Sheep, Scrapie-Confirmed

- LIVE SHEEP
- DEAD SHEEP
- Scrapie Confirmed
- Not Scrapie

---

**Figure 3**

**Figure 4**
SCRAPIE

Figure 1

SCRAPIE REPORTED IN THE UNITED STATES
Fiscal Year 1947–1971

Figure 2
a clinical course of 6 weeks. Figure 6. To date five Targhee sheep have been confirmed at ages ranging from 36 to 41 months. All affected Targhees are sired by the same ram. This ram and four of the dams were purchased from one Montana flock, the other dam was purchased from a second Montana flock. They are the first Targhees known to be affected by scrapie in the United States and in fact the only ones known to be affected in the world. There are 34 lambs still alive from this ram at Mission. The fate of these lambs and their succeeding generations is important. It is interesting to note that Dr. W. J. Hadlow, Hamilton, Montana, has been working with Targhee of the same bloodline background as these animals and has been unable to bring them down with artificial inoculation, regardless of the challenge dose.

Table 2 – Scrapie in Blue Sheep and Goats Born at Mission, Texas

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number Born</th>
<th>Living to 30 Mos.</th>
<th>Dead at 30 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Scrapie</td>
</tr>
<tr>
<td>Goats*</td>
<td>91</td>
<td>48</td>
<td>10</td>
</tr>
<tr>
<td>Rambouillet</td>
<td>160</td>
<td>53</td>
<td>6</td>
</tr>
<tr>
<td>Targhee</td>
<td>146</td>
<td>79</td>
<td>5</td>
</tr>
<tr>
<td>Hampshire</td>
<td>105</td>
<td>30</td>
<td>2</td>
</tr>
</tbody>
</table>

*Does not include blue Angora goats

Table 2 outlines the data for all blue or nonbloodline sheep and goats affected with scrapie at Mission, Texas. This data demonstrates that sheep and goats of various breeds born and raised in contact with scrapied animals developed scrapie by contact exposure and provides conclusive evidence that scrapie can spread by contact from affected sheep and goats to nonbloodline sheep and goats of various breeds. This data confirms the contact transmission work done in Scotland. Using our animals and the agent(s) present in the United States additional observations are needed to determine if the same bloodline picture and incidence of scrapie will evolve in these breeds and species once the disease is introduced into them by contact.

**SUMMARY**

Observations of the Mission Field Trial flock have proved that scrapie can cause substantial losses in sheep and goats, and that the progeny of bloodline animals which do not develop scrapie as well as the progeny of bloodline animals which do develop scrapie will come down with the disease in an infected environment.

The observations have also shown that the progeny of sheep and goats which are from non-scrapie flocks and herds will develop scrapie in significant numbers when bred and raised in an environment where they are in contact with sheep and goats affected with natural scrapie.
are still alive except two animals dying of choke in 1969, and have not exhibited signs of scrapie. Twenty-four blue Suffolk sheep were obtained from New Zealand in May 1969. These were the last blue animals purchased for the contact study. The blue sheep and goats were placed in infected pastures and corrals at Mission upon arrival, and have been subjected to contact with a succession of scrapie-affected sheep and more recently scrapie-affected goats.

These animals are observed daily for signs of disease and upon death tissues are submitted to the laboratory for histopathological examination. Although none of the individual purchased blue sheep or goats have developed scrapie, two of the purchased Hampshires were observed to show signs which could be those of early scrapie. One died and histopathological examination of brain tissue did not reveal lesions. The other one has apparently recovered.

In addition to these two, 55 of the purchased blue sheep and nine of the purchased blue goats have died without clinical or histopathological evidence of scrapie being found.

In 1965 three Nubian and one Toggenburg does were bred to one buck at Mission, Texas, and seven kids from these matings were born in February and March 1966. Of these seven, one died at 5 months of age from causes other than scrapie and five have been confirmed scrapie by histopathological examination. One of the five exhibited no signs of scrapie, while four showed typical signs lasting from 2 to 11 weeks. The seventh goat is still alive at 68 months of age. Figure 3. outlines the breeding of the goats concerned.

To date ten cases of scrapie have been confirmed in these closely related goats. It appears that the same bloodline picture seen in affected Suffolk and Cheviot sheep is developing and extending into the second generation of goats.

The Angora blue goats were not bred until 1966 with the first kids being born in 1967. No signs of scrapie have been observed in any of the Angora goats purchased or born at Mission. Histopathological examination of those that have died have failed to reveal lesion of scrapie.

The first blue sheep to develop scrapie by contact was a 37 month old Hampshire ram born at Mission in the spring of 1967. Figure 4. A second Hampshire ram also born in 1967 developed the disease at 41 months of age. Study of the bloodlines of these animals reveals that they are not related. Their parent stock was derived from Oklahoma snd Montana flocks. We have 17 lambs still alive at Mission from these two rams and it will be important to learn whether they will develop scrapie, and the fate of their succeeding generations.

The next nonbloodline or blue animal to develop scrapie was a Rambouillet ewe born in 1967 and confirmed scrapie by histopathological examination at 39 months of age. To date six Rambouillet blue sheep have contacted the disease at ages ranging from 32 to 40 months. Figure 5. The parent stocks of these animals were derived from two Texas flocks and are not related. There are 42 lambs which are still alive from these mating and it will be important to determine the scrapie incidence in these offsprings and succeeding generations. These are the only known Rambouillets to be affected with scrapie in the United States.

The most recent group of blue animals to acquire scrapie by contact at Mission is a Targhee group. The first Targhee animal to develop the disease was born in 1968 and was confirmed by histopathological examination at 36 months of age after
usable bloodlines and eliminate scrapie by selective breeding: In further consideration of this point it is of interest to note that among the Mission Cheviot and Montadale bloodline animals, only those animals exposed on the premises of origin prior to being taken to Mission have developed scrapie. None of those born at Mission have gone on to clinical disease or been confirmed by histopathological examination.

This phenomenon, of scrapie occurring only in bloodline animals born and exposed on the premises of origin prior to being taken to Mission, has not been observed in the Suffolk sheep at Mission. One hundred and fourteen (95%) of the bloodline animals affected at Mission have been of the Suffolk breed. Forty-five of these 114 affected bloodline Suffolks (40%) were born and raised on infected premises at Mission in contact with scrapie-affected sheep. Of these 45 affected bloodline Suffolks born at Mission, 25 or 55.6% were direct progeny of a scrapied ram, a scrapied ewe, or both a scrapied ram and ewe. However, 20 or 44.4% of these affected bloodline Suffolk born at Mission were not direct progeny of a scrapied ram or ewe. In fact 16 of the 20 animals have no known scrapied animals in their pedigree, but they were second or third generation progeny of apparently refractory bloodline rams and ewes who had sired or lambed other scrapied animals. Three of these animals were grandprogeny of affected rams or ewes and one was a great grandprogeny of a scrapied ewe. Therefore, it appears that even in scrapie bloodline under heavy exposure the disease has ability to skip generations, or refractory animals are produced, and yet show up in later generations. While it is still too early to tell it appears that the apparently refractory animals produce nearly as much scrapie under the same exposure at Mission as do those animals who are themselves affected.

From a program standpoint it would appear these apparently refractory bloodline animals may be more important in the spread of scrapie than those animals that are affected, because they live longer; therefore, produce more progeny which due to the time lag between generations may not be subject to close attention.

These data developed at Mission have demonstrated that significant losses do occur in bloodline animals from scrapie infected and source flocks, further that scrapie continues to occur in the succeeding generations under further exposure. From an eradication program standpoint an important question is Will these animals spread scrapie to healthy animals born and raised in contact with them?

**SPREAD OF SCRAPIE BY CONTACT**

The first nonbloodline and not previously exposed animals (which we call blue animals as they are all tagged with blue ear tags) for use in the field trial study were 17 Angora, Nubian, and Toggenburg goats taken to Mission in June and July 1965. These were followed by 33 Rambouillet, 33 Targhee, and 33 Hampshire sheep in 1965 and 1966. These were purchased at 6–10 months of age and came from Montana, Oklahoma, and Texas flocks. The twins or triplets of these animals were maintained on the premises of origin as control sheep and have been inspected every six months. No signs suggestive of scrapie have been observed in any of these animals or other animals in the flocks. Several tissue for histopathological examination has been obtain from several twins or triplets dying from other causes with no lesions suggestive of scrapie being found.

Nine blue Suffolks were obtained from Wyoming in 1967. The twins of these
Therefore, this committee recommends that the Animal Health Division bring to the attention of the USAHA Board of Directors the deviations from the Uniform Methods and Rules by individual states that have been revealed in this survey.

Further, this committee recommends that the Federal Veterinarians in Charge in the various states who have knowledge of deviations or deficiencies in any cooperative State-Federal disease eradication program forward this information to the Animal Health Division which will immediately so inform the USAHA Board of Directors. It is anticipated that the Board of Directors, in cooperation with the Animal Health Division, will then take prompt action with the states involved to correct these deficiencies.

The Committee accepted proposed questionnaires on both the Cooperative State-Federal Hog Cholera Eradication Program and the Cooperative State-Federal Tuberculosis Eradication Program and asked the Animal Health Division to prepare and distribute these in the same manner as the questionnaire on the Brucellosis Program. It was recommended that both questionnaires be mailed with one letter of transmittal.

A motion was passed that the Committee take under consideration a survey and evaluation of the State-Federal Cattle Scabies Eradication Program during the ensuing year.

The chairman of the committee will appoint a sub-committee to do fact finding on health certificates, accreditation procedures, and similar areas. The sub-committee will report back with the information they find.

The Committee requested information from ANH officials in regard to any proposed rotational system of Veterinarians in charge and urged that rotation without cause be seriously considered before being put into effect because the States feel it could not be in the best interest of the programs.

Again, the committee was quite pleased with the progress made during the past year of its operation and is hopeful that similar progress can be made in the future. The committee extends thanks to all who have contributed so freely of their time and knowledge during the past year.
REPORT OF THE COMMITTEE ON
STATE-FEDERAL RELATIONS

Chairman: W. C. Tobin, Denver, Colorado
W. L. Bendix, Richmond, Virginia, T. A. Ladson, College Park, Maryland,
M. D. Mitchell, Pierre, South Dakota, G. B. Rea, Salem, Oregon, J. C. Schook,
Dover, Delaware.

The State-Federal Relations Committee of the United States Animal Health
Association met in Washington, D. C., on January 25-29, 1971. At this meeting
we were in conference with United States Department of Agriculture Officials,
Directors, and staff of the Animal Health Division, Veterinary Biologics Division,
Veterinary Sciences Research Division, Consumer and Marketing Service, Federal
Extension Service, and the Bureau of Veterinary Medicine of the Food and Drug
Administration. The Committee wishes to express appreciation to all Federal Per-
sonnel who gave freely of their time and efforts at this meeting and we hope that
mutual benefits will result for all agencies and associations concerned. The State-
Federal Relations Committee presents the following statements for mutual consid-
eration and guidance.

VETERINARY BIOLOGICS DIVISION

The committee is appreciative to the Director and his staff for their explana-
tion of the aims, goals, and requirements of the Veterinary Biologics Division. This
committee recognizes the need of the Veterinary Biologics Division for more ade-
quate laboratory and animal facilities for the development and implementation of
testing methods. The United States Animal Health Association wishes to reemph-
asize that "If the Division is ever to reach and maintain a current status", it becomes
imperative that adequate test animal facilities and testing laboratories be acquired.

Since the committee's recommendation for adoption and implementation of
a proposal by Colorado State University to furnish adequate consolidation facilities
on a lease-purchase basis, at least one alternate solution to this problem is recognized.

It is understood by the committee that certain facilities at Fort Detrick, Fred-
erick, Maryland, would become available for purposes other than their present use
in Fiscal Year 1972 — and further, that with only minor adjustment, these facilities
would provide adequate laboratory and animal space to fully meet the current and
projected needs of the Veterinary Biologics Division. This site would also solve
problems of management and coordination which arise due to the distance between
Hyattsville, Maryland, and Ames, Iowa.

The United States Animal Health Association therefore substantiates that an
imperative need for more adequate facilities remains, and recommends adoption
and implementation of a plan to obtain the aforementioned facilities at Fort Detrick,
and transfer the Veterinary Biologics Division laboratory testing and related units
to that site.

30
ANIMAL HEALTH DIVISION

This committee is in complete agreement with ANH Division in their concept of eradication and not control of animal diseases, and our Association (USAHA) is making every effort to support them.

The Hog Cholera review was very encouraging. Financial support to see this program to successful completion is vital, and should be accomplished through adequate financial support, not at the expense of other programs. This committee endorses the discontinuence of salvage as soon as possible and the increase in the Federal indemnity payments on a percentage basis. It is becoming increasingly difficult for individual states with little or no incidence of Hog Cholera to obtain adequate funding to meet emergency situations.

An improved identification system of all species of livestock should continue to be developed. This would then enhance the development of a rapid efficient, effective system of surveillance through new laboratory techniques and positive traceback to herds of origin.

Brucellosis surveillance program needs to be improved particularly in areas of slaughter sample returns with both cattle and swine. We are concerned about the extension of the eradication goal to 1978. If these goals are to be meaningful they cannot be extended indefinitely. The use of Brucellosis Vaccine (S-19) needs continued deemphasis;

While the Tuberculosis program has been successful in lowering incidence of the disease there are two areas of concern: (1) eliminate all M. Bovis herds promptly and trace and destroy all infected animals associated with those herds: (2) The need for better surveillance, particularly though our Meat Inspection Programs increased laboratory support and better animal identification.

Financial support to the Salmonella surveillance should be adequate for the regulatory agencies to advance with a control program when sufficient data and knowledge become available.

Avian leukosis losses are still estimated to be approximately $200,000,000 annually. While the use of Turkey Herpes Virus Vaccine has been an aid in decreasing these losses, greater emphasis on research, diagnostic methods, and nature of the disease warrant more exploration.

We have recognized the seriousness of Equine Infectious Anemia to the equine population. This problem has been compounded by the carrier state of affected equines and lack of a practical diagnostic test. Further support of the Department's research programs is essential before any control programs can be initiated. Any diagnostic methods must be practical, standardized and uniform wherever employed.

The recent ban on the use of many pesticides has created a fear on the part of the pesticide manufacturers and regulatory agencies to continue research. While present caution is justifiable, we must continue to search for safe, effective disease control products.

We are pleased to learn that the movement of the barrier zone to Southern Mexico has progressed.

The creation of the Agricultural Quarantine and Inspection Division is a most agreeable compromise to our recent apprehension of the inspection of animal and animal products imports by non-veterinary personnel.
It has become increasingly evident that the need for additional space has become critical at the National Animal Disease Laboratory. In order to develop better laboratory support disease surveillance programs even to the suggested automated systems, this critical space problem must be solved. This committee recommends strongly that consideration be given to obtaining space at Fort Detrick, Frederick, Maryland for the Veterinary Biologics Division. This would provide that division adequate space to improve the coverage of control on Veterinary Biologics. The space they now occupy, could then be used by the diagnostic service program.

The Implementation of Project IV should be approached with caution. In the interest of efficiency and economy there should be no duplication of effort in any area. Certain programs however still require separate and distinct identity of Federal and State Agencies. In order not to lose uniformity and identify the system must be established so that we do not regress. The present system of State-Federal Cooperation in Animal Health Programs has not been a panacea, but it has proven its effectiveness.

**VETERINARY SCIENCES RESEARCH DIVISION**

We were pleased to learn of the name change from the old Animal Disease and Parasite Research Division to Veterinary Sciences Research Division. It is more meaningful and in keeping with actual division function. Changing the Beltsville facility name to Animal Parasite Laboratory also has merit.

In reviewing this committee's presentation last year, and, when comparing the report with information provided us now – 1971 – it appears that the issues spoken of then are still mostly unresolved.

We were happy to hear that some planning progress has been made with regard to the Toxicology and Entomology research facility at College Station, Texas. We still feel that with a facility of this magnitude, the present staffing is entirely inadequate and special effort should be made to bring the program up to strength at the earliest moment.

With the present public interest in ecology and environment it would seem that the importance of research on pesticides and attendant problems is all the more important.

A priority plan for five major programs was described. This supposedly encompasses seventy five, scientific man years and is estimated at this time to necessitate the expenditure of $5,000,000 annually.

Although we have already acknowledged the need for the complete implementation of the College Station facility, and we desperately need additional work on parasitic diseases of livestock — we cannot ignore the importance of our defenses against invasion by foreign animal diseases, which carries a No. 3 priority in the present planning schedule. With 100,000 ships docking annually at U. S. Ports of Entry, and with 350,000 airplanes and two million people entering our boundaries each year, we cannot afford to withhold any effort in our surveillance against foreign diseases. As important as research on present disease problems is any neglect in the area of suppressing encroachment by any of the 31 known foreign animal diseases not presently in the United States, would not only add to our problems economically, but would increase our control and eradication problems.
The request for importation of semen from countries where exotic disease exist and importation of live animals from countries used as quarantine facilities between the United States and the source country causes a demand for improved laboratory techniques which can only be gained through research. This would indicate a need for continued and expanded effort toward improving the Plum Island capability.

A review of progress of research projects presently underway has caused the committee to wonder concerning the relative national importance of some of this work and to inquire of the methods on criteria which caused some of these efforts to be undertaken.

Could the money and scientific man years being expended be more gainfully applied in other areas and/or could some of these projects not be as successfully pursued by the States and Universities on a local basis as against preempting the use of research facilities on a National level?

CONSUMER AND MARKETING SERVICE
MEAT AND POULTRY INSPECTION PROGRAMS

We appreciate the opportunity to meet with the staff of the Meat and Poultry Inspection Service and to discuss with them the areas where we are mutually involved. We also greatly appreciate the courtesy shown us and the frankness with which the discussion was held.

We believe the new proposed organization is soundly structured and will make for better and quicker communication between the State and Federal personnel. The new proposed Field Operations Divisions we welcome.

The Compliance and Evaluation unit we realize is a necessary function and that the Administration must be kept directly informed as to the entire program. At the same time we would again urge that the states be specifically advised as to the policy controlling the actual methods to be used by the C. & E. people. We do not think these officers should enter plants and begin their investigations without first identifying themselves to both management and the inspection personnel. We also request that such investigations be made in the company of a representative of both management and the inspection service.

Most investigations should involve inspection and plant acceptability and such related matters. We must be aware that occasionally there may be cause to suspect either criminal intent or acts on the part of either party or actual collusion involving both. Most states, we feel, have little or no experience in such investigations, and therefore we request the Federal assistance and firm guidelines be provided in such cases.

We are in an era that is beginning, at least, to be concerned about our environment and more specifically the disposal of waste materials. Packing plants are directly involved and subject to the new Federal regulations in this regard. Non-compliance could affect the "equal to" status of state services. We request early clarification of the regulations and advice as to how the Federal Service plans to proceed in this matter. The time allowed for compliance is none too long and we should start very soon. We are pleased that the final announcements as to State-by-State compliance are to be made at once. It is disappointing that only 35 states will
be classified as "equal to". We are concerned about the 15 states that will go straight Federal or are probables.

We are basically not in accord with the time lag between Federal notice to so designate a state and the actual Federal take over. We feel that this is most unrealistic and will leave a vacuum of 90 days or more which would provoke great criticism. We urge that another approach be developed and quickly.

After "equal to" status is achieved of course periodic review is necessary to maintain compliance. We urge that when state plants in any area are so reviewed that the Federal plants in the same area are also reviewed and in exactly the same manner, and that a full exchange of the results be furnished each party.

We are aware of the reluctance of Congress to grant, "equal to" States, "equal rights", in commerce. We know that the Department has finally supported such a move. The need now becomes critical. With the potential 15 states that may be placed under total Federal inspection, equal rights, in commerce will be automatic and at no cost to the state. Where does this leave the "equal to" states which have complied and whose citizens are directly paying half of the cost of inspection. We need a Federal Policy in this matter quickly and it should reflect recognition of the state in compliance and not put such state at any disadvantage.

There is one other matter about which we are concerned. Laboratory back-up service. There are 8 Federal labs now in operation. Considerable increase in sampling is predicted. Capability and capacity in many states may not develop. Hardware is becoming prohibitively expensive unless justified by volume and continual use. Down time for much of this equipment is out of the question.

We think that serious consideration should be given to enlarge the Federal labs and increase their capacity so that all necessary lab work connected with these programs could be handled. Cost sharing could remain on the present basis. Overall cost should be considerably reduced as volume and usage time increases.
Ecological awareness is the watchword of the present decade, and the Committee on Parasitic Diseases and Parasiticides goes on record, as the year 1971 draws to a close, as cognizant of its civic responsibilities within this frame of reference. The Committee expresses its vital concern with new approaches to the control of all animal parasitisms, with particular emphasis upon arthropod parasites. The chemotherapeutic attack upon livestock parasites is currently being evaluated very critically by environmentalists, and with good reason. Virtually all of our concerted efforts in the past have been directed toward the refinement of techniques involving the chemical control of parasitic diseases. This admission places those of us responsible for the health of American livestock in an uncomfortable position, for it is not inconceivable that by the end of the seventies, most chemotherapeutic measures in vogue today will be totally unacceptable.

During very recent years, topical spray, dips and dust applications for ectoparasite control have fallen into disfavor, since wind-borne sprays and dusts, and the disposal of vat contents, present formidable logistical problems. As a result, investigations are being conducted into the merits of toxicants administered as feed additives and pour-ons, for the control of lice, ticks, cattle-grubs, nose-bots and keds.

Eventually, the Committee acknowledges, we may be obliged to forsake even these procedures in favor of biological control programs, such as the now familiar sterile male technique for the control of flies, the introduction of predators for the suppression of various insect populations, the selection of host strains genetically refractory to the support of specific endo-and ectoparasites, and immunological measures aimed at this achievement. Investigators are already engaged in developing the economic potential of a wide range of novel biological pest control materials aptly described as “third generation pesticides”. These include naturally-occurring hormones and other compounds that play essential roles in the lives of animal (and also plant) pests, as well as synthetic versions of these natural materials.

The pesticides now in general use are mainly non-selective. Present insecticides, for instance, often kill desirable insects and other animals (including livestock), as well as the target parasite. By the same token, anthelmintics are frequently only
slightly less toxic to the host than to gastro-intestinal parasites. Contemporary workers are particularly interested in exploring the use of hormones, chemosterilants, repellants and stimulants, alone or in combination, as well as other chemical compounds that are specific to the parasitic objective, and without direct side effects in other life.

The Committee recommends support of all of the above efforts, and is following with particular interest the production and evaluation of juvenile insect synthetic hormone mimics, now being employed experimentally against a variety of pests, including stable and other flies, and at least two genera of lice of ruminants. This work is being conducted by the pharmaceutical and related industries and by USDA, and other public agencies, and shows every promise of early fruition. A resume of the chemistry, biological activity and potential application of synthetic juvenile hormones was presented to the Committee by Dr. W. F. Chamberlain, of the Entomology Research Division, ARS, USDA, Kerrville, Texas. Dr. Chamberlain advises the Committee that practical application of these compounds may be anticipated within a period of approximately five years.

While hopes run high for the eventual achievement of parasite control by non-chemical means, there are certain problems of the moment which, unfortunately, cannot await the prophetic visions of technologists. The following areas of concern were among those which received primary consideration:

HEEL FLIES, OR CATTLE GRUBS

A case in point involves losses attributed to heel flies (Hypoderma spp.). These flies and their larvae, commonly known as cattle grubs, or ox-warbles, continue to inflict hundreds of millions of dollars of damage annually upon cattle growers, meat packers, hide tanners and eventually the American consumer. Approximately a quarter century of intensive research has gone into the development of remarkable chemical compounds, broadly and popularly described as animal systemic insecticides. In view of the high order of effectiveness, low cost and relative innocuousness of several of these compounds, the Committee suggests that an effort be made to promote interest in cattle grub control, if not on a national level, then at least in those areas of the country—the Midwest, the Northern Plain States and the Southwest east of the Rocky Mountains—where cattle and cattle grub populations are highest. Interest and subsequent voluntary response on the part of cattlegrowers, it is believed, could best be stimulated by providing demonstrations of successful cattle grub control programs. The Committee therefore unanimously approved adoption of the following resolution:

That the USAHA support the establishment of programs designed to demonstrate, on a large scale, the effectiveness of community efforts to suppress heel fly and cattle grub population.

That the USAHA seek the cooperation of the American Association of Bovine Practitioners, American Association of Extension Veterinarians, American National Cattlemen’s Association, Entomological Society of America, Livestock Conservation, Inc., National Livestock and Meat Board, Future Farmers
of America, U. S. Department of Agriculture, and other agencies and institutions, in active or advisory capacity, in promoting and evaluating this demonstration program, and in providing technical assistance.

That such demonstration programs be voluntary in character and be privately funded.

SHEEP SCABIES

In a paper presented by Dr. James L. Hourrigan, Senior Staff Veterinarian, Sheep, Goats, Equine and Ectoparasites Staff; Animal Health Division, ARS, USDA, an announcement was made to the effect that psoroptic scabies of sheep in the U.S.A. was not reported during FY 1971. It was stated that this is probably the first year that the disease was not detected since sheep were introduced, on a permanent basis, into what is now continental U.S.A., and is a landmark of achievement in disease control. The most recent isolation of *Psoroptes ovis* from sheep took place in New Jersey, in January, 1970. This Committee continues to recommend, as it has in the past, the maintenance of inspection programs at a high level of intensity; if psoroptic sheep scabies is not disclosed for a period of at least three years, the nation will be regarded as free of the disease.

The leg mange mite of ruminants and other livestock, *Chorioptes bovis*, was isolated from six flocks of sheep in five states. Disclosure of this parasite associated with sheep scabies inspections is regarded as indication of high quality inspection practices. *Chorioptes bovis*, was also isolated from a single flock of goats.

Federal and State Inspection programs involved the examination of 9,042,397 sheep on farms and ranches, and 3,072,946 at public stockyards during FY 1971.

CATTLE SCABIES

The national cattle scabies control program was marked by an increase in the number of infested lots of cattle detected during the past year; the incidence rose from one in FY 1970, to 11 in FY 1971. Infestations were found in Nebraska, Iowa and Texas. Epidemiological pursuit of these outbreaks failed to reveal source herds. *Sarcoptes scabiei* was isolated from seven herds of cattle in three states; *Chorioptes bovis* was found on 115 lots of cattle widely distributed about the nation, and from a single horse in New Mexico.

A total of 26,163,586 cattle on farms and ranches, and 14,019,291 at public stockyards, were reported to have been examined for the presence of mites responsible for scabies; 150,363 cattle were dipped under the supervision of State or Federal personnel, at various locations.

CATTLE FEVER TICKS

In Texas, efforts to prevent the entry of *Boophilus* spp. from Mexico were continued. During FY 1971, in the Texas buffer zone currently under Federal and State quarantine, 46,962 lots, consisting of 680,360 head of livestock, were inspected for the presence of fever ticks, and 15,751 lots, comprising 84,214 animals were
dipped. Outside of the area under quarantine, 28,111 lots of 963,650 livestock were inspected, and 4,282 lots of 54,206 livestock were dipped or sprayed as required.

SCREWWORMS

During the calendar year 1971, through October 9, screwworms were found in the Texarkana region of Texas and in adjacent areas of Arkansas. This is the first report of screwworm detection in Arkansas since prior to the inception of the eradication program in the Southwest, and the first time that the flies have penetrated so far north in Texas since 1961. The screwworm infestations in this area have persisted despite an intensive sterile fly drop and an elaborate preventative spraying program. _Cochliomyia americana_ infestations have been confined to the region described, and have not advanced into other areas.
REPORT OF THE COMMITTEE ON
BIOLOGIES

Chairman: N. H. Casselberry, Berkeley, Calif.
Co-Chairman: M. J. Twiehaus, Lincoln, Nebr.


Your committee this year is not including information on Veterinary Biologies Division (VBD) licensing. As pointed out last year this information is circulated to you in Vet Topics bimonthly directly from VBD.

There were several significant developments pertaining to biological products which were discussed in our meeting as follows:

1. Venezuelan Equine Encephalomyelitis vaccination.
   This subject will be covered in depth elsewhere in your program. We have nothing to add except a note of satisfaction that the vaccination program utilizing the TC 83 vaccine developed by the Department of Defense seems to prove the vaccine safe and effective in curtailing the outbreak. We hope it is found to have no unfavorable effect on the developing fetus and we should have this information by next spring. The vaccine is now commercially available for next year vaccination.

2. Equine Infectious Anemia
   The agar gel diffusion test to detect antibody to E. I. A. (known as the Coggins Test) was used to screen horses producing Veterinary Antisera this spring.
   Some positive horses were found in some licensees herds. This finding raised the question, "Could serum derived from these animals transmit the virus of E. I. A. to horses treated with such antisera?" It was found by V. B. D. that raw serum from positive horses would transmit the virus. However, final processed serum (which is pasteurized at 58–59°C for one hour) did not. Even though pasteurization did inactivate the virus, it was still deemed advisable to avoid leaning on an inactivation procedure and instead require that equine antisera be produced from horses tested and found negative by the Coggins Test for E. I. A. antibody. These horses must be negative on two test bleedings taken at 8 weeks intervals.

3. Mareks Disease
   The committee has some concern for the large volume of vaccine produced by companies not holding an interstate (Federal) license. Such vaccine can be used only in the state in which it is produced and is not required
to meet the same rigid standards of V. B. D. It is unfortunate that this loophole to escape federal licensing is available, since the federal law doesn't provide authority over products produced for intrastate sale. It is hoped that state requirements will be developed to match federal requirements for proper quality control of these products.

4. Newcastle Disease

This disease has been a problem again and the virus isolated is highly virulent (Velogenic). This has occurred in vaccinated chickens so attention has been focused on (1) Vaccines and vaccination methods (2) The question of duration of immunity following the various vaccination procedures (3) Virulence and antigenic character of the epizootic virus.

It has been shown that presently available vaccines do protect against this strain, but other variables involved in routine vaccination programs need study.

Agriculture Research Service (ARS) is planning an experimental program comparing vaccine and vaccination methods when challenged by this epizootic and highly virulent virus. This challenge work is not to be done at Ames. The epizootic challenge virus is being treated as an exotic virus in view of its highly virulent character.

5. Mixed Bacterins

The V. B. D. has informed all licensees' producing these products that data must be developed to re-evaluate them. Such information should include laboratory animal tests for potency and host animal data for proof of efficiency.

This is an old problem and a difficult one. In fact, to show a positive immune response against some of the organisms in mixed bacterins may be impossible in the present state of knowledge. Maybe some of the organisms used in the present formulas are not needed. There is not good agreement among Veterinary experts on this point as to which organisms are pathogens. Certainly many of them do not produce frank disease experimentally and this makes host animal evaluation difficult. V. B. D. has asked industry to come up with a program of re-evaluation or get rid of the products. There is good reason to believe that we should not make a decision to get rid of them without study and advice from other segments of the Veterinary profession.

Therefore, a subcommittee of the Animal Health Institute (AHI) made up of licensees producing one or more of these products is trying to get the latest data available from diagnostic laboratories in the United States. We are interested in the frequency of isolation of these organisms in the mixed bacterins as listed in various categories. We have also asked for opinions as to the significance of these isolations. We urge you all to participate in this survey and return your questionaires to the A.H.I. office as requested.
This information may provide a basis for disregarding those organisms considered unimportant so effort can be expended on the others which could then be represented in revised formulas. We see no need for the expensive evaluation of some antigens that the informed sources feel are unimportant.

In joint V. B. D.-Industry discussions we have anticipated results to the extent that work is underway to develop the means for evaluation of the efficacy of Pasteurella and Salmonella antigens contained in mixed bacterins.

6. Warning on IBR vaccines to avoid vaccination of suckling calves on non-vaccinated cows.

You have seen a news release recommending caution on this practice because of presumptive field evidence in South Dakota that abortions were caused by IBR virus transmitted from calves to their pregnant mothers.

There still is no proof that this is a hazardous practice, but field evidence has pointed this way.

We learned that V. B. D. has been able to isolate IBR virus from the nasal passages of calves vaccinated intramuscularly.

V. B. D. is now proposing a change in labeling for IBR live attenuated vaccines to warn against vaccination of these suckling calves if the cows are unvaccinated.

Where calves are to be vaccinated with live IBR vaccine in a preconditioning program this danger to the cow can be avoided by vaccination of the cows and heifers while they are open. However, this then could be expected to transmit passive antibody to the calf and the responsiveness of those calves to vaccination at a young age should also be studied.

There is now a killed IBR vaccine available which could be used on calves without transmitting virus to the cow. It requires two doses to induce immunity.

Recommendation:

Last year the committee made the following request, "The committee recommends that the Association re-examine the objectives of the Biologies Committee in light of the rapid changes in this area. Through appropriate officers or a task force the new responsibilities should be defined if it is to continue to serve a useful purpose."

We've received no pearls of wisdom from the Association and we'd appreciate some guidance for the future.
REPORT OF THE COMMITTEE ON MEAT AND POULTRY HYGIENE

Co-Chairman: J. K. Payne, Washington, D. C.

Import and Export:
   Peter Gailiunas, Greenport, L. I., N. Y.
Industry:
Federal:
State-Federal Collaboration:
   D. C. Breeden, Lincoln, Nebr.
State:

Teaching Research and Public Health —
   Universities and Training Instructions:

Mr. Chairman:
   Your Committee reviewed its report of the 74th Annual Meeting of the United States Animal Health Association, Philadelphia, Pennsylvania. A continuing study of all major topics covered in that report has been conducted during the past year.

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

   Your Committee has been reorganized into four subcommittees to permit continuing study in the given areas which your Committee recognizes as its major responsibilities.

1. RESIDUES IN MEAT AND POULTRY PRODUCTS
   Your Committee recommends that the United States Animal Health Association continue to support the establishment of a National Residue Monitoring Program which would determine the presence of biological residues (as defined in the Federal Meat Inspection Regulations) in animals and poultry at the time of slaughter, prescribe and implement regulatory control actions, and assist in correc-
tive procedures to prevent the adulteration from recurring. This monitoring pro-
gram would function through the cooperative efforts of the United States Depart-
ment of Agriculture, Food and Drug Administration, and responsible state agencies,
the livestock and poultry industries, and the livestock and poultry producers. Your
Committee recommends that the United States Animal Health Association rec-
commend the establishment of a national system of gathering, evaluating, and rapidly
dissiminating data from residue monitoring programs of all agencies (State and
Federal) to enable the regulatory control procedures of national residue monitor-
program to be used to its fullest extent for consumer protection.

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

TOXICOLOGY INFORMATION

A Toxicology Information Program was brought to the attention of your
committee by Dr. Fred Clayton, Chief, Toxicology Information Services, National
Library of Medicine, Bethesda, Maryland 20014.

This program disseminates information which is related to meat and poultry
hygiene upon request to the address as indicated above.

2. DISEASE CONTROL AND PRODUCT SAFETY

While the committee recognizes that an increasing effort is being directed to-
ward control of taeniasis-cysticercosis, the incidence of infection shows that further
effort is needed. Your committee recommends a broad based approach encom-
passing such controls as effective meat inspection, public health actions to break the
life cycle at the human carrier stage, research to provide diagnosis of cysticercosis in
the life animal and epidemiologic activities to identify and correct conditions causing
outbreaks.

The committee on Meat and Poultry Hygiene wishes to direct attention to the
seventh resolution of this committee's report of the 74th Annual Meeting of the
USAHA. It must be emphasized again that the most logical control of trichinosis
from a meat hygiene standpoint is to develop a national eradication program. There-
fore, your committee recommends that the USAHA should continue to actively
support the development of such a trichinae eradication program.

The report of the 74th annual meeting of the United States Animal Health
Association recommended that Federal and State inspection regulations be amended
to require all exposed products be protected by an effective covering or container
before being transported, loaded or unloaded. It was reported in the 75th annual
meeting that Federal policy people are considering this requirement at the present
time. It is recommended that encouragement continue to be given to state and
federal groups to establish guidelines for such handling and that implementation be
effected.

3. EDUCATION, TRAINING AND PERSONNEL

Your committee again recommends that the principles of food hygiene be a
required part of the curriculum for professional students at the colleges of veterinary medicine.

Your committee reiterates its recommendation that the Consumer and Marketing Service be encouraged to make available a MPIP veterinarian on a part-time basis to assist in the training of undergraduate students in the colleges of veterinary medicine.

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

It is further recommended that the Consumer and Marketing Service and the State Meat Inspection programs should permit and encourage program veterinarians to attend professional meeting in their vicinity.

4. COOPERATIVE PROGRAMS AND FUNCTIONS

Your committee recommends that MPIP, C&MS clarify present Federal regulations pertaining to the definition of retail stores. Such clarification should include whether or not it is intended that pre-processes, canned and/or otherwise pre-packaged inspected meat and meat food products are to be included in the 24 percent or maximum gross annual HRI sales limitation for the purpose of determining if the retail store is subject to regular inspection. Your committee further recommends that the Federal regulations specifically be amended to require that a reasonable bookkeeping system be maintained by all exempted retail stores which have any sales of meat and meat food products to non-household consumers i.e. hotels, restaurants and institutions.

The committee also recommends that the gross annual limitation of $10,000.00 of sales to non-household consumers (HRI) be raised to $25,000. It is the contention of this committee that some such higher limit is mandatory considering the large number of additional retail stores that are being required to come under regular inspection without an actual expansion of sales volume. This is due to the past inflationary economy affecting retail meat prices.

The National School Lunch Program and its affiliated local School Districts are large consumers of Meat and Poultry products from inspected establishments. There is presently considerable variation in the standards and inspection requirements of contracts let by local school districts in purchasing Meat and Poultry Products and there is a lack of understanding of inspection activities and present labeling and product standards on the part of local school lunch program administrators. The USDA Food and Nutrition Service issued Notice No. 219 on February 22, 1971 which authorizes modification of Section 210.10 of the regulations governing the National School Lunch Program (September 4, 1970) and Section 225.9 of the reg-
ulations governing the Special Food Service Program for Children (April 1970). Said modifications allow the addition of Textured Vegetable Protein (TVP) to meat fed in Type A school lunches and other feeding programs to the extent of 70 parts uncooked meat in combination with not more than 30 parts hydrated vegetable protein product.

Therefore, your committee strongly urges a concerted effort, at the National level, to establish communication between the USDA Food and Nutrition Service and Meat and Poultry Inspection Programs for purposes of accomplishing the following worthwhile goals:

1. To acquaint school lunch program administrators, at all levels, with existing nationally established meat product designations, standards and restrictions on the addition of Textured Vegetable Protein (TVP) to certain products.
2. To acquaint such administrators with Meat Inspection Program determinations regarding labeling of TVP and other forms of soy products.
3. To acquaint school lunch program administrators with the inspection controls routinely applied in the processing of specific products normally purchased by lunch programs, in Federal and "equal to" State inspected establishments.
4. To emphasize the merits of letting school lunch program contracts to inspected establishments wherever possible.
5. To identify for school lunch program administrators, possible contract requirements and product standards which can only be assured by requiring that the establishment seek USDA, or comparable state, Grading Service Certification and acceptance inspection; and to point out the inspection charge incurred by the establishment in providing this assurance.
6. To establish product names, Meat Inspection Program standards, and authorization to produce "new" meat products occasioned by Food and Nutrition Service Notice No. 219.

The California Department of Agriculture and the U.S.D.A. Consumer and Market Service have entered into a cooperative federal-state laboratory agreement.

This is another step forward in a complete cooperative working relationship between state and federal meat and poultry inspection program for the benefit of consumer protection. Sample exchange will be in the areas of chemical, antibiotic, pesticide, and heavy metals.

Federal training is to be provided State personnel, and split sample checks will be made. AOAC methods will be used and checked periodically by the U.S.D.A. Your committee commends the State of California and U.S.D.A. for the cooperative venture and urges other states to move in this direction.

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

Chairman: R. C. Hammond, College Park, Maryland
C. C. Beck, Ann Arbor, Michigan; W. C. Burnet, Kansas City, Missouri;
G. L. Crenshaw, Davis, California; C. Dobbins, Athens, Georgia; N. B. Haynes,
Ithaca, New York; W. L. Henning, University Park, Pennsylvania; R. I.
Hostetler, Pullma, Washington; Moses Simmons, Denton, Texas; T. P. Siburt,
Blacksburg, Virginia; K. Weinland, Lafayette, Indiana.

The Committee on Professional Education and Extension met yesterday after-
noon in Room 1018 of this hotel. Actions resulting from the Committee are as
follows:

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

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

3. The committee feels that the name of this committee does not clearly
   define its function and recommends that it be changed to the Committee
   on Professional Relations.

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

   a. Possibly this should be a charge of this committee.

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

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

Mr. Chairman, these recommendations and decisions constitute the action of the Committee on Professional Education and Extension, and they are respectfully submitted. We recommend their adoption.
REPORT OF THE COMMITTEE ON RABIES

Chairman: R. K. Sikes, Atlanta, Ga.
Co-Chairman: E. M. Joneschild, Pierre, S. Dak.


In 1970, 3,276 laboratory-confirmed cases of rabies were reported to the Center for Disease Control (CDC); this is a 7 percent decrease from 1969, and a 20 percent decrease from the average for the previous 5 years (Table 1 and Figure 1). The most significant decline of rabies in animals during the past few years has been in dogs. In 1970, for the first time since records have been available on animal rabies, there were only 185 laboratory confirmed cases of rabies in dogs throughout the United States.

Forty-seven states reported confirmed cases of rabies in animals, only Delaware, Hawaii and South Carolina reported no cases in 1970 (Figure 2). Puerto Rico reported a total of 49 confirmed cases of animal rabies, the most in over 10 years. Guam and the Virgin Islands remained free of rabies in 1970, as did Washington, D.C.

California reported the largest number of cases with 322. The five most frequently infected species in 1970 were skunks (38%), foxes (24%), cattle (10%), bats (9%), and dogs (6%). It should be noted that for the tenth consecutive year, skunks were the most frequently infected species in the United States. This species of animal also served as the vector for the only two human rabies deaths in the United States in 1970. (Table 2). These two human cases were in boys aged 4 and 11 years, who were bitten during the night while camping in Arizona and South Dakota during the summer of 1970. An episode of at least one rabid skunk being sold by a pet shop was reported in 1970.

Your Committee reviewed several aspects of rabies control and research and endorse the following recommendations:

1. That the second revision of the "Guidelines for the Control of Rabies" be developed to include new recommendations on the use of the 10 types of rabies vaccines currently licensed in the United States.

2. That the states work together through legal and professional means to standardize rabies control practices.

3. Abolish persistent low-intensity population reduction programs over diffuse geographic areas.

4. Intensify population reduction efforts in restricted problem areas. This is especially important in those areas where rabies exposure rate to humans is highest, such as picnic areas and camp sites.
5. That the Departments of Interior and Health, Education, and Welfare, as well as appropriate state agencies and universities, undertake studies to provide the basic biologic data on wildlife rabies vectors needed to develop intelligent control measures.

6. That the Rabies Control Unit of CDC conduct studies on the safety and efficacy of licensed inactivated vaccines in order to recommend their use in vaccinating at least the three most commonly owned species of wild animals in the United States.

ACCOMPLISHMENTS resulting from recommendations made in 1969 and 1970 of this committee:

1. In 1971, a rabies sub-committee from the Animal Health Committee of the National Research Council, National Academy of Sciences, convened with various scientists and experts in the rabies field to evaluate national rabies problems that need greater attention. This Committee was formed as a result of the recommendation made by the Rabies Committee of the USAHA in 1968 and 1969. A final “white paper” report of that sub-committee is expected in 1972.

2. Improved surveillance of animal rabies in the United States has resulted from the recommendations made last year by this committee. This has involved the receipt and publication of monthly instead of quarterly Rabies Surveillance Reports at CDC. The additional information requested on the species of animals diagnosed as rabies has been included in the monthly and will also be reflected in the Animal Rabies Surveillance Reports.

3. Forms have been provided to the states requesting a complete case history on each dog and cat diagnosed with rabies. This will provide better assessment of vaccines under field use.
REPORT OF THE COMMITTEE ON RABIES

CASES OF RABIES IN WILD AND DOMESTIC ANIMALS
1953–1970

TOTAL

WILD

DOMESTIC

0
2,000
4,000
6,000
8,000
10,000

ANIMAL NUMBER OF RABIES CASES REPORTED BY STATE, 1970

TOTAL CASES LABORATORY CONFIRMED – 3,276
REPORT OF THE COMMITTEE ON RABIES

Table 1
INCIDENCE OF RABIES IN THE UNITED STATES BY TYPE OF ANIMAL
1953—1970*

<table>
<thead>
<tr>
<th>YEAR</th>
<th>DOGS</th>
<th>CATS</th>
<th>FARM ANIMALS</th>
<th>FOXES</th>
<th>SKUNKS</th>
<th>BATS</th>
<th>OTHER ANIMALS</th>
<th>MAN</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1953</td>
<td>5,688</td>
<td>538</td>
<td>1,118</td>
<td>1,033</td>
<td>319</td>
<td>8</td>
<td>119</td>
<td>14</td>
<td>8,837</td>
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<tr>
<td>1954</td>
<td>4,083</td>
<td>462</td>
<td>1,012</td>
<td>1,028</td>
<td>547</td>
<td>4</td>
<td>118</td>
<td>8</td>
<td>7,282</td>
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<tr>
<td>1955</td>
<td>2,657</td>
<td>343</td>
<td>924</td>
<td>1,223</td>
<td>580</td>
<td>14</td>
<td>98</td>
<td>5</td>
<td>5,844</td>
</tr>
<tr>
<td>1956</td>
<td>2,592</td>
<td>371</td>
<td>794</td>
<td>1,281</td>
<td>631</td>
<td>41</td>
<td>126</td>
<td>10</td>
<td>5,846</td>
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<tr>
<td>1957</td>
<td>1,758</td>
<td>382</td>
<td>714</td>
<td>1,021</td>
<td>775</td>
<td>31</td>
<td>115</td>
<td>6</td>
<td>4,802</td>
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<tr>
<td>1958</td>
<td>1,643</td>
<td>353</td>
<td>737</td>
<td>845</td>
<td>1,005</td>
<td>68</td>
<td>157</td>
<td>6</td>
<td>4,814</td>
</tr>
<tr>
<td>1959</td>
<td>1,119</td>
<td>292</td>
<td>751</td>
<td>920</td>
<td>789</td>
<td>80</td>
<td>126</td>
<td>6</td>
<td>4,083</td>
</tr>
<tr>
<td>1960</td>
<td>687</td>
<td>277</td>
<td>645</td>
<td>915</td>
<td>725</td>
<td>88</td>
<td>108</td>
<td>2</td>
<td>3,457</td>
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<tr>
<td>1961</td>
<td>594</td>
<td>217</td>
<td>482</td>
<td>614</td>
<td>1,254</td>
<td>186</td>
<td>120</td>
<td>3</td>
<td>3,470</td>
</tr>
<tr>
<td>1962</td>
<td>565</td>
<td>232</td>
<td>614</td>
<td>594</td>
<td>1,449</td>
<td>157</td>
<td>114</td>
<td>2</td>
<td>3,727</td>
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<tr>
<td>1963</td>
<td>573</td>
<td>217</td>
<td>531</td>
<td>622</td>
<td>1,462</td>
<td>303</td>
<td>224</td>
<td>1</td>
<td>3,933</td>
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<tr>
<td>1964</td>
<td>409</td>
<td>220</td>
<td>594</td>
<td>1,061</td>
<td>1,909</td>
<td>352</td>
<td>238</td>
<td>1</td>
<td>4,784</td>
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<tr>
<td>1965</td>
<td>412</td>
<td>289</td>
<td>625</td>
<td>1,038</td>
<td>1,582</td>
<td>484</td>
<td>153</td>
<td>1</td>
<td>4,584</td>
</tr>
<tr>
<td>1966</td>
<td>412</td>
<td>252</td>
<td>587</td>
<td>864</td>
<td>1,522</td>
<td>377</td>
<td>183</td>
<td>1</td>
<td>4,198</td>
</tr>
<tr>
<td>1967</td>
<td>412</td>
<td>293</td>
<td>691</td>
<td>979</td>
<td>1,568</td>
<td>414</td>
<td>250</td>
<td>2</td>
<td>4,609</td>
</tr>
<tr>
<td>1968</td>
<td>296</td>
<td>157</td>
<td>457</td>
<td>801</td>
<td>1,400</td>
<td>291</td>
<td>210</td>
<td>1</td>
<td>3,613</td>
</tr>
<tr>
<td>1969</td>
<td>236</td>
<td>165</td>
<td>428</td>
<td>988</td>
<td>1,156</td>
<td>321</td>
<td>307</td>
<td>1</td>
<td>3,522</td>
</tr>
<tr>
<td>1970</td>
<td>185</td>
<td>135</td>
<td>399</td>
<td>771</td>
<td>1,235</td>
<td>296</td>
<td>252</td>
<td>3</td>
<td>3,276</td>
</tr>
</tbody>
</table>

*Data prior to 1960 from USDA, ARS. Subsequent data from PHS, NCIH.

Table 2
HUMAN RABIES DEATHS, UNITED STATES, 1970

<table>
<thead>
<tr>
<th>Locality</th>
<th>Age</th>
<th>Sex</th>
<th>Nature of Exposure</th>
<th>Brining Animal</th>
<th>Treatment</th>
<th>Length of Illness</th>
<th>Date of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamberlain, S. D</td>
<td>4</td>
<td>M</td>
<td>Bites on arm</td>
<td>Striped Skunk</td>
<td>Antirabies serum</td>
<td>8 days</td>
<td>8/2/70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Scratches on face</td>
<td></td>
<td>1 M + 14 doses D+V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McNary, Ariz</td>
<td>11</td>
<td>M</td>
<td>Bites on shoulder and hand</td>
<td>Striped Skunk</td>
<td>14 doses D+V</td>
<td>10 days</td>
<td>7/30/70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 days after exposure</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
THE ALBION MASTITIS PROJECT

Dr. A. R. Smith, Staff Veterinarian,  
Wisconsin Dept. of Agriculture,  
Madison, Wisconsin

The University of Wisconsin, Veterinary Science Department, the United States Department of Agriculture, ARS, Animal Health Division and the Wisconsin Department of Agriculture, Animal Health Division, entered into an agreement to do applied research on mastitis. The project was to study proposed methods of eradicating Streptococcus agalactiae from herds in an area. The objective was to develop the fastest, most economical, yet workable method under farm conditions. The rate of elimination of the organism from the various groups, the producer's costs and production effects were to be recorded. The acceptance and effectiveness were to be observed for the test and treat approach and the so called "British System."

The area selected was the 200 Grade A and 60 patrons of the Albion Cooperative Creamery, Edgerton, Wisconsin. It was selected because of its size, it involved both fluid milk and manufactured milk producers and all farms had bulk tanks for storage and refrigeration of their milk. The willingness of the management to cooperate also was in their favor.

The bulk tanks were all sampled and cultured using TKT media; first by streaking with .01 ml loop, followed by the cream flotation method. Of the 260 samples, 158 or 60.7 per cent cultured Strep. ag. The following week the negative herds were again sampled and culture repeated. Of the 102 herds, 12 (4.6 per cent of total) were Strep. ag. positive on this sample. Three weeks later a third sample of the negative herds was cultured and all were negative. A meeting was held with the producers and the project was explained. The ones positive on culture were invited to participate further; so that all could cooperate without financial loss, the University of Wisconsin paid out funds for treatment by veterinarians and the milk thereby disposed of because of adulteration.

The patrons were divided into four groups. The method used to divide the herds into groups was by ranking them according to weight of milk shipped to the plant in a 15-day period. Then numbering them 1, 2, 3, 4, 4, 3, 2, 1. The weight of the milk ranged from 47,775 pounds to 2,084 pounds delivered to the plant the first half of November, 1969.

The cows in all the herds were sampled and the Bacteriology Section of the Wisconsin Central Animal Health Laboratory did the culturing. Group 3, the intensive group, was begun on January 5 and first sampling completed by January 16 with one exception. The dates on Group 1, the control, are January 15—February 6; Group 2, culture and treat only. January 19—February 19; Group 4, "British System," January 23—February 18.

Group 1, the control, was sampled by lay personnel who were instructed to give no mastitis information but to ask the herd owner to continue to operate as he had been doing. This group was to be the control group.

Group 2, culture and treat, also was sampled by lay personnel who were again instructed to give no mastitis information. These herds were resampled approximately every 12th working day. The results were telephoned to the herd owner's veterinarian who infused the four quarters of the Streptococcus agalactiae infected animals as soon as possible. The herds were resampled, cultured, and treated until two consecutive negative herd cultures were obtained or 12 herd samplings, whichever came first.

Group 3 was handled by regulatory veterinarians applying the technique known as "Wisconsin Intensive Mastitis Control Program." It is done at milking time when milking procedure is checked, individual udder samples collected, CMT of lactating animals and milking machines are analyzed. Recommendations were made at this time regarding milking procedure and upgrading of the machine. One gallon of Bovadine and oral instructions on its use were given to the owner. He also received order blanks for refills at no charge.

The culture report was also telephoned to the herd owner's veterinarian who did the treating with the antibiotics furnished for the project. All the milk of Groups 2 and 3 which was disposed of because of antibiotics was paid for at blend price plus 50c a day for each infected lactating cow for 72 hours following treatment. Group 3 herds were resampled by division veterinarians approximately every three weeks until two consecutive negative herd cultures were obtained.

The fourth group used the so called "British System." They were sampled by lay personnel who delivered the Bovadine and reorder forms and informed the herd owner to have all cows treated by their veterinarian as they went dry, the cost which was to be assumed by the project.

Each herd owner and/or manager was asked to maintain a calendar furnished to them monthly. The calendar was to keep the number of cow days of production each month. This was done by recording the number of cows milking at start and end of each month and when a fresh or new cow's milk was added to the supply or when a cow dried up, its name or number was recorded as in or out on that certain day. The calendars were picked up by Animal Health personnel on a monthly visit to the farm to obtain bulk tank milk samples for culture. At this time a questionnaire was completed each month regarding milking procedure changes. The weight of the milk received at the plant each month was then used in figuring the production/cow/day.

In January of 1971, Groups 1, the control, and 4, the dry treatment group were resampled and cultured to determine the incidence after one year on the project. Groups 2 and 3 were not resampled unless the monthly bulk tank samples were positive after the two consecutive negative herd tests. These herds were considered negative as most herds had six to eight negative monthly bulk tank cultures. The herd owner was presented with a Strep. ag. free certificate after two negative herd tests and two monthly bulk tank cultures. This certificate is issued in Wisconsin.

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2Bovadine was furnished by the Lazurus Laboratories, Inc., a Division of West Agro Chemical Group.
when any herd meets these requirements. During the 12 months, if herds in the control or dry treatment groups were negative on bulk samples, two consecutive months, the individuals in that herd were resampled. This was to ascertain when these herds were cleaned up of Streptococcus agalactiae.

Table number one relates to size of groups, the initial incidence of infection within a group and at the end of a 12-month period. All groups had about the same number of herds ranging from 32 to 36 in each group. In number of cows the groups were similar ranging from 1,075 to 1,277. The number of infected animals in each group were from 457 to 550. The percent ranged from 39 percent in Group 2 to 43 percent in Group 3. The number of cows at the end of the 12 months stayed about the same in all groups, but the incidence of infection changed in all four groups, dropping 9 percent in the controls down to only 1 percent in the culture and treat only group with complete eradication in the intensive group and in Group 4, the incidence was reduced over 50 percent by treat dipping and dry cow treatment.

### Table I

#### Final

<table>
<thead>
<tr>
<th>GROUP</th>
<th># HERDS</th>
<th># COWS</th>
<th># INFECTED</th>
<th>%</th>
<th># COWS</th>
<th># INFECTED</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>33</td>
<td>1,075</td>
<td>457</td>
<td>42.5</td>
<td>1,078</td>
<td>362</td>
<td>33.5</td>
</tr>
<tr>
<td>II</td>
<td>32</td>
<td>1,186</td>
<td>468</td>
<td>39.4</td>
<td>1,177</td>
<td>14</td>
<td>1.1</td>
</tr>
<tr>
<td>III</td>
<td>36</td>
<td>1,277</td>
<td>550</td>
<td>43.0</td>
<td>1,257</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>IV</td>
<td>34</td>
<td>1,125</td>
<td>468</td>
<td>41.6</td>
<td>1,119</td>
<td>215</td>
<td>19.2</td>
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<tr>
<td>TOTALS</td>
<td>135</td>
<td>4,663</td>
<td>1,943</td>
<td>41.6</td>
<td>4,631</td>
<td>591</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Table number two shows the ranges in size of herds and percent of infection. All four groups were about the same size. The smallest number of animals in the herd was about 14 and the largest herd had 77 cows. The incidence of infection varied more in that the lowest was 2 percent on Group 4 and the highest was 94 percent in Group 2.

### Table II

#### Ranges of Groups

<table>
<thead>
<tr>
<th>GROUP</th>
<th>HERD SIZE</th>
<th>INFECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I</td>
<td>14 - 67 COWS</td>
<td>14 - 77%</td>
</tr>
<tr>
<td>GROUP II</td>
<td>17 - 72 COWS</td>
<td>10 - 94%</td>
</tr>
<tr>
<td>GROUP III</td>
<td>14 - 64 COWS</td>
<td>7 - 83%</td>
</tr>
<tr>
<td>GROUP IV</td>
<td>14 - 77 COWS</td>
<td>2.2 - 75%</td>
</tr>
</tbody>
</table>
Table number three shows comparison of the rate of elimination in the herds of Groups 2 and 3 where eradication was accomplished with no information to the dairyman and the group where all aids were used. The organism was considered eradicated when the herd passed two consecutive negative tests. This chart is the cumulative rate of Strep. ag. eradication. The solid line is the Group 2 herds where eradication was achieved by culture and treatment only. The circled dot on the twelfth herd which is when eradication was achieved on the ninth test, this is the median herd in the group. The median herd on Group 3 would be the 18th herd which was achieved on the eighth test, whereas the 12th herd in Group 3 was reached on the seventh test. The effectiveness of the first treatment was 51 percent on Group 2 and 56 percent on Group 3. These animals never again cultured Strep. ag. during the project.
Table number four shows the accumulated cost per cow for each round of sampling for Groups 2 at the top and 3 in the lower line, and the 24 herds in Group 2 in the middle that eliminated Streptococcus agalactiae leaving out the eight herds that did not attain it. This cost includes drugs, veterinary fee and dumped milk. The cost of sample collection and culturing is not included.

The intensive group costs can be used as a guide to herd owners as their anticipated costs for eradication. The $8.20 multiplied times the number of animals in their herd would give them the cost as it occurred in the Albion Mastitis Control Project. As is shown on the graph, the fewer the number of cultures necessary, the faster and more economical eradication would be achieved.

**TABLE IV**

![Graph showing accumulated cost per cow for each round of sampling for Groups 2 and 3, and the 24 herds in Group 2 that eliminated Streptococcus agalactiae, and the 8 herds that did not attain it. The cost includes drugs, veterinary fee and dumped milk. The graph shows that the fewer the number of cultures necessary, the faster and more economical eradication would be achieved.]
Table number five compares the mean production per cow day in all four groups. Although three of the four groups show an increase in production at the end of the year, the increase is not statistically significant from the controls. Group 3, the intensive one, as was anticipated, began to pull away in late summer above the other groups, but in October the control group for an unknown reason did not follow the seasonal drop shown by Groups 2 and 4.
Table number six is very similar yet there is a variation in the method of graphing. This shows the average group production compared to the control. The control, or Group 1, is the base line. It should be pointed out in this graph that in January of 1970, Group 3, the intensive, was the only group where the initial test was completed and complete records were kept during this month. To clarify this graph, go to July and the lowest, Group 2, shows that they were 1½ pounds below that of the control. Group 3, the average production was slightly two pounds over the control and Group 4, dry treatment, was almost 3½ pounds above the production of the controls. In November and December it shows the seasonal drop of the three groups, but the control does not go along with it and almost attains the level of the intensive group in December.

**TABLE VI**

![Graph showing average group production compared to control]
Table number seven reports on teat dipping and dry treatment, Group 4, as to the percent of the herds treated and the gallons of Bovadine used. Of the 36 herds, 20 herds treated over 50 percent of the cows, 16 treated less. You will note five herds treated no animals at all. They used 500 gallons of Bovadine which is approximately 40 percent of company recommendations. Three herds did not use any Bovadine. Only about 58 percent of all the animals were treated in this group, yet this group reduced the Strep. ag. incidence from 41 percent to 19 percent.

**TABLE VII**

**ALBION**

**GROUP IV – DRY COW TREATMENT**

<table>
<thead>
<tr>
<th>TREATMENT %</th>
<th>NO. HERDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>90</td>
<td>4</td>
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<td>70</td>
<td>9</td>
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<tr>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

20 HERDS OVER 50% TREATED
16 HERDS UNDER 50% TREATED
500 GAL. BOVADINE USED
40% OF COMPANY RECOMMENDATIONS

In conclusion, it is possible to eradicate Streptococcus agalactiae from an area. The speed of eradication and cost is affected by the information gotten across to the producer. Milking procedure, teat dipping, and professional supervision are essential also. The Group 3, intensive herds, all achieved eradication and have with only one exception remained free for at least nine months following completion of the project. Eradication in this group, I believe, would have been even faster if the herd owner had assumed all or part of the cost. The “British System” does reduce the incidence of Streptococcus agalactiae in herds where it is practiced. But when the cost was eliminated, as in this project, it still was not acceptable as they would not all do it. The effect on production when Streptococcus agalactiae was eliminated in the project is not statistically significant due to the fact that the project was run under practical conditions. So the results of other researchers studying mastitis where strict control was maintained can be used for production benefits.
For area testing, it is necessary to have a method of surveillance and in this project, the monthly sampling and culturing by the cream flotation method worked very well. It could be a supplemental test applied to the quality taken monthly. After the completion of the project, a questionnaire was sent to the participants to obtain their views on the project. The last two questions were:

1. Do you feel a statewide Streptococcus agalactiae eradication program would be a benefit to the dairy industry and to you as an individual? (Eighty-nine percent answered "yes.")

2. Would you favor a program of this type if the culture and treatment cost were furnished but not reimbursed for milk withheld? (Seventy-seven percent answered "yes" to this question.)

Summarizing the project then, is that it can be done on an area basis and the dairymen await it.
REPORT OF THE MASTITIS COMMITTEE

Chairman:  J. S. McDonald, Ames Iowa  
Co-Chairman:  D. S. Postle, Ithaca, New York


The annual open meeting of the U.S.A.H.A. Mastitis Committee was held on October 27, 1971. Thirty-two interested persons, including 5 committee members, were in attendance.

INFORMAL REPORTS

Dr. Thelma Njaka discussed the results of seasonal bacteriological studies on milk bulk tank samples in West Virginia. Streptococcus agalactiae was recovered more frequently during winter months than during July through September.

Mr. Lee Williams discussed the activity report of the Connecticut Mastitis Control Program. A total of 513 herds are included in the voluntary program. Of the 513 herds, 380 or 73% are free of Str. agalactiae. The Connecticut program has been operational since 1940.

Dr. A. R. Smith discussed area-wide eradication of Str. agalactiae in Wisconsin. A total of 152 herds including control herds were observed over a 12-month period. In one group of herds where aggressive eradication procedures were carried out, 100% of the herds were freed of Str. agalactiae. The cost of this aggressive eradication was $8.20 per cow which included drug costs, veterinary fees, and dumped milk. The figure does not include the cost of laboratory diagnostic procedures. Eighty-nine percent of cooperators felt they had derived marked benefit from the program.

STREPTOCOCCUS AGALACTIAE ERADICATION

A motion unanimously accepted by the Committee directed that a subcommittee be appointed by the chairman to draft uniform methods and rules for the eradication of Str. agalactiae in dairy herds. It is intended that these methods and rules will be used as a guide by the states in development of their mastitis control programs.

As Str. agalactiae is an eradicable, infectious and contagious pathogen that causes serious economic loss to the dairy industry, the Committee strongly feels that infected animals should not be moved intrastate or interstate. Therefore, the following resolutions were unanimously adopted.
Be it resolved that the U.S.A.H.A. recommends:

1. Adoption, by each state, of a program for the eradication of Str. agalactiae infection in dairy herds, and
2. That each state prohibit importation of preparturient and lactating dairy cattle unless such cattle are shown to be free from Str. agalactiae.

INTRANAMMARY MEDICATIONS

With regard to FDA rules pertaining to drugs used for intramammary medication, we are fully cognizant of and appreciate the need to safeguard human health. However, we strongly urge that the veterinary profession not arbitrarily be deprived of therapeutic agents that have been proven to be efficacious in eradication of udder infection. In the treatment of infection caused by the common pathogens, no drugs or combinations thereof have been proven to be more efficacious than penicillen and streptomycin in adequate dosage. Where the use of antibiotics or other drugs in animals has been shown, on the basis of adequate valid evidence, to present a human health hazard, we recommend that such usage be limited to veterinary prescription only. Inherent in this concept is the understanding that the prescribing veterinarian shall be responsible for proper use and observation of designated withdrawal times of any drug so prescribed.

TEAT DISINFECTANTS

Some chemical agents, when used as post-milking teat disinfectants, have been proven to be efficacious in reducing the rate of new udder infection. The Committee feels that any product offered for sale for use as a teat disinfectant should be subject to adequate quality control at the point of manufacture. In addition, the manufacturer should be required to demonstrate effectiveness and safety of his product. The label on the product should include at least the amount and kind of active ingredient(s), actual and residual acidity or alkalinity, and precautions, if any, to be observed in use of the product.

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

This constitutes the report of the Mastitis Committee.
REPORT OF THE 1971 COMMITTEE ON LIVESTOCK IDENTIFICATION

Chairman: S. H. Flora, Brownsville, Texas
Co-Chairman: John F. Quinn, Lansing, Michigan


The Livestock Identification Committee of the U.S.A.H.A. met in Room 1017 of the Skirvin Hotel in Oklahoma City October 27 and again on October 28 in the Balinese Room in the same hotel. Committee members present: S. H. Flora, Chairman; Leo G. Berg, Norman Powers, Elmer Haas, L. B. Barnes, J. K. Atwell, Burton Eller, Norman Swanson, J. R. Bishop and Keith Farrell.

The Committee heard reports from researchers on progress made in development of temporary and permanent identification marks and systems including a report on work from the Las Alamos Scientific Laboratory that shows great promise in the future.

The Committee suggests the following as criteria for the development of identification marks. The mark should be a permanent part of the animal, inexpensive, easy to apply, relatively painless, adaptable to modern data retrieval systems, visible from a distance of several feet, acceptable to all segments of the industry and not damage the animal or interfere with other identification programs.

Insufficient research has been done on swine tattooing. The Committee recommends that the Livestock Conservation, Incorporated, swine identification project of backslap tattooing be continued and broadened. Research must be accomplished to establish the duration of a tattoo, Food and Drug Administration approval of the ink used and location of the tattoo. The Committee also recommends that ear tagging of feeder pigs in interstate commerce be continued as required by existing State and Federal regulations.

The Committee recommends that the federal regulation contained in Title 9, Part 71.18 of the Code of Federal Regulations be strengthened in regard to cattle covered and in required documentation, and that greater effort and uniformity be used in the enforcement of the regulation.

The Committee further recommends that those states who have not done so adopt legislation or regulations governing the intrastate identification of cattle and that such legislation or regulation include requirements that all cattle moving through markets or concentration points be identified by backtags or other official identification with penalty provisions for removal by improper or unauthorized persons.

On behalf of the committee on infectious diseases of equines, Dr. Baldwin advised the committee that New York is going to freeze brand EIA reactors and requested that the committee suggest a location on the animal for this identification.
REPORT OF THE COMMITTEE

Inasmuch as Australia and New Zealand are identifying Thoroughbreds and standardbreds with freeze brands and the Arabian Horse Association in the United States is embarking on a similar program and all are using the right side of the neck, the committee recommends that the right side of the neck be reserved for official identification purposes.

Various proposals for coding herds of origin are in existence and others are being proposed. The Committee recommends that for the present various states accept the responsibility for establishing herd identification systems and preclude the establishment of different incompatible systems of limited usefulness. It further recommends that, for the time being, such codes or numbers include the prefix currently in use in the national tag system.

The Committee commends Mr. Lee Garner and the New Mexico Lifestock Board for their program of individual identification of horses now in effect in New Mexico. It was pointed out that certain other states have adopted a similar life-time identification and brand inspection form in horses.

The Committee heard a proposal, presented by Dr. Crouch, from the American Association of Bovine Practitioners, and a proposal on swine identification from Dr. Alan Morrow, Assistant State Veterinarian of Minnesota. In the opinion of the committee, neither proposal, at its current stage of development, meets the criteria referred to earlier in this report. The Committee wished to commend these people for their intense interest and efforts, and it is hoped that they will continue their research and development.

The Committee appreciates that much research is being accomplished in animal identification and strongly recommends that funds be made available for further research in this field. The need for more research in swine identification is critical and particularly noted.

Further development of methods of livestock identification are essential if progress in livestock disease control and eradication is to continue.

The Committee recommends that all in attendance, when they return home, emphasize the great need for total animal identification. It was agreed that all segments of the livestock industry must be made aware of the need for and the benefits of such an identification program in their own production efforts.
A small boy was asked "if you had a quarter in one pocket and a quarter in another pocket, what would you have?" The young boy thought for awhile, then replied, "somebody else's pants." His perception was somewhat different than that of the person who asked the question. Several of my colleagues made a point of questioning the validity of the title of this paper. One colleague said "what do you mean, getting one good idea is not enough?" We then embarked on a discussion about the purposes of educational meetings and certain aspects of learning and behavioral change. The title did cause some active discussion rather than merely passive acceptance. Curiosity was stimulated. It also showed that each of us often perceives things differently. Many of us have stated that if we got one good idea from a meeting it was worth the time and expense. There are other conclusions. The fact that we feel satisfied with a minimum of one good idea from a meeting may be a reflection on our ability to select the program that best fits our needs. Or it could be that we have attended so many meetings that did not meet our expectations, that when we did discover a meeting that gave us one good idea, we were overwhelmed by this fact and used this as an indicator of a successful meeting. We need to improve the design of programs to meet individual needs of the learner and leave him with a positive attitude about the subject matter so that he actually applies the newly acquired knowledge and skills, or if you will, ideas from the meeting.

Educational researchers have questioned whether many of our continuing education offerings do, in fact, affect behavioral change. (1,2) Mark Twain made a statement that fits some of our problems about lifelong learning habits. He said, "a cat which jumps on a hot stove will never jump on a hot stove again; nor," he added, "will he ever jump on a cold one." Many of us can recall how we were "turned off" by a boring lecture or lecturer on a very important subject. Our attitude about the lecture or lecturer caused us to shy away from him and his topic from then on. Professor Cyril Houle of the University of Chicago indicated that "much of the attitude of the beginning professional toward continuing education and his ability to pursue it has already been set at the time he enters service. The major frontier of continuing education is in the undergraduate classroom... facts must be taught, but always within the context of a constant and continuing exploration of the unknown. If you teach a person what to learn, you are preparing him for the past, if you teach him how to learn, you are preparing him for the future." (3) Indeed, our educational methods need to be improved. (4) We need to study and apply well established principles of adult education psychology and methodology in our continuing education programs. We must assess individual needs of the learner and evaluate progress toward pre-determined objectives.

According to Jack London, by 1974 there will be more people involved in adult education programs then young people in the formal educational systems in the United States. He indicated that the trend is clear and signifies a need to develop
new conceptions about educational training at all levels for all clientele of all ages. Adult educators, learning specialists, and educators from formal educational establishments must begin to rethink the character of education and training with these facts in hand. The public, state legislators, and the Congress must also understand these requirements.

Each of us needs a personal self-assessment program to aid us in making plans to direct our learning efforts. Regardless of a veterinarian's professional area of "practice", time is his most valuable commodity. He cannot increase the number of hours in a 24-hour day, but he can increase the efficiency by which it is used. The public is demanding competence. The cries of accountability, cost-effectiveness and relevance for education are upon us. You have read and heard much of this in the past year.

TRADITION, CHANGE, AND THE FUTURE

In coping with societal problems, the outreach of the Land-Grant University has been primarily through its service by continuing education and extension programs. Programs of continuing education and extension are affected by numerous factors such as human and animal population locations and trends. In 1960 two-thirds of Missouri's population lived in urban areas. This proportion is predicted to increase to 85 percent by 1990. Anticipating these trends, the University of Missouri Board of Curators, in 1960 established a single Extension and Continuing Education Program supported by the total resources of the University, thus becoming the first Land Grant University to do so. As veterinary medicine continues to serve a changing and specializing agriculture, so it is increasingly becoming aware of its role in a rapidly urbanizing society. Our programs in Missouri have attempted to reflect these changes.

VETERINARY MEDICAL EXTENSION ROLE AND SCOPE

Veterinary medicine serves society through protection of animal health, relief of animal suffering, the conservation of livestock resources, the promotion of public health, and the advancement of medical knowledge.

Veterinary Medical Continuing Education and Extension programs function primarily in four of six extension education program areas, none of which are mutually exclusive. They are: (1) Continuing Education for Professionals (veterinarians and other health and agricultural professionals), (2) Food and Fiber, (3) Environmental Quality, and (4) Quality of Living. To date our main thrust has been in program areas 1 and 2 with increasing emphasis being given to Environmental Quality and Quality of Living (youth, 4-H, etc.)

MAJOR PROGRAM OBJECTIVES

1. Provide continuing education programs for veterinarians and related health and agricultural professionals.

2. Provide extension education programs for Missouri citizens, urban and rural, that promote proper utilization of veterinary medical knowledge and services to safeguard the health of food producing and companion animals including prevention of
diseases transmissible from animals to man (zoonotic diseases).

3. Provide continuing education and extension programs that emphasize learning instead of teaching.

4. Encourage evaluation of educational programs.

WHAT ARE SOME PROGRAM EXAMPLES?

Equine Census-VEE: As part of the Food and Fiber program of the Cooperative Extension Service, we participated with State, Federal, private practicing veterinarians, and Livestock Specialists in taking an equine census in Missouri because of the Venezuelan Equine Encephalomyelitis program.

4-H Veterinary Science Projects: Dr. Bonnard Moseley, Extension Veterinarian, advised several students, one of whom won first place in local competition for a display about the status of Venezuelan Equine Encephalomyelitis. These programs are most valuable for both urban and rural youth.

Publications: MU Guides and Monographs are published by Missouri Extension veterinarians. A recent MU Guide was entitled ‘‘Disposal of Dead Animals in Missouri’’. It was a cooperative effort between University of Missouri-Columbia Extension faculty and State and Federal Animal Health and Public Health veterinarians. One of the questions raised was ‘‘why do we have dead animals in the first place?’’ With proper utilization of preventive medical practice and veterinary medical services a large proportion of deaths could have been avoided (we have little control over lightning strike).

The Continuing Education Unit (CEU) And Missouri Academy of Veterinary Medicine: Extension faculty have participated in organizing the Missouri Academy of Veterinary Medicine. One of the objectives is to assess continuing education needs. With this information, meaningful priorities can be established for cooperative programs between the Academy and School of Veterinary Medicine. The Academy will provide a mechanism for recognizing continuing education participation. A given amount of formal and informal continuing education must be completed for Academy membership. The Missouri Veterinary Medical Association has adopted the Continuing Education Unit (CEU) as a uniform unit of measurement for non-credit continuing education programs as proposed by a National Task Force. (7) Academy membership is open to all veterinarians. It is a Section of the Missouri Veterinary Medical Association.

Independent Study Program (Correspondence Course): In keeping with the philosophy that learning should be continuing and convenient, Dr. C. J. Bierschwal, Extension Veterinarian and Chief of Theriogenology, and colleagues of the American Veterinary Society for Study of Breeding Soundness have developed a correspondence course entitled, ‘‘Infertility and Breeding Soundness Examination of the Bull’’. It consists of 12 lesson plans. Audio-visual materials including a tape-cassette, slides and slide projector (called Mo-Av-Pac) are loaned to each enrollee. A certificate is awarded after successful completion of the course. Hopefully, this type of learner-oriented approach will be utilized by other veterinary medical specialty groups.

New Segmented Curriculum and Continuing Education Opportunities: A new curriculum was initiated on June 2, 1971. It primarily involves the last two years of
the professional curriculum. The student is required to select and successfully complete eight instructional blocks (out of a choice of 10) in a 24 month period. Each block is 2 months in length. Because of the concentration on specific subject matter areas for this period, veterinarians are presented with a greater opportunity to utilize faculty and facilities of the School for continuing education purposes.

The first practitioner is presently enrolled in the Medical Services Block which includes Radiology (Dr. E. A. Corley, Chief) and Anesthesiology (Dr. C. E. Short, Chief). Dr. D.C. Blenden is Chairman of the Epidemiology and Public Health Block. Several State and Federal Veterinarians have assisted in on-and off-campus teaching as a cooperative effort. Auto-tutorial programs are used with instructional objectives of what the student will learn. (8) An example of an A-T program suitable for this type of continuing education effort was reported at the 1970 U.S.A.H.A. meeting by Dr. James Libby of the University of Minnesota. He described his A-T program on meat inspection for contract veterinarians. (9)

Seminars, Workshops, Conferences: Examples of these types of programs recently sponsored include: (1) District 7, American Association of Bovine Practitioners, (2) North Central Conference of Veterinary Laboratory Diagnosticians and the North Central Poultry Disease Conference, (3) Workshop on Equine Anesthesiology and Surgery, (4) 47th Annual Conference for Veterinarians. A total of 240 veterinarians participated in this Conference in 1971. The program format is one of four rotating seminars. This format allows participants to select those topics of interest. Each session is followed by a question and answer period. All papers presented at the Conference are given to veterinarians at the time of registration. The fourth seminar was added this year. An ad hoc committee of State and Federal veterinarians assisted in program planning for panel discussions about biological and pesticide residues as problems in the live animal and trends in humane animal care legislation. Sequential learning programs have been initiated between the University of Missouri School of Veterinary Medicine and the Missouri Veterinary Medical Association. A large proportion of Missouri veterinarians attend both the School’s Annual Conference and M.V.M.A. State Convention. Two part programs on environmental pollution and pesticide residues have been organized.

Educational Resources Program: The School of Veterinary Medicine and University Extension Division have cooperated in developing this program for undergraduate, post-graduate and continuing education and extension programs. By encouraging faculty to use newer instructional methods and media, resources developed (i.e. auto-tutorial programs) can be further utilized by veterinarians for continuing education purposes. A single concept program describing and illustrating surgical procedures used for small and large animals was developed by Dr. R. E. Brown and Dr. P. D. Garrett. Several other faculty have participated by developing these programs which are also used in the undergraduate professional curriculum. Every attempt is being made to provide recognition to faculty for producing innovative instructional materials.

Tele-lecture, FM Radio and Amplified Telephone Communication Systems: Early in 1972, we will have available FM radio and Amplified telephone systems to aid us in reaching off-campus audiences. Dr. L. G. Tritschler, recently participated with a physician in presenting a tele-lecture on “Equine Encephalitis in Relation to Human Health” to 35 human hospitals in Missouri.
Urban Extension and Veterinary Medicine: Because of the large urban population in Missouri, an urban extension and continuing education program is being developed. The need for cooperative projects of education and use of veterinary medical services is being emphasized. (10) Dr. C. R. Dorn, University of Missouri faculty member, studied the utilization of veterinary medical services by dog and cat owners between 1954 and 1964 and found that as many as 49 percent of dog owners and 73 percent of cat owners in certain areas of the United States had never used veterinary services. (11) Education of prospective and new pet owners on selection and maintenance of healthy animals is needed. We have proposed a position for an Urban Extension Veterinarian. (10) He would serve as a "change agent" by working with veterinary societies and citizens interested in keeping pets healthy, in the prevention of environmental nuisances, and in the exploitation of the beneficial role of the pet in child development and mental health.

Time-Function Studies of Large and Small Animal Practitioners in Missouri: Extension faculty have participated with graduate students (M.S.P.H.) in determining how large and small animal practitioners spend their professional time. These data are valuable in assessing those professional areas that continuing education efforts might be emphasized. (12,13) One study revealed that 25 percent of a small animal practitioner's time was spent on activities directly related to public health. (12)

Newsletters: Timely information is sent to Missouri veterinarians in a newsletter—Lifelong Learning in Veterinary Medicine. Information from the School of Veterinary Medicine Veterinary Medical Diagnostic Laboratory is also included and an Annual Report from the Laboratory is sent to Missouri veterinarians.

Undergraduate Student Participation: Whenever possible, veterinary medical students are encouraged to attend on- and off-campus continuing education programs. We feel that these experiences provide excellent motivation for the concept of lifelong learning.

SUMMARY

Lifelong learning habits must be encouraged. It follows that the more we can understand and apply the principles of adult learning processes, the more effective will be our continuing education programs. Nattress has described a model of continuing professional education in which he emphasizes the importance of the individual's readiness to change. He states that "Although the avenues to change have been opened, without willingness or readiness on the part of the professional person to learn and change his behavior, programs of continuing education will have little or no impact." (14) He feels that adult educators know far too little about readiness to change, especially about how to create or develop it. Because many of you in this room are directly or indirectly involved with in-service training programs, I would suggest you read Nattress' article in its entirety. Several adult educators have studied about why people participate in formal continuing education programs. (15,16,17). We need studies about why veterinarians participate in continuing education programs to aid us in curriculum design.

The solution of many societal problems can be aided by veterinary medical talents. It will require the cooperation of both private and public veterinary medical organizations and agencies and other health and agricultural professionals.
Continuing education programs must reflect these trends.

We must realize that the differences in urban versus rural thinking, public health versus animal health thinking, private versus public veterinary medical practice thinking are really less of differences in people and problems than they are of differences in our perception or understanding that the problems are ours and not theirs.

Learning through meaningful continuing education experiences and acceptance of new ideas involves risks for us—it often requires the unlearning of obsolete attitudes, skills, and knowledge. In the book, Future Shock, Toffler describes the problems man faces because of the rapidity of change. (18)

However, change we must if we are going to meet our personal and professional obligations to society. By meeting these obligations we will also gain a feeling of personal self-fulfillment—and isn’t this really what it’s all about?

REFERENCES


SUGGESTED READING

CLINICAL ASPECTS OF BLUETONGUE IN OREGON CATTLE

by
Guy E. Reynolds*, D.V.M.

Summary

The definitive diagnosis of Bluetongue (BT) is difficult but necessary in order to develop preventive programs to stop future losses. Since BT has clinical similarities to Foot and Mouth Disease, Rinderpest, Infectious Bovine Rhinotracheitis (IBR) and Bovine Virus Diarrhea (BVD), an effective test should routinely be used to identify the disease.

The agar gel precipitin test has been used effectively by the Oregon State Veterinary Diagnostic Laboratory for two years. A survey of 300 blood samples indicates 7.9% of cattle in Oregon have been exposed to BT virus.

History

Bluetongue was first recognized as a definite disease entity in the United States by McKercher and co-workers in California in 1952. Since that time, the BT virus has been isolated from livestock in 16 states, e.g. Arizona, California, Colorado, Florida, Idaho, Kansas, Minnesota, Missouri, Montana, Nebraska, New Mexico, Oklahoma, Oregon, Texas, Utah and Wyoming.11, 18 In addition, verbal reports indicate it has been found this year in Louisiana, Georgia, Tennessee, North and South Carolina.

Clinical Signs

Technically, Bluetongue is one of the mucosal disease complex which includes a number of symptomatically related diseases such as foot and mouth, rinderpest, vesicular stomatitis, IBR and BVD. In the western United States, BVD is widespread among cattle populations and may have clinical signs very similar to BT. Both viruses are epitheliotropic and may produce oral lesions and lameness. The mucosal form of BVD tends to follow a prolonged course with cyclic temperature rises to 106°F. The elevated temperatures with BT occur early in the course of the disease and approach normal by the time clinical signs are observed.2

* Dr. Reynolds formerly practiced at Caldwell, Idaho, and is now the Extension Veterinarian at Oregon State University.
Differential Clinical Features

<table>
<thead>
<tr>
<th></th>
<th>BT</th>
<th>BVD</th>
<th>IBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>104°, early &amp; transient</td>
<td>106°, chronic &amp; recurrent</td>
<td>106°, acute</td>
</tr>
<tr>
<td>Mouth Lesion</td>
<td>anterior 1/3, extensive necrosis</td>
<td>diffuse throughout</td>
<td>seldom</td>
</tr>
<tr>
<td>Lameness</td>
<td>common &amp; acute</td>
<td>occasional &amp; chronic</td>
<td>none</td>
</tr>
<tr>
<td>Eyes</td>
<td>clear</td>
<td>chronic &amp; severe</td>
<td>conjuncto-conjunctivitis</td>
</tr>
<tr>
<td>Skin Lesions</td>
<td>pityriasis, common</td>
<td>occasional</td>
<td>none</td>
</tr>
<tr>
<td>Herd Incidence</td>
<td>low, 10% or less</td>
<td>usually low but variable</td>
<td>high in feedlot</td>
</tr>
<tr>
<td>Occurrence</td>
<td>seasonal-associated</td>
<td>any time</td>
<td>any time</td>
</tr>
</tbody>
</table>

The clinician must keep in mind the disease pattern in an outbreak will vary considerably depending upon the susceptibility of the host, the virulence of the viral strain and the type of secondary bacteria which may predominate in a given herd.

No attempt is ordinarily made to differentiate foot and mouth disease, rinderpest, or vesicular stomatitis from Bluetongue. These diseases are not believed to occur in the Pacific Northwest. However, should vesicles or other unusual signs be noticed, regulatory officials should be immediately notified and aid requested.

The incidence of BT in cattle herds in our experience has been quite low; in an outbreak, less than 10% show clinical signs of the disease. However, a survey of blood sera following a herd outbreak will reveal up to 60% have been exposed to the viral agent, and have developed a demonstrable antibody titer.

One of the most striking lesions is frequently found on the dental pad. Massive necrosis occurs which suggests the area has been touched with a hot branding iron. Similar but less severe necrotic areas may be observed on the lips, behind the incisor teeth and on the anterior dorsal aspect of the tongue. Mouth lesions are usually restricted to the anterior third of the oral cavity. The eyes are generally clear with little or no ocular discharge.

Extensive mouth lesions may prevent grazing, and supplemental feeding of chopped hay and grain is necessary during the convalescent period. The mucosa of the dental pad and muzzle may slough but is rapidly replaced by granulation tissue. Diligent nursing care and proper feeding may be necessary for a week to ten days.

Lameness is a common observation in cattle herds affected with BT. Many individuals may be lame when first aroused from the bedground. The majority will "warm out" of the lame condition with moderate exercise. All limbs may be involved and affected animals will move very much like a foundered horse. The cause of lameness in some individuals is coronitis. The coronet is inflamed and swollen and cracking has been observed in the interdigital area and around the
coronary band. Extensive damage to the coronet has caused claws to slough in some individuals.

Pityriasis is usually observed in cattle BT when a number of sick animals in an area are examined. The skin will be thickened with cracking and scaling apparent. Areas of the neck and perineal region are most severely affected. The lesion is not unlike that which occurs following extensive radiation. It is possible that peripheral thrombosis results from BT viremia and impairs circulation and nutrition to the skin. The improperly nourished skin is more susceptible to solar radiation irritation. Extensive pityriasis may cause large patches of skin to slough. When this occurs several weeks are required for the areas to heal. Cattle with skin lesions should be protected from the sun to prevent exacerbation of skin damage. Cattle with non-pigmented skin are more prone to pityriasis than those with skin pigment.

Beef cows nursing calves may develop burned teats which become sore to the extent they will prematurely wean their calves. This has been observed to cause considerable economic loss in some herds. Serologic evidence suggests the BT virus is involved in hydranencephaly of newborn calves.

Bluetongue Outbreaks in Oregon

Bluetongue in cattle was first identified in Jackson County in September 1959. The virus was isolated from sick cattle near Eagle Point. This was the first confirmed report of BT causing clinical disease of cattle in the United States. The disease apparently became enzootic in the area since a highly susceptible cattle population developed by 1968 when a second epizootic occurred. Dr. Hugh Metcalf of the Denver BT laboratory made epidemiological studies of the 1968 outbreak. Eighteen herds representing over 1,200 cattle were investigated. Virus isolation was made in eight herds from cattle sick with a disease suggestive of BT. Approximately 158 head of cattle were reported to have undergone clinical disease. The deaths of four cattle were attributed to acute effects of the disease. Six additional cattle were salvaged two to four months later when their hoofs started to slough.

Bluetongue virus was isolated from sick cattle along the Oregon-Idaho border in 1960.3 It had been recognized in my practice as a clinical disease for several years prior to that time, but had not been properly identified until virus isolations were accomplished. Each year a few sporadic cases were observed and as might be expected in an endemic area, these were usually in young individuals under two years of age. The stage becomes set for epizootic outbreaks in cattle when the following conditions exist:

1. Proper environmental conditions for large numbers of insects.
2. Conditions conducive for exposure of cattle to the insects.
3. Existence of a large population of susceptible animals.

The disease is observed in the summer and early fall and subsides a few days after the first killing frost. The biting midge, Culicoides veripennis, is reported as the most significant insect for transmitting BT virus.2,9 Dr. R. Goulding, entomologist at Oregon State University, informs me that this insect species is present in all geographical regions of Oregon. The disease potential exists and when the above criteria are met we can anticipate serious outbreaks. In one geographical region there is a cyclic pattern that recurs every four to eight years.
CLINICAL ASPECTS OF B.T. IN OREGON CATTLE

Diagnostic Methods

Virus isolations should be made when Bluetongue is suspected in a new area. If personnel and facilities are unable to accomplish this within the state, the USDA-ARS supervisor of your state should be contacted. If, in his opinion, the seriousness of the situation warrants, he may solicit assistance from the USDA Denver BT Laboratory. In lieu of this he may provide you with specimen kits for submitting appropriate blood samples to the Denver laboratory.

Virus isolation attempts are time consuming and expensive whether they are accomplished by sheep inoculation or in incubating chick embryos. The Agar Gel Precipitin (AGP) test provides meaningful diagnostic assistance and has been used in our state diagnostic laboratory for two years.\(^4\),\(^8\),\(^13\) The AGP Test is inexpensive to perform and one can obtain rapid and significant results. Highly skilled technicians are unnecessary for accomplishing this work. We are indebted to the Denver BT laboratory for providing the antigen necessary for performing this test.

The AGP Test is group specific and provides qualitative evidence of past infection.\(^4\) We advise practitioners to collect clotted blood samples from acutely ill cattle. Convalescent samples are then collected in three weeks. Both samples are submitted to the diagnostic laboratory. In addition to determining exposure to the BT virus, we are frequently requested to process these paired samples for IBR and BVD by other appropriate test methods.

If we are fortunate and can demonstrate negative results on the acute sera and positive for the convalescent samples, we have proved a recent experience with the BT virus. Under experimental conditions, positive reactions have been obtained 14 days post-inoculation.\(^13\) Clinicians should keep in mind that many BT infected cattle have been exposed ten to 18 days previously and samples taken on the initial visit may be positive. One should remember that the AGP test is qualitative only and reflects past viral experience. Known infected cattle will remain positive to the test for at least two years.\(^13\)

Blood samples collected from mule deer in Oregon have also showed positive reactions to the AGP test.

In addition, the availability of this test has made it possible for us to conduct a modest survey of cattle blood samples throughout the state of Oregon. Cooperating with the regulatory veterinarians of the State Department of Agriculture and the Denver BT laboratory, we collected 300 cattle blood samples to determine their experience with the BT virus. Findings of this survey are as follows:

1. BT virus exists in at least 13 of 31 Oregon counties (5 counties were not sampled).
2. Twenty-four of 303 samples (7.8%) reacted to the AGP test.
3. BT was not found in the coastal area, the lower Columbia River region, or the upper portion of the Willamette Valley.

It should be noted that BT does occur in sheep within our state. It exists as a mild disease of this species but does occur in areas where it has been identified in cattle. Veterinarians are reluctant to report this disease of sheep due to export restrictions.

Discussion

Bluetongue is a significant but not a major disease of the cattle industry in
Oregon. The veterinary clinician should be aware of the signs of this disease in cattle and be able to differentiate BT from other infectious diseases which may cause similar symptomatology. The Agar Gel Precipitin Test is adaptable to state veterinary diagnostic laboratories and is a useful tool in diagnosing the disease in cattle.
REFERENCES

Parainfluenza-3 Virus in Intercurrent Infections Causing Bovine Respiratory Disease

M. L. Frey and R. A. Ball*

INTRODUCTION

Parainfluenza-3 (PI-3) virus is often mentioned as a possible predisposing agent to infection with bacterial and other microbial agents, the combined infection then causing pneumonia or other severe respiratory signs. However, the previously reported experimental work is limited largely to studies in which Pasteurella spp. were used as the secondary agents. Hermodsson did provide evidence of the ability of PI-3 virus to act either as an accentuating or interfering agent for other viral agents in cell culture systems. Hamdy et al. used PI-3 virus and several other agents in combination infections in calves, but their infection experiments were complicated by the addition of temperature stressors. Also, it happened that clinical signs were seen only when PI-3 virus was combined with infectious agents which by themselves appeared to be capable of causing clinical signs.

In naturally occurring cases of bovine respiratory disease where PI-3 virus is incriminated, there are almost always accompanying physiologic as well as infectious stressors. In the combination infection studies which we undertook, we used only agents that by themselves (because of their nature or attenuation) caused few or no clinical signs in normal unstressed colostrum-deprived calves. In this way, we hoped to determine those combination infections which would be pathogenic even in animals handled under optimum conditions. From this we hoped to make a better determination of the relative value of immunization procedures as compared to improved management practices. Most important of all, we hoped to define some of the precautions necessary in administration of PI-3 or other vaccines by the respiratory route.

MATERIALS AND METHODS

Experimental calves. Calves used for infection experiments were high grade Herefords from the Veterinary Medical Research Institute herd. They were removed from their dams immediately after calving and raised in isolation on a milk replacer diet until they were at least 6 weeks of age. A blood sample was drawn prior to infection and the serum was tested for absence of HI antibodies to PI-3 virus. Temperatures were taken once daily for 1 to 3 days prior to infection and twice daily after infection, at which time the calves were also observed for other clinical signs.

Infectious agents. A strain of PI-3 virus (SF-4) which had been passed 22 or 23 times in Madin-Darby bovine kidney (MDBK) cell line cultures was used as viral inoculum. One ml of serum-free maintenance medium (Eagle's MEM) containing approximately $10^7$ TCID$_{50}$ was diluted to 5 ml with maintenance medium and inoculated intratracheally.

* Veterinary Medical Research Institute, Iowa State University, Ames, Iowa 50010.
PI-3 VIRUS IN INTERCURRENT INFECTIONS

Strain HB-24 (BP-5)\(^4\) chlamydial agent, isolated in Wisconsin from nasal exudate of a calf with respiratory disease, was passed 10 times via chick embryo yolk sac and 9 times via chick embryo via chick embryo allantoic sac. The allantoic fluid, which titrated \(10^{4.0}\) ELD\(_{50}\) (via chick embryo yolk sac), was frozen and used in 1 ml aliquots. The 1 ml inoculum was diluted to 5 ml with serum-free cell culture maintenance medium, and was inoculated intratracheally.

*Mycoplasma bovigenitalium* was grown in M-96 broth\(^5\) supplemented with 15% swine serum. One ml of 24 hour culture titering between \(10^8\) and \(10^9\) colony forming units was diluted to 5 ml with 0.01 m K\(_2\)HPO\(_4\) buffer\(^2\), and inoculated intratracheally.

RESULTS

As shown in table 1, there was little or no febrile or clinical response in any of the calves infected with a single agent. Gross lung lesions observed at necropsy were also of minimal severity, and were limited almost entirely to small areas of consolidation in anteroventral portions of the lungs. In calves infected with the chlamydial agent, there also was usually a mild enteritis present at necropsy.

<table>
<thead>
<tr>
<th>INFECTION(S) AGENT(S)</th>
<th>NO. CALVES INFECTED</th>
<th>AVERAGE OF HIGHEST TEMP.(F)</th>
<th>AVERAGE HOURS OF TEMPERATURE (&gt;104.0)</th>
<th>NO. CLINICALLY ILL/ TOTAL INFECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI-3 VIRUS</td>
<td>3</td>
<td>103.2</td>
<td>8</td>
<td>0/3</td>
</tr>
<tr>
<td>CHLAMYDIA</td>
<td>2</td>
<td>NFR*</td>
<td>0</td>
<td>0/2</td>
</tr>
<tr>
<td>MYCOPLASMA BOVIGENITALIUM</td>
<td>1</td>
<td>NFR</td>
<td>0</td>
<td>0/1</td>
</tr>
<tr>
<td>PI-3 VIRUS + CHLAMYDIA</td>
<td>7</td>
<td>106.2</td>
<td>80**</td>
<td>7/7</td>
</tr>
<tr>
<td>PI-3 VIRUS + M. BOVIGENITALIUM</td>
<td>2</td>
<td>105.6</td>
<td>78***</td>
<td>2/2</td>
</tr>
</tbody>
</table>

* NFR = No febrile response
** Two calves killed with temperature \(>104.0\)
*** One calf killed with temperature \(>104.0\)

The calves infected with either combination of agents had lung lesions which were much more extensive than those seen in monoinfected calves. In most cases there were large plum-colored or consolidated areas. Some had a pleuritis as well.

Microscopic examination revealed little evidence of pulmonary lesions in calves which were infected with either the chlamydial agent or mycoplasma alone. A very low grade interstitial pneumonia was evident histologically in the calves mono-infected with PI-3 virus.

Early microscopic lesions in calves receiving PI-3 plus a second agent were primarily those of a fairly extensive interstitial pneumonia with broncho-pneumonic lesions appearing as the disease progressed.
DISCUSSION

These experiments were limited in number, particularly in the case of the mycoplasma infections. They do indicate, however, that an attenuated strain of PI-3 virus may act in combination with at least two agents to produce severe respiratory disease in cattle.

There are many serotypes of mycoplasmas which infect cattle and, in our experience, well over half of Iowa cattle carry one or more serotypes in their nasal exudate. No generalizations can be made concerning the involvement of given mycoplasma serotypes in bovine respiratory disease until more serotype identification has been done, and more experimental infection studies completed.

Chlamydial agents are harder to isolate than mycoplasmas, so even fewer data are available concerning their frequency of occurrence in normal cattle and involvement in cattle with respiratory disease. They have been isolated on one or more occasions from cattle in most Midwestern states, and there are sufficient reports of their occurrence in cattle in many parts of the world to indicate that they are probably ubiquitous in the bovine population. In addition, several experimental studies have been done in which it was demonstrated that some strains of chlamydia in monoinfections may be significant pathogens of the bovine respiratory tract.

Administration of attenuated PI-3 virus by aerosols has been proposed as an effective means of stimulating local immunity of the bovine respiratory tract to natural infection. On the basis of the experimental data and judging from the experience gained with aerosol vaccines in human populations in Eastern Europe, one concludes that aerosol administration of respiratory virus vaccines may become much more popular.

Calves which are being shipped from one area to another, in addition to be stressed physiologically, presumably are continually exposed to new infectious agents. This is especially true when they are exposed to or combined with other cattle. We feel that exposure to aerosol PI-3 vaccines as part of a preconditioning program is probably a very sound procedure. But with our present knowledge, it is probably contraindicated at time of shipment, except in calves which are kept well-isolated.

SUMMARY

An attenuated Parainfluenza-3 virus strain was used in experimental combination infections with (1) a chlamydial agent and (2) Mycoplasma Bovigenitalium. None of the three agents caused clinically observable respiratory disease or serious lung lesions when injected alone into the trachea of calves (PI-3 into 3 calves, chlamydia agent into 2 calves, and M. bovigenitalium into 1 calf). Either of the bacterial agents combined with PI-3 virus caused a marked febrile response, signs of respiratory disease and rather widespread lung lesions of moderate to high severity. The implications with regard to aerosol immunization with attenuated PI-3 virus are discussed.
REFERENCES

Immunogenicity in Calves of a Bovine Viral Diarrhea Vaccine Inactivated with Beta-propiolactone


SUMMARY

An inactivated vaccine against bovine viral diarrhea was evaluated for safety and immunogenicity in 35 calves aged 5 to 11 months. The calves were vaccinated either once or twice at an interval of 14 days. There was no evidence of undesirable post-vaccinal reactions in any of the calves. The immunity of these calves and of 9 nonvaccinated control calves was challenged with homologous virulent NADL strain of virus at either one or three weeks after the last vaccination.

An Index of Illness scheme based on the number of days that each calf had fever, leukopenia, diarrhea, respiratory distress, depression, anorexia and viral shedding was used to evaluate the postchallenge clinical responses. The mean Index of Illness values of vaccinated calves varied from 1.3 days in a group of 9 naturally-born, twice-vaccinated calves to 4.8 days in a group of 5 specific-pathogen-free, once-vaccinated calves. In contrast, after challenge, naturally-born and specific-pathogen-free, nonvaccinated control calves had mean Index of Illness values of 11.7 days and 24.5 days respectively.

Bovine viral diarrhea (BVD) accounts for substantial losses annually to the livestock industry despite the availability commercially of attenuated live-virus vaccines. Limitations in the use of such vaccines have stimulated research attempts to develop a safe, potent, inactivated BVD vaccine.2, 3 This report concerns additional studies at this laboratory on the immunogenicity of an experimental BVD vaccine which was inactivated by Beta-propiolactone and used with an aluminum hydroxide adjuvant.

MATERIALS AND METHODS

Vaccine – The experimental vaccine was prepared from the NADL strain of BVD virus and was inactivated by methods described previously.2, 3 The vaccine was administered intramuscularly in 5-ml doses.

Serology – Sera from experimental calves were tested for the presence of neutralizing antibodies using methods described previously.4 Serum samples were collected before vaccination and at weekly intervals after vaccination and challenge.

Experimental Calves – Forty-four Holstein-Friesian calves from 5 to 11 months of age at the time of vaccination were utilized in 2 experiments. In the first experiment, 22 calves were collected by a modified specific-pathogen-free (SPF) technique.1, 5 Calves were colostrum-deprived and maintained in a separate barn throughout the experiment. All calves were serologically negative for BVD-neutralizing antibodies prior to vaccination. They were assigned to 1 of 4 treatment
groups (1, 2, 3 or 4) but were housed together. In the second experiment, 22 naturally-born calves were premitted dam's colostrum for 2 days, then weaned and assigned to 1 of 3 treatment groups (5, 6, or 7). They were not housed separately.

**Group 1** — Five calves were vaccinated once and their immunity was challenged with the virulent homologous NADL strain of BVD virus at 21 days after vaccination.

**Group 2** — Five calves were vaccinated twice at a 14-day interval and their immunity was challenged similarly with homologous NADL virus at 7 days after the second vaccination.

**Group 3** — Seven calves were vaccinated twice at an interval of 14 days and their immunity was challenged at 21 days after the second vaccination.

**Group 4** — Five calves were not vaccinated and served as contact and exposure controls.

**Group 5** — Nine calves received 1 dose of vaccine. Their immunity to BVD was challenged at 21 days after vaccination.

**Group 6** — Nine calves received 2 doses of vaccine at an interval of 14 days. Their immunity was challenged at 7 days after the second vaccination.

**Group 7** — Four calves were not vaccinated and they served as contact and exposure controls.

**Immunology** — The immunity of all calves was challenged by exposure to the homologous NADL strain of virulent BVD virus. This virus had been passaged 3 times in embryonic bovine kidney cells since the original isolation.

Challenge material consisted of 20 ml. of BVD viral fluids containing approximately $10^6$ cell culture infectious doses per ml. One-half of the dose was given orally and 5 ml. was instilled into each nostril.

**Clinical Responses** — Body temperatures of the calves were recorded twice daily for 7 days after each vaccination and for 14 days after challenge. The erythrocyte counts as well as total and differential leukocyte counts were performed on all calves daily for 14 days after challenge. The calves were observed daily for signs of anorexia, diarrhea, nasal discharge, respiratory distress, lameness and depression. The number of days of viral isolation or recovery were recorded also. These data were used to evaluate the clinical responses employing an accumulative Numerical Index of Illness based upon duration in days of these clinical signs.

**Virology** — Attempts to isolate the BVD virus after challenge of both the vaccinated and control calves were done by taking nasal and rectal swabs at daily intervals. Blood samples were collected at the same time for evidence of viremia. The procedures have been described previously.

**RESULTS**

There was no evidence of systemic reaction in any of the calves after vaccination. Neither persistent swellings nor abscesses occurred at the sites of vaccination. Contact controls remained serologically negative during the prechallenge period. The postchallenge results are summarized in Table I.

All of the nonvaccinated control calves had elevated body temperatures from 1 to 7 days after challenge. Elevated body temperatures from 1 to 5 days' duration were detected after challenge in 26 of the 35 vaccinated calves.

Postvaccinal leukopenia was not detected in any of the calves. Postchallenge leukopenia was detected from 1 to 7 days in all of the nonvaccinated control calves.
TABLE 1 — SUMMARY OF POSTCHALLENGE RESULTS

<table>
<thead>
<tr>
<th>GROUP AND NO. OF CALVES</th>
<th>PERSISTENCE OF CLINICAL SIGNS (TOTAL DAYS)</th>
<th>RECOVERY OF VIRUS (MEAN NO. OF DAYS)</th>
<th>INDEX OF ILLNESS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NUMBER OF TIMES VACCINATED</td>
<td>VACCINATION INTERVALS (DAYS)</td>
<td>ELEVATED BODY TEMPERATURE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPF</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
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<tr>
<td></td>
<td>5</td>
<td>9</td>
<td>1</td>
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<tr>
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<td>7</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural born</td>
<td>5</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

and in 26 of the 35 vaccinated calves.

All of the nonvaccinated control calves had diarrhea from 1 to 6 days after challenge. In contrast, 2 of 35 vaccinated calves had diarrhea after challenge.

Eight of 9 nonvaccinated control calves had excessive nasal discharge and respiratory distress of varying severity up to 4 days after challenge. In contrast, 4 of 35 vaccinated calves had excessive nasal discharge and signs of respiratory distress after challenge.

All of the nonvaccinated calves were depressed from 1 to 5 days after challenge; whereas 1 of 35 vaccinated calves was depressed at this time.

Anorexia was observed in 7 of 9 nonvaccinated calves from 1 to 2 days after challenge. Anorexia was not observed in any of the vaccinated calves.

Neither control nor vaccinated calves had signs of laminitis or lameness after challenge.

Virulent BVD virus was recovered from 5 of the 9 nonvaccinated control calves up to 34 days after challenge. In contrast, BVD virulent virus was recovered from 2 of 35 vaccinated calves on day 1 only after challenge. The BVD virus was isolated from the leukocyte layer (buffy coat) of the blood and from nasal and rectal swabs.
The postvaccinal viral-neutralization titers ranged from 4 to 8192 at the time of challenge. Calves given a single vaccination did not attain prechallenge titers as high as calves receiving two inoculations of vaccine. The prechallenge serologic data are correlated with the mean Index of Illness for each group and are shown in Table 2.

**TABLE 2 — SUMMARY OF CORRELATION OF PRECHALLENGE SERUM NEUTRALIZATION TITERS AND INDEX OF ILLNESS**

<table>
<thead>
<tr>
<th>Group and no. of calves</th>
<th>Prechallenge serum-neutralization titers (Mean)</th>
<th>Postchallenge Index of Illness (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 5</td>
<td>9.0</td>
<td>4.8</td>
</tr>
<tr>
<td>2 - 5</td>
<td>53.0</td>
<td>4.6</td>
</tr>
<tr>
<td>3 - 7</td>
<td>2240.0</td>
<td>3.7</td>
</tr>
<tr>
<td>4 - 5</td>
<td>&lt;4</td>
<td>26.4</td>
</tr>
<tr>
<td>5 - 9</td>
<td>21.8</td>
<td>4.4</td>
</tr>
<tr>
<td>6 - 9</td>
<td>171.0</td>
<td>1.3</td>
</tr>
<tr>
<td>7 - 4</td>
<td>4</td>
<td>11.7</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The vaccine was developed primarily for safety, potency and economic reasons. The safety of the vaccine was demonstrated in both SPF and in naturally-born, colostrum-permitted calves. There was no evidence of postvaccinal systemic reactions in any of the calves after the first or second inoculation. Safety of the vaccine was reconfirmed since the contact controls remained serologically negative throughout the prechallenge period.

Antigenicity or potency of the experimental vaccine was demonstrated by the magnitude of the viral-neutralizing antibodies in the vaccinated calves. The maximum prechallenge titer was 8192 with a median titer of 64. When the data included only calves challenge-exposed 3 weeks after the 2nd vaccination, the range of titers at time of challenge was from 64 to 8192.

The immunogenicity of the vaccine was demonstrated by comparing the postchallenge results of the vaccinated and control calves. When the Index of Illness, as proposed by Fernelius et al., was applied to these data, the immunogenic merits of the vaccine were apparent. Generally, after challenge, elevated body temperature and leukopenia were the only clinical signs observed in the vaccinated calves whereas the nonvaccinated controls additionally had diarrhea, respiratory distress, depression, and anorexia.

BVD virus was isolated from 2 of the 35 vaccinated calves on the first day only.
after challenge-exposure. In contrast, isolation of BVD virus from 5 of the 9 nonvaccinated controls was possible for varying periods up to 34 days. The results suggest that this experimental vaccine could offer a safe, serviceable level of protection against virulent BVD and should be considered as an adjunct to our present control programs.

Although detectable protection from one injection was apparent, the additional protection offered by the second or "booster" inoculation was obvious in both SPF and conventionally-born calves.
REFERENCES


REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF CATTLE

John F. Hudelson

The committee on Infectious Diseases of Cattle met with some 50 persons in attendance. Last year's committee report was reviewed, and we commend the president of the USAHA on the appointment of a committee on livestock identification.

Through the efforts of the subcommittee on artificial insemination, this committee and the USAHA, the initial proposed rules for the interstate movement of bovine semen have been published in the federal register. The subcommittee on artificial insemination reviewed these proposed rules and realized, this being the first draft, that there would be a need for changes prior to the final draft being published. This committee recommends that we adopt in principle the proposed rules but recognize that changes should be submitted. We urge all persons wishing to make suggestions and changes to do so in writing to the Director of the U.S. Animal Health Division prior to November 30, 1971.

A report was given on the “Sudden Death Syndrome” of feedlot cattle followed by a discussion. This syndrome is of great economic importance throughout the United States and especially in the large feedlot areas of the Midwest and Western areas. This syndrome is associated with heavy feedlot cattle that are found dead without clinical signs. The major necropsy lesion is that of peritracheal hemorrhage and edema. Rapid decomposition is usually observed. Many signs of clostridial infections are often noted; however, at this time it has not been determined whether the primary cause of death is physiological or infectious.

This committee is submitting a resolution to the resolutions committee requesting the U.S.D.A. to provide funds for this costly syndrome of feedlot cattle.

If this resolution is approved, it is recommended that copies of the resolution be sent to the Secretary of Agriculture, the appropriate Congressional Committee Chairman, the American National Cattlemen's Association, Livestock Conservation Incorporated, American Veterinary Medical Association, and the Director of the Animal Health Division.

The committee heard brief reports on each of the following papers and recognized the importance of each of these disease conditions.

"Intercurrent infections in Experimental Bovine Respiratory Diseases"
"Clinical Aspects of Blue Tongue in Oregon Cattle"
"Immunogenicity of Inactivated BVD Vaccinated Calves"

The committee also heard a report on infectious conditions caused by Pasteurella and Hemophilus somnus. The committee urged that research be continued on these conditions.

The committee was advised that the National Animal Disease Laboratory had research projects on neonatal diseases of the bovine and on infectious Keratitis. The committee urges continued research efforts on these important disease conditions.

Discussions were heard regarding I.B.R. vaccine. The committee recommends that the committee on Biologics review these vaccines in regard to production methods, the virus antigenicity, the immune response and the possible transmission
of vaccine virus from vaccinated animals to nonvaccinated animals.

This committee requests that members of this association submit problems to
this committee for discussion next year.

We respectfully submit this report for your consideration and recommend its
approval.
PROMISING THERAPEUTIC AGENTS FOR THE ELIMINATION OF *ANAPLASMA MARGINALE* IN THE CARRIER ANIMAL

K. L. Kuttler, D.V.M.

The need for and the implementation of an effective program for the reduction of losses due to anaplasmosis has been discussed for years by both the veterinarian and the livestock producer. The continuation of research progress inevitably brings the goal of anaplasmosis control nearer. In recent years significant progress has been made in the area of vaccine production\(^3\), \(^11\), \(^14\) and in the development of new and effective diagnostic procedures\(^1\). Both of these advances can perform an important function in the reduction of animal losses due to anaplasmosis. The persistant nature of *Anaplasma* infections, even after apparent recovery, creates serious problems when eradication programs are considered. A therapeutic approach which would effectively eliminate the disease agent from infected animals will probably play an important role in control programs of the future.

For many years the tetracyclines have been the drugs of choice in treating clinical anaplasmosis and in eliminating the carrier state of infection. A number of workers\(^4\), \(^6\), \(^7\), \(^13\) have in the past reported on the efficacy of these drugs showing them to be useful and effective. The elimination of the carrier status with tetracyclines usually requires either daily injections for 10 or more days or a low level feeding program for 30 to 60 days. Both procedures are time consuming and expensive. A more efficient regime to eliminate carrier infections would significantly contribute to the control of anaplasmosis.

A program of test and treatment appears practical for the eradication of anaplasmosis in some areas of the country\(^5\). The problem of eradication is more difficult in those areas where ticks serve as biological vectors and wildlife are a reservior of infection.

During the past few years a new drug, in addition to the tetracyclines, has attracted some attention as a possible therapeutic agent. In 1965 the effectiveness of an a-dithiosemicarbazone (356 C 61)* in the treatment of anaplasmosis, was reported\(^2\). Since that time serveral reports have been published confirming the effectiveness of this agent\(^5\), \(^9\), \(^10\), \(^12\). More recently favorable results have been reported using the a-dithiosemicarbazone with oxytetracycline**8. The results suggest that the simultaneous use of these drugs was more effective than either alone.

In addition to the dithiosemicarbazone a second material, Imidocarb (3, 3'-Bis-(2-imidazolin-2-yl) - carbanilide dihydrochloride) or 4A65,* has been tested

Acknowledgements:

This work was supported by a grant from the U.S. Agency for International Development to the Institute of Tropical Veterinary Medicine, Texas A&M University.

* Burroughs-Wellcome and Co., Inc., Raleigh, North Carolina

** Liquamycin injectable: Chas. Pfizer & Co., Inc., New York, N.Y.
ELIMINATION OF ANAPLASMA MARGINALE

and shows promise in the treatment of anaplasmosis.

This report will review results of studies dealing with these 3 agents, oxytetracycline, 356 C 61, and 4A65, when used for the elimination of anaplasmosis carrier status in splenectomized calves.

Splenectomized calves have proven useful in these preliminary studies largely because of the characteristic A. marginale relapses which occur in treated animals that retain a non-apparent infection. In these experiments, calves failing to show a relapse within 62 days of treatment have been shown to be free of infection by subsequent infectivity tests, and in many instances by later re-challenge. Splenectomized calves are an abnormal system, and these results should not stand alone without further trials in intact cattle. These results are useful to delineate the relative activity of various treatment schedules, and provide the basis for further studies.

MATERIALS AND METHODS

Treatment was administered to 59 splenectomized, A. marginale-infected dairy type calves of mixed breeding ranging in age from 5-21 months with an average of 10.8 ± 4 months.

Groups I, II, and III consisted of 14 calves treated with 356 C 61 only (Table 1). Calves in Group I were treated 5 times at 24 hour intervals with 5mg/kg injected intravenously (I.V.). Calves in Group II were treated 10 times at 24 hour intervals with 5 mg/kg injected I.V. A total of 8 calves in Group III were injected intramuscularly (I.M.) either 3 or 4 times with 5 mg/kg 356 C 61 at varying intervals. Treatment was administered to 1 calf, 3 times, at 48 hour intervals; to 3 calves, 4 times, at 3 and 4 day intervals (twice a week); to 3 calves, 3 times, at 7 day intervals; and to 1 calf, 3 times, at 14 day intervals.

Groups IV, V, VI, VII, and VIII consisted of 33 calves treated with 356 C 61 oxytetracycline (Table 2). Calves of Group IV were treated 3 times at either a 24 or 48 hour interval with 2 mg/kg 356 C 61 and 11 mg/kg oxytetracycline. Calves of Group V were treated 3 times at either a 24 or 48 hour interval with 5 mg/kg 356 C 61 and 11 mg/kg oxytetracycline. Calves of Group VI were treated 3 times at 72 hour intervals with 5 mg/kg 356 C 61 and 11 mg/kg oxytetracycline. Calves of Group VII were treated 3 times at either 24 or 48 hour intervals with 10 mg/kg 356 C 61 and 11 mg/kg oxytetracycline. Calves of Group VIII were treated 2 times at either a 24 or 48 hour interval with 5 or 10 mg/kg 356 C 61 and 11 mg/kg oxytetracycline. The 356 C 61 and oxytetracycline were mixed in and diluted with 150 ml 0.85% NaCl (PSS) and injected I.V.

Groups IX and X consisted of 12 calves treated with 4A65 alone and in combination with 356 C 61 and oxytetracycline (Tables 3 and 3A). Calf 405 was treated 1 time with 4 mg/kg 4A65, 15 mg/kg 356 C 61 and 44 mg/kg oxytetracycline. The 4A65 was injected I.M., and the 356 C 61 and oxytetracycline were diluted in PSS and injected I.V.

Calves 135 and 219 were each treated 1 time, with 6 mg/kg 4A65 I.M., and 15 mg/kg 356 C 61 I.V. Calves 424 and 431 were each treated 1 time with 15 mg/kg 4A65 I.M. (Table 3).

Calves 217 and 248 were each treated 3 times at 24 hour intervals with 2 mg/kg 4A65 I.M. and 5 mg/kg 356 C 61 I.V. Calf 450 was treated 3 times at 24 hour intervals with 5 mg/kg 4A65 I.M., and 2 mg/kg 356 C 61 I.V. Calf 245 was treated
3 times at 24 hour intervals with 4 mg/kg 4A65 I.M. Calves 405 and 413 were each treated 3 times at 24 hour intervals with 5 mg/kg 4A65 I.M. Calf 265 was treated 3 times at 24 hour intervals with 6 mg/kg 4A65 I.M. (Table 3A).

All animals were monitored for changes in percent parasitemia, packed cell volume (PCV), and serum complement-fxation titers for anaplasmosis. Blood samples were collected for this purpose twice a week a minimum of 14 days before and 60 days after treatment. The relapse period is expressed as days following treatment required for the development of a 1% or greater A. marginale parasitemia. Except for 3 calves in Table 3A, infectivity trials were conducted on all animals, not showing an A. marginale relapse, by an I.V. injection of 200 ml of whole blood from the treated animal into a susceptible splenectomized calf. With the exception of Group I and II the time at which infectivity trials were conducted was never less than 60 days and on an average was 87 days. Groups I and II were tested for infectivity 5 to 20 days after treatment. In some instances blood from 2 and 3 calves was pooled and injected into 1 splenectomized calf.

RESULTS

The results following the use of 356 C 61 alone was presented in Table 1. Treatment with 5 mg/kg of 356 C 61 for 5 consecutive days failed to eliminate the A. marginale infection. The use of 5 mg/kg 356 C 61 injected 3 and 4 times at 2, 3-4, 7 and 14 day intervals was equally unsuccessful. Treatment for 10 consecutive days with 5 mg/kg 356 C 61 was apparently successful in removing evidence of A. marginale based on infectivity tests, but resulted in fatal toxicosis in every animal.

The results of combined therapy with 356 C 61 and oxytetracycline are presented in Table 2. Group IV, using only 2 mg/kg 356 C 61, in combination with a constant of 11 mg/kg oxytetracycline, failed to prevent an A. marginale relapse. Groups V and VII, using 5 or 10 mg/kg 356 C 61 in combination with oxytetracycline for 3 times at either a 24 or 48 hour interval eliminated evidence of infection based on the absence of a relapse and negative infectivity trails. Group VI given similar drug levels 3 times at 72 hour intervals was less effective, and 1 of the 3 treated calves developed an A. marginale relapse 39 days after the last treatment. Group VIII given only 2 injections of similar drug levels at either 24 or 48 hour interval failed in every case to prevent relapse infection.

The results of 1 injection of 4A65 alone and in combination with 356 C 61 and oxytetracycline are presented in Table 3. All calves receiving only 1 injection, showed a relapsing infection an average of 42 ± 7 days after treatment.

The results of 3 injections at 24 hour intervals of 4A65 alone and in combination with 356 C 61 are presented in Table 3A. In every instance evidence is present to indicate an elimination of A. marginale. Infectivity trials are complete in 4 of 7 calves, and the 3 remaining animals have shown no sign of relapse for over 60 days.

DISCUSSION AND CONCLUSIONS

A combination of oxytetracycline and 356 C 61 appears more effective than either given alone. It would appear from these results that this action represents more than just an additive effect, and that there may be a synergistic effect in this instance. Using the drug levels reported, 4A65 appears equally as competent to eliminate infection when given alone or in combination with 356 C 61. This drug
when given I.V. was responsible for excessive salivation, lacrimation and labored breathing. These side effects appear less severe when the drug is administered I.M. or S.C., and the efficacy was not altered.

Relapses when they occur usually do so between 30-45 days. The longest relapse period observed to date has been 62 days. The occurrence of a diagnostic relapse negates the need for infectivity trials. Our experience has been that treated calves, failing to show a relapse within a 62 day period are consistently free of infection as determined by infectivity trials.

These studies suggest that marked improvements are possible in treatment techniques, however much needs to be done before these drugs can be freely used and dispensed. Toxic side effects, tissue residues, as well as trials in intact animals are indicated. Hopefully such trials will result in the evolution of more effective and efficient drugs for the elimination of infection.

SUMMARY

Two new drugs, dithiosemicarbazon (356 C 61) and 3, 3'-Bis (2-imidazolin-2-yl) - carbаниlide dihydrochloride (4A65) have been successfully used to treat splenectomized calves with anaplasmosis. Carrier infections were eliminated with 5 or 10 mg/kg 356 C 61 and 11 mg/kg oxytetracycline when given 3 times at either a 24 or 48 hour interval. In addition 5 mg/kg 356 C 61 plus 2 mg/kg 4A65 given 3 times at 24 hour intervals was effective in eliminating A. marginale infections. Levels of 4 and 6 mg/kg of 4A65 given 3 times at 24 hour intervals has proven successful in eliminating A. marginale infection.
Table 1

Use of 356 C 61 to Eliminate Carrier Status in Splenectomized Calves

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Dose Rate mg/kg</th>
<th>Route of Inoculation</th>
<th>Number of Inoculations</th>
<th>Interval of Inoculations</th>
<th>Results of Treatment</th>
<th>Infectivity Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>3</td>
<td>I.V.</td>
<td>5</td>
<td>24 hrs</td>
<td>Failed</td>
<td>Positive</td>
</tr>
<tr>
<td>Group II</td>
<td>3</td>
<td>I.V.</td>
<td>10</td>
<td>24 hrs</td>
<td>Successful</td>
<td>Negative *</td>
</tr>
<tr>
<td>Group III</td>
<td>8</td>
<td>I.M.</td>
<td>3 and 4</td>
<td>2, 3-4, 7 and 16 days</td>
<td>Failed</td>
<td>Relapse: 60 2.11 ds K.T.</td>
</tr>
</tbody>
</table>

* All calves treated with 356 C 61 - 10 times at the 5 mg/kg level died, apparently due to drug toxicity.

N.T. - No Test  I.V. - Intravenous  I.M. - Intramuscular

Table 2

Use of 356 C 61 in Combination With Oxytetracycline to Eliminate Anaplasma Carrier Status of Splenectomized Calves

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Dose Rate mg/kg</th>
<th>Route of Inoculation</th>
<th>No. of Inoc.</th>
<th>Interval of Inoc. (hrs)</th>
<th>Results of Treatment</th>
<th>Relapse Time Days</th>
<th>Infectivity Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group IV</td>
<td>3</td>
<td>I.V.</td>
<td>11</td>
<td>3</td>
<td>24 or 48</td>
<td>3/3 Failed</td>
<td>31.7 ± 18.1</td>
</tr>
<tr>
<td>Group V</td>
<td>11</td>
<td>I.V.</td>
<td>11</td>
<td>3</td>
<td>24 or 48</td>
<td>11/11 Successful</td>
<td>--</td>
</tr>
<tr>
<td>Group VI</td>
<td>3</td>
<td>I.V.</td>
<td>11</td>
<td>3</td>
<td>72</td>
<td>2/3 Successful</td>
<td>39</td>
</tr>
<tr>
<td>Group VII</td>
<td>8</td>
<td>I.V.</td>
<td>11</td>
<td>3</td>
<td>24 or 48</td>
<td>8/8 Successful</td>
<td>--</td>
</tr>
<tr>
<td>Group VIII</td>
<td>8</td>
<td>I.V.</td>
<td>11</td>
<td>2</td>
<td>24 or 48</td>
<td>8/8 Failed</td>
<td>41.9 ± 9.6</td>
</tr>
</tbody>
</table>

I.V. - Intravenous  N.T. - No Test
ELIMINATION OF ANAPLASMA MARGINALE

Table 3
Use of 4A65 Alone and in Combination With 356 C 61 to Eliminate The Anaplasma Carrier Status of Splenectomized Calves

Group IX

<table>
<thead>
<tr>
<th>Calf Number</th>
<th>4A65 mg/kg</th>
<th>Route</th>
<th>No. of Inoc.</th>
<th>356 mg/kg</th>
<th>Route</th>
<th>No. of Inoc.</th>
<th>Result of Treatment</th>
<th>Relapse Time In Days</th>
<th>Infectivity Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>405 *</td>
<td>4</td>
<td>I.M.</td>
<td>1</td>
<td>15</td>
<td>I.V.</td>
<td>1</td>
<td>Failed</td>
<td>42</td>
<td>N.T.</td>
</tr>
<tr>
<td>135</td>
<td>6</td>
<td>I.M.</td>
<td>1</td>
<td>15</td>
<td>I.V.</td>
<td>1</td>
<td>Failed</td>
<td>54</td>
<td>N.T.</td>
</tr>
<tr>
<td>219</td>
<td>6</td>
<td>I.M.</td>
<td>1</td>
<td>15</td>
<td>I.V.</td>
<td>1</td>
<td>Failed</td>
<td>43</td>
<td>N.T.</td>
</tr>
<tr>
<td>424</td>
<td>15</td>
<td>S.C.</td>
<td>1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Failed</td>
<td>38</td>
<td>N.T.</td>
</tr>
<tr>
<td>631</td>
<td>15</td>
<td>S.C.</td>
<td>1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Failed</td>
<td>35</td>
<td>N.T.</td>
</tr>
</tbody>
</table>

* Treated simultaneously with 44 mg/kg oxytetracycline I.V.

I.M. = Intramuscular I.V. = Intravenously N.T. = No Test S.C. = Subcutaneously

Table 3A
Multiple Injections of 4A65 Alone and in Combination With 356 C 61 to Eliminate The Anaplasma Carrier Status of Splenectomized Calves

Group X

<table>
<thead>
<tr>
<th>Calf Number</th>
<th>4A65 mg/kg</th>
<th>Route</th>
<th>No. of Inoc.</th>
<th>356 C 61 mg/kg</th>
<th>Route</th>
<th>No. of Inoc.</th>
<th>Results of Treatment</th>
<th>Relapse Time In Days</th>
<th>Infectivity Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>217</td>
<td>2</td>
<td>I.M.</td>
<td>3</td>
<td>24 hrs</td>
<td>5</td>
<td>I.V. 3</td>
<td>Successful</td>
<td>&gt;120</td>
<td>Negative</td>
</tr>
<tr>
<td>248</td>
<td>2</td>
<td>I.M.</td>
<td>3</td>
<td>24 hrs</td>
<td>5</td>
<td>I.V. 3</td>
<td>Successful</td>
<td>&gt;120</td>
<td>Negative</td>
</tr>
<tr>
<td>430</td>
<td>5</td>
<td>I.M.</td>
<td>3</td>
<td>24 hrs</td>
<td>2</td>
<td>I.M. 3</td>
<td>Successful</td>
<td>&gt;60</td>
<td>N.T.</td>
</tr>
<tr>
<td>245</td>
<td>4</td>
<td>I.M.</td>
<td>3</td>
<td>24 hrs</td>
<td>--</td>
<td>--</td>
<td>Successful</td>
<td>&gt;120</td>
<td>Negative</td>
</tr>
<tr>
<td>405</td>
<td>5</td>
<td>I.M.</td>
<td>3</td>
<td>24 hrs</td>
<td>--</td>
<td>--</td>
<td>Successful</td>
<td>&gt;90</td>
<td>N.T.</td>
</tr>
<tr>
<td>413</td>
<td>5</td>
<td>I.M.</td>
<td>3</td>
<td>24 hrs</td>
<td>--</td>
<td>--</td>
<td>Successful</td>
<td>&gt;90</td>
<td>N.T.</td>
</tr>
<tr>
<td>265</td>
<td>6</td>
<td>I.M.</td>
<td>3</td>
<td>24 hrs</td>
<td>--</td>
<td>--</td>
<td>Successful</td>
<td>&gt;120</td>
<td>Negative</td>
</tr>
</tbody>
</table>

I.M. = Intramuscular I.V. = Intravenous N.T. = No Test
REFERENCES

EXCERPTS FROM A REPORT ON THE EVALUATION OF AN ATTENUATED ANAPLASMA MARGINALE VACCINE

Fred C. Neal and George T. Edds
University of Florida
Gainesville, Florida 32601

An experimental *A. marginale* vaccine attenuated by irradiation and serial passage through deer and sheep was evaluated for safety and efficacy. (lots 175, 20, 21, 27 and 28). Poor results with lot 175 necessitated further evaluation studies.

Trial I* — Evaluation Goals.
1. Development of a CF positive titer in mature cattle after receiving a single intramuscular injection of 5 ml of vaccine indicated a protective level of immunization.
2. Stability of the vaccine after storage for 1 year at -120 C.
3. Reactions observed in cattle receiving such vaccine.
4. Protection of vaccinates against a strong “standardized” Florida isolate challenge, 0.5 ml of acute *A. marginale* 70% infected erythrocytes, suspended in 4M DMSO and preserved in liquid nitrogen. Groups of vaccinates were challenged at 63, 90, 180 or 365 days after vaccination. A CF negative control animal was challenged with each group.
5. Reversion to virulence of the vaccine strain by serial transfer of blood from vaccinates to splenectomized calves and to intact, susceptible mature cattle.

Trial II** — Further reversion to virulence studies by serial passages of Lot 28 vaccine from vaccinates 8 months after vaccination into susceptible cattle. Sponsored by the Department of Veterinary Science.

Results and Discussion — Trial I.

Experiment 1. Doses of an original experimental Lot 175 injected into 18 mature CF negative cattle. Parasitemia, drop in PCV, and a positive CF titer developed in 11; 7 showed no changes, remained CF negative and were fully susceptible on challenge. This suggested only 61.1% protection. Because of the poor results, the USDA provided 2 new lots of vaccine, 20 and 21.

Experiment 2. Doses of Lot 20 were injected into 10 susceptible CF negative cattle; 3 became CF positive. Lot 21 was used to vaccinate 5 additional cattle; only 1 became CF positive. Thus, based on CF reaction only 30 and 20% would have been protected.

† The detailed report has been submitted to the American Journal of Veterinary Research.
* Partially supported by the USDA, ARS, Biological Division.
** Supported by the Florida Agricultural Exp. Station.
Experiment 3. The USDA provided 2 new lots, 27 and 28. Five mature, susceptible CF negative cattle were injected with each lot. All 10 animals became CF positive with presumed 100% protection.

Experiment 4. Lots 27 and 28 were again tested for potency after 1 year storage at -120°F by injection of 5 CF negative cattle with each lot. Positive CF titers were induced in all 10 vaccinates.

Those vaccinates becoming CF positive in Experiment 1-4 generally showed a drop in PCV associated with development of a parasitemia. One lactating dairy cow developed a 16% parasitemia and severe drop in PCV. Two, 2 year-old, dairy bulls were anorectic, moderately depressed following vaccination; one required tetracycline therapy.

On challenge, a virulent *A. marginale* dose of infected RBC's caused little change in the vaccinates with positive CF titers in Experiments 1-4. However, the vaccinated cattle, remaining CF negative, were equally as susceptible as the CF negative controls. Deaths occurred in several of the challenged control cattle even though treated with tetracycline after the PCV dropped below 10 percent.

Experiment 5. Reversion to virulence studies were first performed wherein blood from vaccinates was transferred to splenectomized calves. All 7 calves receiving such blood died with acute anaplasmosis.

Experiment 6. Further reversion studies were done using CF negative mature cattle as recipients of "vaccinate" blood. Five mls of blood from each of 5 vaccinates, after 8 months, were transferred to 5 CF negative cattle. High parasitemia, marked drop in PCV, anorexia and marked weight loss developed in all recipients; one died. Another group of 4 susceptible cattle individually received 0.1 ml transfer from these identified remaining animals — severe anaplasmosis developed with 1 death. Again, 0.1 ml was transferred from the 3 recovered cattle to 3 CF negative cattle. One of the recipients died with acute anaplasmosis, one was acutely ill, the other remained apparently normal. This apparently normal animal showed no parasitemia and remained CF negative. It was believed that serial passage at this level occasionally may be below a minimal infective dose. A fourth transfer from the 2 surviving cattle gave similar results as the 3rd transfer, i.e. the recipient of 0.1 ml blood from the apparently healthy cow remained normal, but the recipient of 0.1 ml blood from the surviving cow that had anaplasmosis developed high parasitemia and the PCV dropped below 10%.

Trail II — 1969-70.

Further trials were done to evaluate this apparent reversion to virulence. Seven CF negative cattle were selected from an anaplasmosis CF negative herd. Four were vaccinated with Lot 28 experimental vaccine, three animals were left as negative contact controls. All 7 animals were isolated from other cattle on premise for the next 8 months.

The 4 vaccinates became CF positive; and 3 non-vaccinates remained CF negative throughout this period. After 8 months, 1.0 ml of blood from each animal was transferred to individual CF negative cattle. Those cattle receiving blood from the Lot 28 vaccinates developed clinical anaplasmosis, with high parasitemia and marked drop in PCV while no changes occurred in the 3 cows receiving blood from the original 3 controls. A second transfer of 1.0 ml blood from the recovered
vaccinate recipients into 4 CF negative cattle induced acute anaplasmosis, high 
parasitemia and low PCV. Tetracycline dosage was necessary; — even then 1 animal 
of this group receiving the 2nd serial transfer of blood died from anaplasmosis.

Summary:
Five lots of an attenuated Anaplasma marginale vaccine were evaluated for 
safety and efficacy. Three lots, 175, 20 and 21 protected only 61, 30 and 20 
percent of the vaccinates; lots 27 and 28 protected 100 percent.
These lots of vaccine appeared to be safe in mature, non-lactating cattle 
obscerved for a period of 1 year.
Vaccinated cattle, developing parasitemia and a CF positive titer resisted severe 
challenge, while those that remained CF negative were fully susceptible on 
challenge.
Transfer of blood, as low as 0.1 ml, from recently vaccinated cattle into 
splenectomized calves or from cattle vaccinated 8 months or more into adult CF 
negative cattle resulted in acute anaplasmosis and frequently deaths. The 
experimental vaccine evidently reverts to virulence and vaccinated animals should 
be considered similar to those premunized by other methods.
REPORT OF THE ANAPLASMOSIS COMMITTEE

Chairman: T. F. Zweigart, Raleigh, N.C.
Co-Chairman: J. Lee Alley, Auburn, Ala.

Research:

State-Federal Regulatory:

Biologics and Pharmaceuticals:
J. C. Trace, Fort Dodge, Iowa; Dreyfus Froe, Terre Haute, Ind.

Livestock Industry:

Sub-Committee:
J. Lee Alley, Chairman, Auburn, Ala.

The Anaplasmosis Committee accepted with some modifications, the "Recommended Standards for Anaplasmosis-Free Herds" worked out by the subcommittee appointed at the last annual meeting. A resolution asking that the Director of the Animal Health Division, ARS, USDA, be petitioned to participate in recognition of Anaplasmosis-Free herds and encourage recognition by the various states, and the countries which import cattle from the United States, has been submitted to the Committee on Nominations, Resolutions and Internal Affairs. The recommended standards for voluntary development of Anaplasmosis-Free herds follow:

RECOMMENDED STANDARDS FOR ANAPLASMOSIS-FREE HERDS

I. Definitions
1. Anaplasmosis Free Herd
   A herd in which no anaplasmosis infected or reactor animals (or animals) has been detected on at least two consecutive herd tests. The qualifying or second consecutive negative herd test must be at least six months from the time of the first negative test and not more than eighteen months. All herd additions must be made in accordance with Section IV.

2. Herd Test
   A. Qualifying Test
      A test of all cattle in the herd over twelve months of age. When infection is revealed on a herd test, animals less than twelve months of age should be included on subsequent qualifying tests.
   B. Annual Retest
      A test of all cattle in the herd over twenty-four months of age.
C. Identification

All animals included in a herd test must be identified with an eartag, tattoo, or other form of permanent identification.

3. Negative Animal

An animal that has been tested and found to be negative to the complement fixation test or other official test for anaplasmosis.

4. Suspect Animal

An animal that has been tested and found to reveal a suspicious response on the complement fixation test or any other official test for anaplasmosis.

5. Reactor Animal

An animal that has been tested and classified as positive to the complement fixation test or other official test for anaplasmosis.

II. Individual Anaplasmosis Free Herd Plan

The herd must be under the supervision of a full-time State and/or Federal veterinarian and may qualify as an Anaplasmosis Free Herd upon compliance with the following provisions:

1. Herd test must be conducted and if such test reveals no evidence of infection on the initial test, the herd may be designated as Anaplasmosis Free when it has passed one additional negative herd test not earlier than six months and not more than 18 months from the date of the initial negative herd test.

2. Herds revealing infection on the initial herd test may either sell the infected animal (or animals) for slaughter or follow a recommended treatment program for anaplasmosis infected animals. The herd should be retested at intervals of not less than 60 days if the infected animals are removed from the herd. In those herds where infected animals are treated, the herd retest interval should be not less than 120 days following treatment. The herd retesting should be continued until all evidence of infection has been eliminated. The herd may then be designated as Anaplasmosis Free when it has passed at least two consecutive negative tests, with the first negative test and the qualifying test not less than six months nor more than 18 months apart. Additional herd tests may be conducted on herds if the owner so desires or if the supervising agencies deem it advisable.

3. There should be no feeding of low level broad spectrum antibiotics to animals in herds in the process of qualifying for free status.

III. Maintaining Anaplasmosis Free Herds

1. Upon evidence of a negative herd test at the end of one year, and with adequate fencing, the herd may be designated as an Anaplasmosis-Free Herd for an additional twelve months. If the herd test is not accomplished before the anniversary date, it may be reinstated if the retest is not delayed more than three months beyond the anniversary date. In these cases, the free status will be twelve months from the anniversary date and not twelve months from the date of the retest.

2. If retest of a free herd reveals reactors, or other evidence of infection becomes available at anytime, the free status of the herd will be removed and it must requalify for free status.

IV. Herd Additions

1. Anaplasmosis Free Herds and Herds in the Process of Establishing an Anaplasmosis Free Herd
REPORT OF THE COMMITTEE

a. natural herd increases
b. from Anaplasmosis Free Herds
c. other additions must have a negative test within 30 days prior to addition, then held separate for not less than 60 days and retested negative before addition to the herd.

In view of the recent controversy concerning the proposed licensing of a living, attenuated anaplasmosis vaccine and the unresolved doubts concerning its safety, the Committee passed a resolution urging the Veterinary Biologics Division of the United States Department of Agriculture to deny licensing of any living, attenuated anaplasmosis vaccine until such time as possible transmittal factors, adverse vaccine reactions, interference with diagnostic procedures to complicate interstate and international movement of cattle, and other problems of concern to the industry and regulatory officials are satisfactorily resolved, and that no action be taken in any event before the USAHA Annual Meeting in 1972. A copy of the resolution has been submitted to the Committee on Nominations, Resolutions and Internal Affairs.

The Committee recognizes that limited research on anaplasmosis is being conducted at several locations in the United States. However, this Committee feels that the research expenditures for anaplasmosis are by no means in keeping with the economic importance of this disease. In past years the Committee has urged without success that more research be conducted in this area.

The Committee recognizes the importance of establishing whether Anaplasmosis Free herds can be maintained in certain Western States where the infection is prevalent in deer and biological vectors are present. The Committee at the 1970 Annual Meeting urged the USDA to inaugurate pilot projects in several herds in representative states of the Northwestern region to determine the feasibility of establishing and maintaining Anaplasmosis-Free herds for periods of five or more years by the use of recommended procedures. The committee requests that the USDA make available a progress report on these proposed projects, and that summaries of anaplasmosis research in progress be made available to the committee at its annual meetings.

A favorable preliminary report supporting the feasibility of establishing and maintaining Anaplasmosis-Free herds by the use of the Rapid Card Test and antibiotic therapy was presented by Dr. Lee Alley, Extension Veterinarian, Auburn University.

A favorable statement concerning the safety and efficacy of Diamond Laboratories' attenuated live *Anaplasma marginale* vaccine under development was read by Dr. D. A. Fuller of Diamond Laboratories.

Dr. Fred C. Neal, University of Florida, presented data from his work with Diamond Laboratories' live attenuated anaplasmosis vaccine. Dr. Neal's findings cast doubt concerning the safety of this vaccine. A summary of his report will be published in the proceedings.
SUPPLEMENTAL NEGATIVE CREAM BRT 
IN WISCONSIN

Gordon C. Janney, D.V.M., M.S.
USDA, ARS, Animal Health Division
Madison, Wisconsin

The Brucella abortus ring test (ABR) now called the Brucella ring test (BRT) was first described by Fleischauer in Germany in 1937 and 1938.

Later, as a result of studies by Roepke, Bruhn, Huddleson, and others in the United States, this test was integrated into the State-Federal Cooperative Brucellosis Eradication Program.

On July 14, 1951, Wisconsin officially launched a statewide program to eradicate brucellosis in cattle. Under this program, all dairy herds were ring tested at least once every six months and all herds reacting to the ring test were to be blood tested. The first BRT round, July – December, 1951, disclosed 55,003 (41.5%) suspicious herds out of a total of 132,536. The 44th round disclosed 20 (.03%) suspicious herds out of a total of 61,850 herds. Table I shows the BRT round summary from Round 1 through Round 44.

The BRT program in the state of Wisconsin began with the collection of milk from each can of milk sent to the dairy plant by a farmer. No more than three cans, estimated to contain the milk of no more than 15 cows, could be combined into one sample. At the laboratory, 2 ml of milk was poured into a 10 mm x 75 mm tube and 2 drops of brucella ring test antigen* was added and mixed. The test results were observed after an incubation period of 1½ hours at room temperature. The test procedures were modified to achieve better standardization. Measured quantities of milk (1 ml) were mixed with 1 drop of antigen and the incubation period was adjusted to 1 hour at a controlled 37°C.

In the mid and late 1950's, bulk milk handling at the farm started to replace milk cans as the method for shipping milk to the dairy processing plants. The BRT was tried and found to work satisfactorily with samples kept at the dairy plant for the Babcock butterfat test, provided it contained more than five days samplings. Wisconsin, on Round 12 (January - June, 1957) changed over to the Babcock method of ring testing. This resulted in the reduction of the total number of laboratories and men needed to conduct the tests and permitted increasing the frequency of BRT tests to three per year. The use of the Babcock test sample also resulted in the reduction in percentage of positive tests.

In July, 1965, the Wisconsin Mastitis Program was started. If the BRT sample was fresh milk, it could also be used for the catalase test as part of the Mastitis Program. Half of the samples collected on this round were fresh and half Babcock test samples. It was shown that use of the fresh milk samples resulted in a more sensitive test with an increased number of apparently false positive results. The

* Produced by USDA, ARS, ANHD, NADL, Ames, Iowa.
Negative Cream Brucellosis Ring Test (NC BRT) was developed. This paper is a report of the studies made and follow-up testing of the NC BRT.

MATERIALS AND METHODS

Fresh milk samples were collected by the Animal Health BRT field men at each dairy processing plant and brought to the BRT laboratory for testing. Approximately 4 oz. of fresh milk was taken from the weighing tank at the dairy processing plant or from the bulk tank at the farm in the case of bulk pickup. Each sample was identified with the patron’s number and cooled with ice during holding and shipping to the laboratory. Current rapid data processing records of the patron lists for each dairy processing plant were used in an attempt to get a sample from every shipper each round. When fresh milk was not available for sampling, a preserved

<table>
<thead>
<tr>
<th>Date</th>
<th>Round</th>
<th>Intakes</th>
<th>Patrons Sampled</th>
<th>Patrons Not Sampled</th>
<th>Total Patrons</th>
<th>% of Herds Susp.</th>
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<td>1951(July-Dec.)</td>
<td>1</td>
<td>1,901</td>
<td>132,536</td>
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<td>132,536</td>
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<td>130,413</td>
<td>2,237</td>
<td>132,650</td>
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<td>(July-Dec.)</td>
<td>3</td>
<td>1,871</td>
<td>125,899</td>
<td>3,690</td>
<td>129,589</td>
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<td>1953(Jan.-June)</td>
<td>4</td>
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<td>126,667</td>
<td>2,105</td>
<td>128,772</td>
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<td>(July-Dec.)</td>
<td>5</td>
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<td>124,723</td>
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<td>2,428</td>
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<td>(July-Dec.)</td>
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<td>123,379</td>
<td>1,951</td>
<td>125,330</td>
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<td>1955(Jan.-June)</td>
<td>8</td>
<td>1,729</td>
<td>121,197</td>
<td>2,650</td>
<td>123,847</td>
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<tr>
<td>(July-Dec.)</td>
<td>9</td>
<td>1,709</td>
<td>120,203</td>
<td>1,564</td>
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<td>1956(Jan.-June)</td>
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<td>1,691</td>
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<td>2,476</td>
<td>119,827</td>
<td>12.06</td>
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<td>(July-Dec.)</td>
<td>11</td>
<td>1,651</td>
<td>116,254</td>
<td>1,233</td>
<td>117,487</td>
<td>8.59</td>
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<td>1957(Jan.-June)</td>
<td>12</td>
<td>1,600</td>
<td>109,596</td>
<td>2,351</td>
<td>111,947</td>
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<tr>
<td>(July-Dec.)</td>
<td>13</td>
<td>1,557</td>
<td>110,224</td>
<td>673</td>
<td>110,897</td>
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<td>1958(Jan.-June)</td>
<td>14</td>
<td>1,524</td>
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<td>910</td>
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<td>(July-Dec.)</td>
<td>15</td>
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<td>586</td>
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<td>2.62</td>
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<td>1959(Jan.-June)</td>
<td>16</td>
<td>1,508</td>
<td>101,425</td>
<td>1,111</td>
<td>100,314</td>
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<td>(July-Dec.)</td>
<td>17</td>
<td>1,486</td>
<td>100,279</td>
<td>517</td>
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<td>1960(Jan.-June)</td>
<td>18</td>
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<td>97,042</td>
<td>1,042</td>
<td>98,084</td>
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<tr>
<td>(July-Dec.)</td>
<td>19</td>
<td>1,452</td>
<td>95,649</td>
<td>512</td>
<td>96,161</td>
<td>1.17</td>
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<tr>
<td>1961(Jan.-June)</td>
<td>20</td>
<td>1,431</td>
<td>93,093</td>
<td>746</td>
<td>93,839</td>
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<td>(July-Dec.)</td>
<td>21</td>
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<td>93,503</td>
<td>378</td>
<td>93,881</td>
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<td>1962(Jan.-June)</td>
<td>22</td>
<td>1,411</td>
<td>92,158</td>
<td>103</td>
<td>92,055</td>
<td>0.338</td>
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<tr>
<td>(July-Dec.)</td>
<td>23</td>
<td>1,361</td>
<td>90,340</td>
<td>62</td>
<td>90,278</td>
<td>0.251</td>
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<tr>
<td>1963(Jan.-June)</td>
<td>24</td>
<td>1,349</td>
<td>88,802</td>
<td>144</td>
<td>88,946</td>
<td>0.284</td>
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<tr>
<td>(July-Oct.)</td>
<td>25</td>
<td>1,343</td>
<td>86,996</td>
<td>154</td>
<td>87,150</td>
<td>0.199</td>
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<tr>
<td>1963-64(Nov.-Feb.)</td>
<td>26</td>
<td>1,350</td>
<td>85,098</td>
<td>502</td>
<td>85,600</td>
<td>0.155</td>
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</table>
### SUPPLEMENTAL NEGATIVE CREAM BRT IN WISCONSIN

<table>
<thead>
<tr>
<th>Date</th>
<th>Round</th>
<th>Plants</th>
<th>Patrons Sampled</th>
<th>Patrons Not Sampled</th>
<th>Total Patrons</th>
<th>Susp. Herds</th>
<th>Blood Reactor Herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1964 (Mar-June)</td>
<td>27</td>
<td>1,368</td>
<td>84,336</td>
<td>379</td>
<td>84,715</td>
<td>0.112</td>
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<tr>
<td></td>
<td>28</td>
<td>1,371</td>
<td>83,483</td>
<td>237</td>
<td>83,720</td>
<td>0.108</td>
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</tr>
<tr>
<td>1964-65 (Nov.-Feb.)</td>
<td>29</td>
<td>1,368</td>
<td>81,704</td>
<td>527</td>
<td>82,231</td>
<td>0.154</td>
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<tr>
<td>1965 (Mar.-June)</td>
<td>30</td>
<td>1,356</td>
<td>80,605</td>
<td>424</td>
<td>81,029</td>
<td>0.140</td>
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<tr>
<td>(July-Oct.)</td>
<td>31</td>
<td>1,359</td>
<td>78,888</td>
<td>250</td>
<td>79,138</td>
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<td>1965-66 (Nov.-Feb.)</td>
<td>32</td>
<td>1,363</td>
<td>76,737</td>
<td>619</td>
<td>77,356</td>
<td>0.082</td>
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<tr>
<td>1966 (Mar.-May)</td>
<td>33</td>
<td>1,356</td>
<td>74,766</td>
<td>502</td>
<td>75,268</td>
<td>0.062</td>
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<tr>
<td>(June-Sept.)</td>
<td>34</td>
<td>1,380</td>
<td>73,492</td>
<td>210</td>
<td>73,702</td>
<td>0.096</td>
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<tr>
<td>1966-67 (Oct.-Jan.)</td>
<td>35</td>
<td>1,374</td>
<td>71,827</td>
<td>521</td>
<td>72,348</td>
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<tr>
<td>1967 (Feb.-May)</td>
<td>36</td>
<td>1,356</td>
<td>70,306</td>
<td>624</td>
<td>70,930</td>
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</tr>
<tr>
<td>(June-Sept.)</td>
<td>37</td>
<td>1,327</td>
<td>69,686</td>
<td>205</td>
<td>69,891</td>
<td>0.181</td>
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<tr>
<td>(Oct.-Jan.)</td>
<td>38</td>
<td>1,297</td>
<td>68,382</td>
<td>473</td>
<td>68,755</td>
<td>0.067</td>
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<tr>
<td>1968 (Feb.-May)</td>
<td>39</td>
<td>1,282</td>
<td>67,110</td>
<td>525</td>
<td>67,635</td>
<td>0.040</td>
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<tr>
<td>(May-Aug.)</td>
<td>40</td>
<td>1,256</td>
<td>66,220</td>
<td>202</td>
<td>66,422</td>
<td>0.054</td>
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</tr>
<tr>
<td>(Sept.-Dec.)</td>
<td>41</td>
<td>1,225</td>
<td>65,143</td>
<td>288</td>
<td>65,431</td>
<td>0.153</td>
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<tr>
<td>1969 (Jan.-April)</td>
<td>42</td>
<td>750</td>
<td>63,727</td>
<td>610</td>
<td>64,337</td>
<td>0.0295</td>
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<tr>
<td>(Apr.-Aug.)</td>
<td>43</td>
<td>743</td>
<td>62,653</td>
<td>259</td>
<td>62,912</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>(Aug.-Nov.)</td>
<td>44</td>
<td>733</td>
<td>61,850</td>
<td>275</td>
<td>62,125</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Babcock sample was taken.

At the laboratory, each sample was mixed thoroughly before a portion was removed for the catalase test and one ml removed for the BRT, which was conducted by adding one drop of BRT antigen (0.03 ml) to the milk, inverting the tube to assure mixing and then incubating at 37°C for 30 minutes. The test was read as described by Roepke et al. A 10 ml syringe was filled with milk and held overnight in an upright position from all fresh milk BRT positive samples. The next day 0.9 ml of skim milk was taken from the sample and placed in a BRT tube, 5 drops of negative cream were added, along with 1 drop of BRT antigen. This was mixed thoroughly and incubated for 1 hour at 37°C. Readings were made in the same manner as for the fresh milk BRT. Herds with positive ring test results on this procedure were classified as suspicious and were authorized to be blood tested at program expense.

The herd owner was notified of the suspicious BRT results by mail and was contacted by a State-Federal veterinarian to schedule his herd for testing. Blood samples were obtained from all lactating cattle and from all non-vaccinates over eight months of age. Composite milk samples (equal quantities of milk from each quarter) were collected from each lactating animal in the herd. Samples were mailed to the Central Animal Health Laboratory (CAHL).

The standard tube test and the complement-fixation were conducted on all blood samples. A serially diluted individual BRT was performed on all milk samples. Highly suspicious animals that reacted to the standard test and were negative or had low titers to the supplemental tests were bled again in 15-30 days.
and quarter milk samples collected for culture studies. All reactors were rebled and quarter milk samples for culture were taken at the time the animal was branded and appraised for slaughter.

The fresh negative cream used at each of the three BRT laboratories was obtained weekly at the University of Wisconsin Dairy processing plant which was supplied by 10 private herds and the University herds. The temperature of the cream was about 35° C. at the time of collection immediately after separation.

Aseptically collected quarter milk samples used for culture were centrifuged at about 6000 g. The cream layer and the sediment were mixed with a “Q” stick** and streaked onto two serum albimi*** agar plates and two “W” agar plates.7 One serum and “W” plate were incubated at 37° C. with 10% CO2 and the other serum and “W” plate were incubated without CO2. The culture plates were checked in three days and daily thereafter for seven days.

Colonies with the growth characteristics of the Brucella group were selected for subculture and were sent to USDA Brucellosis Laboratory, Moore Air Force Base, Mission, Texas, for typing.

All herds with positive samples on the negative cream BRT were blood and milk tested. In addition, 142 herds which were positive to the fresh milk BRT but negative to the NC BRT were similarly tested. These data cover a period from June 5, 1967 to November 26, 1969, Rounds 37 through 44 (Table IIA).

### TABLE II-A

**Summary of the Fresh Milk Positive, Negative Cream Negative Herds Tested**

<table>
<thead>
<tr>
<th></th>
<th>38th BRT Round</th>
<th>39th BRT Round</th>
<th>41st BRT Round</th>
</tr>
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<tbody>
<tr>
<td>Total Herds</td>
<td>76</td>
<td>14</td>
<td>52</td>
</tr>
<tr>
<td>Total Animals Tested</td>
<td>1,350</td>
<td>239</td>
<td>1,228</td>
</tr>
<tr>
<td>No. Animals Titer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over 1/25 on Tube Test</td>
<td>220</td>
<td>44</td>
<td>142</td>
</tr>
<tr>
<td>Cows with Milk Titers</td>
<td>230</td>
<td>27</td>
<td>211</td>
</tr>
<tr>
<td>Dry Cows</td>
<td>393</td>
<td>58</td>
<td>325</td>
</tr>
<tr>
<td>Suspects</td>
<td>67</td>
<td>16</td>
<td>43</td>
</tr>
<tr>
<td>Reactors</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>

### RESULTS

During the eight BRT rounds, a total of 6,869 herds were positive to the fresh milk BRT, but only 324 were also positive to the negative cream BRT. The follow-up blood and milk testing of these 324 herds resulted in 22 infected herds being located. Follow-up blood and milk testing of 142 herds showing positive

** “Q” Stick Trademark
*** Albimi Laboratories, Brooklyn, N.Y.
results on the fresh milk BRT but negative results on the negative cream BRT did not reveal any infected herds. Of these 142 herds, 96 were positive to the fresh milk BRT more than once. (Table IIB). A breakdown and summary of the numbers of test results by round can be seen in Table III.

TABLE II-B

<table>
<thead>
<tr>
<th>Times Herds Were Fresh Milk Positive</th>
<th>Herds 38th Round</th>
<th>Herds 39th Round</th>
<th>Herds 41st Round</th>
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<tbody>
<tr>
<td>1 time positive</td>
<td>46</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 times positive</td>
<td>18</td>
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<td>0</td>
</tr>
<tr>
<td>3 times positive</td>
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<td>13</td>
</tr>
<tr>
<td>4 times positive</td>
<td>7</td>
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<td>25</td>
</tr>
<tr>
<td>5 times positive</td>
<td>0</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>6 times positive</td>
<td>0</td>
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<td>4</td>
</tr>
<tr>
<td>7 times positive</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL HERDS</td>
<td>76</td>
<td>14</td>
<td>52</td>
</tr>
</tbody>
</table>

TABLE III

<table>
<thead>
<tr>
<th>Summary of All Herds by Round</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROUND</td>
<td>37</td>
</tr>
<tr>
<td>FM Pos</td>
<td>1,282</td>
</tr>
<tr>
<td>NC Pos</td>
<td>126</td>
</tr>
<tr>
<td>FM Pos</td>
<td>76</td>
</tr>
<tr>
<td>NC Neg</td>
<td></td>
</tr>
<tr>
<td>Infected Herds</td>
<td>6</td>
</tr>
<tr>
<td>Avg. Herd Size</td>
<td>17.6</td>
</tr>
</tbody>
</table>

A total of 466 herds were tested during this project and of these, 193 were ring test positive only once, that is at the time of test. Of the 22 infected herds, only two had a fresh milk positive ring test the previous round and one had a fresh milk positive test on an earlier round. These three herds proved to be infected with Strain 19 Brucella abortus and contained only one infected animal each.

DISCUSSION

The NC BRT cannot be applied satisfactorily in all states at the present time.
because the cost and time of running this additional step is not justified where the area has a high infection rate.

It should be satisfactory in areas with conditions similar to those found in Wisconsin herds, namely a very low incidence of infection and a high level of vaccination. Also the laboratory facilities, time and personnel must be available to be able to concentrate on herds disclosed as suspicious by this procedure.

The negative cream used is from a mechanical separator and the temperature of the cream is 35°C at time of collection. This cream was classified as 2½ dilutions less sensitive by Dr. Roepke than the cream used for the standard in his studies. There appears to be a significant difference between gravity and mechanically separated cream samples with regard to the sensitivity in ring test procedures. The cream is the important factor in the NC BRT and in Wisconsin, represents milk collected from many herds. The cream is mechanically separated and is subjected to some heat. The cream used in Wisconsin is obtained after separation at the University of Wisconsin Dairies. An investigation on the sensitivity of creams from different sources has not been done in this laboratory but a project on this subject is now in progress at NADL. The test as performed in Wisconsin reduced the number of suspicious BRT herds without apparent adverse effect. The number of infected herds has continued to decrease during the two years this study has been going on.

A seasonal variation in the number of positive BRT’s has been observed using fresh milk samples with the high point occurring in July and August. Paradoxically, at the peak of suspicious BRT’s the results of the catalase test conducted on the same samples reaches its lowest level. The proposal has been made that milk will not lose its false positive agglutination factor if handled under ideal conditions. These proposals have not been supported by results of other studies. Retesting samples the next day and/or even testing them several hours later has resulted in a reduction of the total number of suspicious herds. Heating the samples to 37°C for one hour also significantly reduced the number of detectable suspicious BRT’s.

SUMMARY

The use of the Negative Cream Brucella Ring Test (NC BRT) significantly reduced the number of suspicious results during the past eight BRT rounds (two years) in the state of Wisconsin. Use of a cream negative for brucella antibody and obtained following mechanical (not gravity) separation resulted in a BRT with a lowered sensitivity.

REFERENCES

6. NADL Diagnostic Reagents Manual 65 D & E.
7. Kuzdas and Morse — Alfimi W. Selective Media
8. Jones and Hendricks — CF Test.
PROPOSAL FOR A SWINE HERD VALIDATION PROGRAM – VIA MARKET SWINE TESTING

Paul B. Doby, D.V.M., Division of Meat, Poultry and Livestock Inspection
Illinois Department of Agriculture

Swine brucellosis can be eradicated with the initiation of a practical nationwide eradication program. The incidence of this disease has been reduced in recent years even though a national eradication program has not been in effect. Presently, the only swine brucellosis regulatory programs in effect in most states concern State-Federal validated brucellosis-free swine herds and brucellosis test requirements to qualify breeding animals for sale. There is a consensus of opinion that “down-the-road” testing is not the feasible method to achieve final eradication of swine brucellosis. The validated herd concept was developed in 1949 as a disease control measure, and it is a recognized fact that the validated herd is an important tool in eradicating swine brucellosis. (Provisions for certified brucellosis-free swine herds, later renamed validated brucellosis-free swine herds, were approved by the Executive Committee of this Association in 1949 and published as a part of the report of the Committee on Brucellosis.) It appears, however, that the majority of validated herds are maintained for economic reasons and not for disease prevention and control, i.e., reduction in expense to the owner for veterinary fees for brucellosis testing and an increase in sale value when animals are advertised as members of a validated herd. I believe it is important that members of this Committee consider these facts when contemplating adoption of the proposals of officials of the Illinois Department of Agriculture. As an added incentive for maintaining a validated herd, officials of that Department formulated regulations utilizing market swine testing (MST) as a means for qualifying and maintaining herds as Illinois MST validated brucellosis-free. These regulations as adopted effective November 23, 1970, and as amended effective July 18, 1971, are as follows:

"(Swine from herds that qualify for validation or revalidation under this regulation are eligible for movement within the State of Illinois only. Swine destined for interstate shipment from such herds must be accompanied by a negative brucellosis test conducted within 30 days prior to date of shipment.)

"1. Herds may be validated upon the completion of one negative test of all swine in the herd 6 months of age or older, except barrows. An Illinois MST validated brucellosis-free swine herd certificate, which shall be valid for a period of 1 year, unless revoked, will be issued by the Division of Meat, Poultry and Livestock Inspection, upon declaration of the herd owner that he desires to follow this plan for validation.

"2. Validation may be extended for a period of 1 year upon evidence of negative brucellosis test of ten percent (10%) of the breeding animals in the herd, with a minimum of 5 animals, during the preceding 12 months through a Market Swine Testing (MST) Program, except that twenty-five percent (25%) of the swine shipped to slaughter for validation qualification must have been slaughtered in the quarter of the year immediately preceding the renewal date. Swine tests to be credited back to the herd
under the MST Program shall be counted only on boars over 6 months of age or sows that show evidence of having farrowed. If sufficient MST credits are not available to revalidate the herd, or if the owner so desires, validation may be extended for a period of 1 year upon completion of a negative test of fifty percent (50%) of all swine in the herd 6 months of age or older, except barrows. (Italic portion by amendment effective July 18, 1971.)

“3. If reactors are disclosed as a result of the MST Program, herd validation shall be suspended immediately and may be renewed only by completion of a negative test of all animals in the herd 6 months of age or over. Such test may be made at State expense, provided funds are available.

“4. ADDITIONS TO MST VALIDATED HERDS shall be:
   a. Animals originating directly from a validated herd in good standing, without test (owner shall furnish proof of same to the Department),
   b. Animals from non-validated herds in Illinois, provided they have passed a negative test within 60 days, or
   c. Animals from out-of-state accompanied by an approved interstate health certificate showing compliance with Illinois entry requirements.

“All swine brought on to the farm for feeding purposes shall be segregated from the breeding herd.”

I believe the test requirements for maintenance of the Illinois MST validated herd will enable us to detect brucellosis in a herd very shortly after invasion. When a breeding herd operates on a non-validated basis, generally only young animals are brucellosis tested. In my opinion, these are not the prime animals to test when monitoring a herd; the testing of cull breeding animals increases the probability of locating brucellosis infection in a herd.

As of October 15, 1971, 14 herds have been validated under Illinois regulations governing MST validated brucellosis-free herds. Members of an Illinois MST validated herd are qualified to move only intrastate without additional brucellosis tests. If the majority of sales from a herd are to other states, it is more practical to operate under the State-Federal cooperative validated brucellosis-free herd plan. For this reason, the Illinois MST Validation Program has expanded slowly. If this Committee approves provisions for MST validation, it would gain national status and animals from such herds should qualify for shipment to other states without additional brucellosis tests. This alone would instantly increase the value and popularity of MST validation.

I am aware that, at the present time, not all states have MST Programs and that it is necessary that a MST slaughter program be in operation before officials of a state can adopt the MST type of validation. I believe the practical approach to eradicating swine brucellosis from this Country is the formation of a nationwide MST Program.

In summary, officials of the Illinois Department of Agriculture propose that: (1) the USAHA Committee on Brucellosis approve a system for validation of swine herds as brucellosis-free, utilizing the MST concept, and (2) this Committee, through the Committee Report, urge officials of all states to accept swine from such herds as complying with brucellosis entry requirements of the respective state.
PROGRESS OF THE STATE-FEDERAL
BRUCELLOSIS ERADICATION PROGRAM

by
H. C. King, D.V.M.1

Progress during the past year in the Cooperative State-Federal Brucellosis Eradication Program, although not spectacular, has been substantial. The program has competed in many areas with other programs, for funds, manpower, and attention; but in spite of these complications, it has moved forward in most States.

Some of the progress can be ascribed to improved and reemphasized procedures, but most of it must be credited to the dedicated and committed individuals in each of the States.

Before specific examples of progress that has been made are reviewed, it may be better to review some of the individual components which taken together make up the total program. A number of charts will better illustrate these components.

Blood Testing Cattle (Figure 1)

Brucellosis Eradication

BLOOD TESTING: CATTLE

FISCAL YEAR

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE

1 Chief Staff Veterinarian, Brucellosis Eradication, Animal and Plant Health Service, United States Department of Agriculture, Hyattsville, Maryland.
Prior to this past year, there had been a decrease in total numbers of cattle tested each year for the past 2 years. This downward trend was interrupted with 10.5 million head tested during fiscal year 1971.

For the first time in the program, more cattle were tested under market cattle testing than on farms and ranches. Tests of samples collected at slaughter and markets under the market cattle testing program totalled 5.4 million compared to 5.1 million farm and ranch tests.

Encouragingly, the downward trend in total reactors found continued for the third consecutive year with a total of 116,000 reactors found.

*Market Cattle Testing Program (Figure 2)*

For the past several years, the number of tests under the market cattle testing program had "plateaued" at just under the 5 million level. It has now broken through this level and hopefully will continue to increase. Increases occurred during the past year in both parts of the program. Samples collected at packing plants increased by about 300,000 and at markets by 200,000. During the year, increased emphasis was placed on collection of samples from all cows and bulls at slaughter, including samples from unidentified animals. Authorization was granted to utilize full-time employees and contract personnel in blood collection at high-volume slaughter plants. Although a substantial increase occurred in market cattle testing samples collected, the 3 million samples collected at slaughter plants represents less
than one-third of the cows and bulls slaughtered annually. A surveillance program to be effective in locating all infected herds, must include sampling of close to 100 percent of these cattle. Preliminary information on the results of samples collected from unidentified cattle indicates that the infection rate in this population may be as much as three times greater than in the identified population. It is also becoming evident that a rather high percentage of the reactors found in this group may be traced to herds of origin through various market and slaughter records. Experience gained in several States indicates that it is most important to followup every possible lead in order to find the herds of origin.

Milk Ring Testing: Percent of Suspicious Tests (Figure 3)

The other, and equally important, surveillance procedure is the milk ring test. The test is conducted three to four times each year on milk samples and since becoming an official part of the eradication program in 1952 has been primarily responsible for the relative freedom from brucellosis of the dairy population of this country. The percentage of suspicious tests fell to 0.3 percent in 1971 after remaining constant at 0.4 percent for the previous 3 years.

Milk Ring Test Results (Figure 4)

Over 2,800 followup blood tests were made of herds suspicious to the ring test. Of these, 801 herds were found to contain reactors or about one herd was found infected for every three and a half herds tested. This compares with about one infected herd found for every two and a half herds or origin tested under the
market cattle testing program. The 801 infected dairy herds found on followup tests are only about one-half the number found 4 years ago and represents a significant reduction in the amount of infection remaining in the dairy population.

_Total Brucellosis Infected Herds_ (Figure 5)

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Infected Herds</th>
<th>MCI</th>
<th>BRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1967</td>
<td>21,255</td>
<td>7,117</td>
<td>1,353</td>
</tr>
<tr>
<td>1968</td>
<td>20,662</td>
<td>6,172</td>
<td>1,386</td>
</tr>
<tr>
<td>1969</td>
<td>18,088</td>
<td>5,375</td>
<td>1,641</td>
</tr>
<tr>
<td>1970</td>
<td>15,476</td>
<td>4,796</td>
<td>1,107</td>
</tr>
<tr>
<td>1971</td>
<td>14,812</td>
<td>4,768</td>
<td>9,006</td>
</tr>
<tr>
<td>1972</td>
<td>14,801</td>
<td>801</td>
<td></td>
</tr>
</tbody>
</table>

*ON INITIAL FOLLOW-UP BLOOD TESTS

The total number of infected herds found in fiscal year 1971 was reduced by about 600 to slightly less than 15,000 in spite of increased efforts in many States to locate such herds. About a third of these herds were found on the initial followup herd tests of market cattle testing reactors and brucellosis ring test suspicious results. In evaluating the effectiveness of the market cattle testing program in locating infected herds, it maybe compared to the brucellosis ring test as a standard. On the average, 3.5 percent of the cattle in each of the 801 infected brucellosis-ring test herds were found to be reactors while 13 percent were reactors in each of the 4,005 infected herds of origin of market cattle test reactors. It may be concluded that the market cattle testing program, as presently conducted, is frequently failing to pinpoint infected herds in the initial stage of infection. It indicates that the effectiveness of the program must be increased by improved identification and sample collection.
The distribution of infected herds according to the number of such herds per State defines to some degree the States in which increased program activities need to be concentrated. One State accounted for almost half of all infected herds. An additional two States accounted for over 17 percent of herds with from 1,000 to 3,000 infected herds each. If we add the six States with 300 to 1,000 infected herds each, we find that nine States were responsible for over 85 percent of all infected cattle herds while 29 States accounted for less than 2 percent of the infected herds. Five States and the Virgin Islands recorded no infection. In each of an additional 10 States, less than 10 infected herds were found.

**Brucellosis Infected Herds Found** (Figure 7)

The distribution of infected herds in relation to the certification status of States is one measurement of progress. Last year 219 infected herds were found in the 22 certified-free States, an increase of 35 herds over 1970. However, there were three additional States in this category. In the 27 modified certified States, 7,224 infected herds were found, an increase of almost 1,200 herds indicating increased program efforts in several States. Only one State, Texas, remained as noncertified and in that State over 7,000 infected herds were found reflecting the continued all out efforts to qualify as modified certified.
FIGURE 7

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

NUMBER INFECTED HERDS

STATES WHERE INFECTED HERDS FOUND
- Certified-Free
- Modified Certified
- Noncertified

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE

Brucellosis Reactor Herd Rates (Figure 8)

BRUCELLOSIS REACTOR HERDS
IN NONCERTIFIED, MODIFIED CERTIFIED and CERTIFIED-FREE STATES
(Rates Per 10,000 Herd Population)
Perhaps a more realistic evaluation of the comparative infection in States in relation to their certification status is a comparison of the number of infected herds per 10,000 herds. Last year this rate was 3.8 infected herds per 10,000 herds in the Certified Brucellosis-Free States compared to 44.2 in the Modified Certified Brucellosis States and 345.4 in the one remaining noncertified State. It is encouraging to note that the infected herd rate in the certified-free States has declined in each of the past 3 years from 6.6 percent in 1968.

Increasingly problem herds are being depopulated. The depopulation of complete herds seems justified and, in many cases, is necessary in order to eliminate remaining foci of infections which may post a threat to other herds, particularly in free areas. Last fiscal year, 19 herds were depopulated in nine States with payment of Federal and State indemnity. However, depopulation should not be used as a substitute for good eradication procedures.

*Calves Vaccinated (Figure 9)*

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Calves Vaccinated (Million)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1953</td>
<td>1</td>
</tr>
<tr>
<td>1955</td>
<td>2</td>
</tr>
<tr>
<td>1957</td>
<td>3</td>
</tr>
<tr>
<td>1959</td>
<td>4</td>
</tr>
<tr>
<td>1961</td>
<td>5</td>
</tr>
<tr>
<td>1963</td>
<td>6</td>
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<tr>
<td>1965</td>
<td>7</td>
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<tr>
<td>1969</td>
<td>5</td>
</tr>
<tr>
<td>1971</td>
<td>4</td>
</tr>
</tbody>
</table>

At some point in the effort to reach the goal of total eradication of brucellosis, the use of Strain 19 vaccine must stop. Vaccination is on the decline but not at a desirable rate. Last year almost 4.3 million calves were vaccinated which was only 11 percent less than in 1970. This rate of decrease must be drastically increased particularly in those Certified Brucellosis-Free States with little remaining exposure. The continuing problem of diagnosis caused by vaccination as well as the infections produced can only delay final eradication.

Records of attempted isolations of brucella organisms from reactors show that of 3,213 isolations made, 145 or 4.5 percent were typed as Strain 19. Among the
708 isolates from vaccinated animals, 94 or 13.3 percent were typed as Strain 19. Although the majority of isolates have been from 2 or 3 year old animals, Strain 19 infection has been also found in animals up to 8 years of age.

Situation Map – June 30, 1971 (Figure 10)

During the year, three States, (Minnesota, North Carolina, and North Dakota) reached a Certified Brucellosis-Free status so that on June 30, 1971, 22 complete States and the Virgin Islands were Certified Brucellosis-Free. Florida and South Dakota qualified during the year as Modified Certified Brucellosis States leaving only 13 noncertified counties in Texas.

The 3,140 certified counties represents an increase of 36 compared to a year earlier. A better measurement of progress is to look at the number of areas qualifying as Certified Brucellosis-Free. During the year, there was an increase of only 157 counties compared to 173 in 1970. With a continuation of this low rate of accomplishment in freeing areas of infection, it will take more than 9 years or until 1980 to qualify the remaining 1,498 counties as Certified Brucellosis-Free. It is imperative that the program be speeded up to at least double the present rate of progress.

During the year, six Modified-Certified Brucellosis Areas and one Certified Brucellosis-Free Area lost their status for various reasons all involving failure to comply with the requirements of the Uniform Methods and Rules.
Although much has been said about the need to start a swine brucellosis eradication program, there still has been little coordinated effort directed at this problem. The total number of swine tested this past year was just over one-half million, about the same as has been tested each of the past 5 or 6 years. The 0.32 percent reacting animals to tests was only slightly less than a year ago. The percentage of lots tested containing a reacting animal was 0.98 percent, the first time it has been less than 1 percent.

Validated Swine Herds (Figure 12)

At the end of the year there were 2,658 validated herds. The number of Validated Brucellosis-Free Areas decreased by six to 186 and included three complete States (Utah, Arizona, Vermont) and the Virgin Islands. Nevada lost its validation status during the year but has since been reinstated. All California counties except one are Validated Brucellosis-Free.

Last Year there were 224 reported cases of human brucellosis. Most of the cases occurred in packinghouse workers, and among these cases swine were the source of infection in 67 percent of the cases. The eradication of swine brucellosis from the United States has been too long delayed. All indicators point to a low incidence of swine brucellosis. The relatively few infected herds can be located if an identification program is started and sows and boars are tested as they move to markets or to slaughter. California has already adopted regulations to become
effective January 1, 1973, that no swine may enter the State unless they are from a validated-free herd or area.

The time that remains to eradicate both bovine and swine brucellosis is short. We cannot expect money for a never-ending eradication program to be continuously appropriated by the State Legislatures and the Congress, and we cannot expect the public and the industry to continue to support the current program unless more rapid progress can be demonstrated. The date on which we reach eradication will be determined by the quantity and quality of effort and the commitment of all who have a part in the program.
CURRENT PUBLIC HEALTH PROBLEMS OF SWINE BRUCELLOSIS

by
Stanley L. Hendricks*, D.V.M., M.P.H.

In recent years swine brucellosis has caused an increasing percentage of the human brucellosis in the United States. For a number of years the Center for Disease Control, Public Health Service, U.S. Department of Health, Education and Welfare, has assembled epidemiological data for a large portion of the human cases reported each year in the United States. Figure A and Table 1 show the numbers of cases reported, those with surveillance data and the cases that probably were due to swine for each year during the period 1957 through 1970. The proportion of swine origin cases was about three times as high for the years 1968, '69 and '70 as for the years 1957, '58 and '59.

During the years 1968, 1969 and 1970 a total of 706 human cases were reported in the United States (Table 2). Surveillance data are available on 596 cases of which 286 or 48% were most probably due to infected swine. Bacteriologic studies during the same three year period resulted in recovery of brucella organisms from 135 human cases of which 84 or 62% were Br. suis (Table 3). Thus both epidemiologic data and bacteriologic findings suggest one-half or more of the human cases in the United States in the past 3 years were due to the infection in swine.

Swine have been an even more important source of human cases in Iowa in recent years. In the ten year period 1961 through 1970, brucella organisms were recovered from 99 human cases of which 95 were B. suis and 4 were B. abortus. Of the 135 human cases reported in the years 1968, 1969 and 1970, epidemiologic data indicate hogs were the most probable source of 115 or 85%.

Geographic distribution, and probably source of infection of the Iowa cases for the years 1968, 1969 and 1970 are shown in Figures B, C and D. In 1968 (Fig. B) 26 of the 32 cases were packing house employees. An abattoir in Scott County that slaughters only hogs reported 12 of the cases. Four Marion County cases were farmers who had purchased breeding hogs from a local livestock auction market operator. The fifth case was a trucker who had hauled from this auction market. Late in 1968 a Polk County farmer purchased 100 wet sows for fattening from the Marion County auction market operator. The farmer changed his plans and bred the sows. Early in 1969 he observed clinical manifestations in the sows suggestive of brucella infection and blood tests confirmed the diagnosis. The sows were marketed at an abattoir in Polk County. In the ensuing weeks the farmer and 6 abattoir employees became ill with brucellosis. Thus 7 cases are shown in Polk County in Figure C. Other cases in 1969 were scattered throughout the state except 15 cases in Scott County again in the hog abattoir. Of the 41 cases reported in 1969, 8 were not abattoir related. Two of the 8 were due to accidental injection of Str. 19

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vaccine. The Marion County case was an employee of the auction market mentioned above.

Sixty-two human cases were reported in Iowa in 1970 and they were unusual in several respects. All of them were packing house workers (includes 2 U.S. Gov't inspectors). All were related to swine and 53 of the sixty-two occurred in one plant (Figure D)\(^2\). This large outbreak occurred in the abattoir in Dubuque County in which there had been only a total of 4 cases in the two previous years. This was a large plant with a work force of 2,700 employees that slaughters 10,000 hogs, 1,600 cattle and a few sheep and calves daily.

The cases occurred in all months except February, with most of the cases in the March through September period. The attack rates were highest in the hog-kill, casings, pork cut and offal departments but cases also occurred in curing, pork boning, canning, inedible, sausage, beef boning; beef cooler, freezer, hide, lard, maintenance, receiving, shipping, smokehouse and box departments. There were no cases among employees from the cattle kill despite the fact that the plant regularly slaughtered 30 to 50 bovine brucellosis reactors per week.

During the outbreak, blood specimens were collected from 750 sows and 750 butcher-hogs prior to slaughter on five separate days. They were tested by the State Federal Brucellosis laboratory and all were negative to the card test. Also during the outbreak, tissue specimens for bacteriologic culture were collected from 20 bovine brucellosis reactors. B. abortus type 1. organisms were recovered from 9 of the animals.

Hogs for slaughter in this plant were procured from the same geographic areas in 1968, 1969 and 1970. There is much overlapping of procurement areas by various plants and a producer may market his hogs at one plant at one time and at another plant at another time. Conceivably hogs from infected farms might have been slaughtered in the Scott Co. plant in 1968 and 1969 and hogs from the same infected foci in the Dubuque Co. plant in 1970. However a review of the plants procurement records failed to yield facts to support this possibility.

The distribution of cases in the various departments of the plant suggests swine were the source of the cases as employees of the cattle kill were not affected. Food and water as possible vehicles of infection were ruled out epidemiologically and by bacteriologic tests. The fact that cases developed over a period of several months suggests multiple exposures probably occurred.

Studies indicate the reservoir of brucella infection in swine in Iowa has declined in recent years and is at a low level. No infected animals were found in the sample of hogs tested in the Dubuque County plant. A decline in the prevalence of brucellosis in Iowa hogs was noted in a previous report\(^3\). In an extensive survey in a western Iowa packing plant over a 46 week period in 1967 and 1968 almost 18,000 sows representing 4,251 lots were tested.\(^4\) Thirty animals in 9 lots were positive to the card test. Thus 1.6 per 1,000 sows tested were positive and 2 lots per 1,000 lots contained reactor animals.

A review of results of routine brucella agglutination tests conducted at the State Federal Brucellosis laboratory in Iowa in recent years shows a marked decline in positive reactors\(^5\). Table 4 and Figure E indicate the reactor rate dropped from 9 per 1,000 animals in 1965 to only 1 reactor per 1,000 in 1971. The routine testing conducted at the State Federal Brucellosis Laboratory may or may not be a true indicator of the prevalence of the disease since the animals that are tested are
selected for various reasons and do not constitute a random sample. However comparisons from year to year during 1965-1970 seem valid. There were no major events during this period which would have caused a change in selection of animals for test nor was there a change in interpretation of test results as occurred in previous years. Beginning in April 1971 a change of some significance did occur however in that the card test was gradually phased into use because of a change in the state law. During the 1965-70 period the number of validated herds increased about 22%. (Table 4 and Figure F). The total number of animals in validated herds increased from 15,000 and 23,000 or 50% because the size of the herds increased from an average of 27 animals to 33.

While serologic studies indicate a low prevalence of brucella infection in Iowa swine it must be recognized that even a small reservoir of infection constitutes a human health risk. Scores of workers possibly could be exposed to brucellosis by direct or indirect contact with infectious tissues by slaughter and processing of a single infected animal in a large slaughter house. For prevention of human brucellosis, the reservoir of this disease in swine must be eradicated.

TABLE 1.
TOTAL HUMAN BRUCELLOSIS CASES REPORTED IN THE UNITED STATES, CASES WITH SURVEILLANCE DATA, AND CASES WITH SWINE AS PROBABLE SOURCE 1957-1970*

<table>
<thead>
<tr>
<th>YEAR</th>
<th>TOTAL CASES REPORTED</th>
<th>CASES WITH SURVEILLANCE DATA</th>
<th>CASES WITH SWINE AS PROBABLE SOURCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO.</td>
<td>PERCENT</td>
<td>NO.</td>
</tr>
<tr>
<td>1957</td>
<td>983</td>
<td>13</td>
<td>64</td>
</tr>
<tr>
<td>1958</td>
<td>924</td>
<td>11</td>
<td>40</td>
</tr>
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<td>751</td>
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<td>1961</td>
<td>636</td>
<td>30</td>
<td>122</td>
</tr>
<tr>
<td>1962</td>
<td>409</td>
<td>31</td>
<td>85</td>
</tr>
<tr>
<td>1963</td>
<td>407</td>
<td>41</td>
<td>106</td>
</tr>
<tr>
<td>1964</td>
<td>411</td>
<td>38</td>
<td>103</td>
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<tr>
<td>1965</td>
<td>262</td>
<td>30</td>
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<td>1966</td>
<td>262</td>
<td>29</td>
<td>64</td>
</tr>
<tr>
<td>1967</td>
<td>248</td>
<td>28</td>
<td>58</td>
</tr>
<tr>
<td>1968</td>
<td>251</td>
<td>43</td>
<td>90</td>
</tr>
<tr>
<td>1969</td>
<td>232</td>
<td>58</td>
<td>114</td>
</tr>
<tr>
<td>1970</td>
<td>224</td>
<td>42</td>
<td>82</td>
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*Source: Ref. #1.
### Table 2.


<table>
<thead>
<tr>
<th>Source</th>
<th>1968</th>
<th>1969</th>
<th>1970</th>
<th>Total</th>
<th>% of Total</th>
</tr>
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<tr>
<td><strong>Swine</strong></td>
<td>90</td>
<td>114</td>
<td>82</td>
<td>286</td>
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<tr>
<td><strong>Cattle</strong></td>
<td>26</td>
<td>20</td>
<td>19</td>
<td>65</td>
<td>11</td>
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<tr>
<td><strong>Sheep &amp; Goats</strong></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>0.6</td>
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<tr>
<td><strong>Cattle or Swine</strong></td>
<td>21</td>
<td>16</td>
<td>31</td>
<td>68</td>
<td>11</td>
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<tr>
<td><strong>Cattle, Swine, or Sheep</strong></td>
<td>3</td>
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<td><strong>Raw Milk</strong></td>
<td>18</td>
<td>5</td>
<td>6</td>
<td>29</td>
<td>5</td>
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<tr>
<td><strong>Str. 19. Vaccine</strong></td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>0.8</td>
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<tr>
<td><strong>Accident or Lab Acquired</strong></td>
<td>10</td>
<td>1</td>
<td>7</td>
<td>18</td>
<td>3</td>
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<tr>
<td><strong>Other &amp; Unknown</strong></td>
<td>40</td>
<td>31</td>
<td>45</td>
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<tr>
<td><strong>Total Reviewed</strong></td>
<td>207</td>
<td>195</td>
<td>194</td>
<td>596</td>
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<tr>
<td><strong>Total Reported</strong></td>
<td>251</td>
<td>231</td>
<td>224</td>
<td>706</td>
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*Source: Ref. #1.*
### TABLE 3.

**SPECIES OF BRUCELLA RECOVERED FROM 135 HUMAN CASES DURING THE PERIOD 1968 THROUGH 1970**

<table>
<thead>
<tr>
<th>BRUCELLA SPECIES</th>
<th>1968</th>
<th>1969</th>
<th>1970</th>
<th>TOTAL NO.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suis</td>
<td>25</td>
<td>33</td>
<td>26</td>
<td>84</td>
<td>62.2</td>
</tr>
<tr>
<td>Abortus</td>
<td>8</td>
<td>6</td>
<td>10</td>
<td>24</td>
<td>17.8</td>
</tr>
<tr>
<td>Melitensis</td>
<td>8</td>
<td>3</td>
<td>11</td>
<td>11</td>
<td>8.2</td>
</tr>
<tr>
<td>Suis &amp; Melitensis</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Unknown</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>13</td>
<td>9.6</td>
</tr>
<tr>
<td>Canis</td>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td>47</td>
<td>46</td>
<td>42</td>
<td>135</td>
<td>100.0</td>
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</table>

*Source: Ref. #1.

### TABLE 4.

**ANIMALS AND LOTS TESTED, POSITIVE REACTORS AND VALIDATED HERDS IN IOWA 1965-1971**

<table>
<thead>
<tr>
<th>YEAR</th>
<th>NO. TESTED</th>
<th>POSITIVE REACTORS</th>
<th>VALIDATED HERDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANIMALS</td>
<td>ANIMALS</td>
<td>LOTS</td>
</tr>
<tr>
<td></td>
<td>LOTS</td>
<td>NO.</td>
<td>%</td>
</tr>
<tr>
<td>1965</td>
<td>187,548</td>
<td>26,602</td>
<td></td>
</tr>
<tr>
<td>1966</td>
<td>207,619</td>
<td>29,916</td>
<td></td>
</tr>
<tr>
<td>1967</td>
<td>200,886</td>
<td>27,596</td>
<td></td>
</tr>
<tr>
<td>1968</td>
<td>199,390</td>
<td>26,281</td>
<td></td>
</tr>
<tr>
<td>1969</td>
<td>186,549</td>
<td>22,978</td>
<td></td>
</tr>
<tr>
<td>1970</td>
<td>213,951</td>
<td>25,956</td>
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</tr>
<tr>
<td>1971*</td>
<td>136,770-13,704</td>
<td>115</td>
<td>0.08</td>
</tr>
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</table>

*Jan. thru Sept.*

Data Source: Ref. #5.
PERCENTAGE OF CASES PROBABLY DUE TO SWINE*
Figure B.

HUMAN BRUCELLOSIS CASES IN IOWA IN 1968
BY COUNTY AND BY PROBABLE SOURCE

Figure C.

HUMAN BRUCELLOSIS CASES IN IOWA IN 1969
BY COUNTY AND BY PROBABLE SOURCE

H=Ruman case with hogs as probable source
C=Ruman case with cattle as probable source
X=Ruman case with history of contact with cattle & hogs
V=Ruman case = SW 19 vaccine
Figure D.

HUMAN BRUCELLOSIS CASES IN IOWA IN 1970
BY COUNTY AND BY PROBABLE SOURCE.

H = Human case with hogs as probable source
C = Human case with cattle as probable source
X = Human case with history of contact with cattle & hogs
FIGURE E.

SWINE BRUCELLOSIS TESTS
NUMBER OF REACTOR ANIMALS PER 1000 HOGS TESTED IN IOWA 1965-1971*

YEAR

1965 '66 '67 '68 '69 '70 '71

*Source: Ref. 3
**Data for 1971 is for months Jan. through Sept.
REFERENCES

REPORT OF THE COMMITTEE ON BRUCELLOSIS

Dean E. Flagg, Bismark, N.D., Chairman
H. G. Wixon, Sacramento, Calif., Co-chairman

The brucellosis committee met in open session on Tuesday with over 100 persons in attendance. Everyone was given the opportunity for free expression on the progress made, proposed improvements, problems and objectives of the brucellosis eradication program.

A brucellosis progress report was given by Dr. H. C. King, Chief Staff Veterinarian, brucellosis eradication for U.S.D.A. This report will appear in the annual proceedings of this Association.

The following made formal reports:

Dr. Ken Hook, Asst. State-Federal Veterinarian in Wisconsin delivered a report on supplemental negative cream BRT in Wisconsin as prepared by Dr. Gordon C. Janney, Animal Health Division, Wisconsin.

Dr. John L. O'Hara submitted a report of the committee on evaluation of State-Federal programs on tabulations on responses to the questionnaire on the cooperative State-Federal brucellosis eradication program.

Dr. Paul L. Doby, Superintendent of Livestock and Poultry, Springfield, Ill. submitted a proposal for a swine herd validation program-via Market Swine Testing.

Dr. Arthur L. Starkey, Ill. Department of Agriculture, reported on work performed in Illinois on Market Swine Testing.

These presentations will appear as part of the proceedings of this convention.

The open meeting closed at about 4:00 P.M. and the committee went into executive session.

Total Eradication Goal Reaffirmed

By unanimous decision, the Committee reaffirmed and established December 31, 1975, as the goal for complete eradication of brucellosis from the United States.

In order to achieve this goal the Committee recommends that the Uniform Methods and Rules must be amended and strengthened, as follows:

“These are the minimum standards for initiating and improving the various plans for certifying or for validating areas or herds.”

Reactor

On or after January 1, 1973, the one dilution titer tolerance for official vaccinates be eliminated.
Herd Test
The age of animals in a herd test be changed from over 6 months to 12 months and over for all tests except recertification of certified brucellosis free herds. The Committee accepted the Pennsylvania recommendation that the recertification of brucellosis free herds should include only animals 24 months of age and over, that individual animals for additions are negative to test within 60 days instead of 30 days, and that the annual recertification test be conducted within a period of 10 to 14 months instead of 9 to 15 months.

Official Vaccinates
That dairy heifers be vaccinated when from 3 to 6 months (90-179 days) of age and beef heifers when from 3 to 8 months (90-239 days).

Exposed Animals
Animals that have been held in direct contact with brucellosis reactors in marketing channels for periods longer than 24 hours, or less than 24 hours if the reactor has been recently aborted, calved, or has a vaginal discharge, are considered to be exposed regardless of the blood test results and must be placed under quarantine and restricted pending testing, slaughter, or moved to a quarantined feed lot.

Quarantine and Retesting of Infected Herds
That the quarantine period be extended to 120 days, and retest of infected herds reflect this change.

Branding Reactors
That reactors must be branded no later than 15 days after the date the blood samples were collected.

Reinstatement of Certification or Validation
When herds or areas lose certification or validation status because of deficiencies in levels of surveillance or in procedures necessary for eliminating infected animals as prescribed under the various plans and procedures, the certification or validation status may be reinstated upon presentation of sufficient evidence showing that the deficiencies have been corrected. Areas in which the deficiencies have not been corrected within 6 months of the termination of the status must qualify as initial certification or validation.

Testing BRT Suspicious Herds
A blood test of all herds suspicious on BRT is required within 30 days.

Testing of MCT Positive Herds
Herds of origin of MCT reactors shall be tested within 30 days, otherwise placed under quarantine until tested and found to be negative.

Random Testing
Eliminate the graph for random testing of eligible cattle in a herd.
**BRUCELLOSIS**

**Modified Certified Areas**

(a) That by July 1, 1972, the herd infection rate in Modified-Certified areas be decreased to 3 percent and the animal infection rate by decreased to ½ of 1 percent, and thereafter be continuously reduced to achieve eradication by December; Furthermore the wording “extenuating circumstances” be eliminated.

(b) Requirements concerning effective screening programs and extensive epidemiological investigations, to locate infection and to eradicate disease shall be the same for Modified-Certified areas as for Certified-Free areas.

**Movements of Animals**

All sections pertaining to movements of animals be amended to reflect the committee recommendations pertaining to the Federal Regulations for the movement of animals interstate.

**Validated Brucellosis-Free Herd Plan**

The committee studied the Illinois Validated Herd plan, and from this plan recommends the following:

Swine herds may be validated or revalidated following one complete negative herd test of the breeding animals 6 months of age and older. Herds may also be revalidated through the MST program by testing a minimum of 20 percent of the breeding animals from the herd each year with no disclosure of infection.

The committee further recommends that Federal Interstate Shipment Regulations be amended in order to achieve the goal of total eradication in 1975.

The Committee recommends an amendment to the Federal Interstate Shipment Regulations to require that cattle originating from other than Certified Brucellosis-Free herds or states be tested and found negative within 30 days prior to shipment or be tested immediately upon arrival in the state of destination.

The Committee also recommends that the USDA adopt interstate shipment regulations to become effective, January 1, 1973 to require that all swine moved in interstate commerce originate from Validated Brucellosis-free herds or areas or be negative to test within 30 days prior to shipment.

**Indemnity for Swine**

The Committee recommends that indemnity provisions be made for reactor swine and infected herds.

**Enforcement of Uniform Methods and Rules**

The Committee urges the Animal Health Division to remove the certification status from any area or herd not complying with the minimum standards outlined in the Uniform Methods and Rules. Furthermore, the Committee strongly urges the Division to establish penalties for removal of MCI tags or any other form of animal identification.

The Committee strongly urges the United States Park Service to be notified by the Secretary of the Interior to cooperate in the eradication of brucellosis in bison in Yellowstone National Park and in any other National Parks where this infection exists. This is necessary to accomplish the goal of eradication of brucellosis in the United States by December 31, 1975.
The Committee directed the Chairman to forward copies of this recommendation to the Congressional delegates of Idaho, Wyoming, and Montana.

All proposals and resolutions received were duly considered by the Committee.
EVALUATION OF THE RESPONSES TO THE QUESTIONNAIRE ON THE COOPERATIVE STATE-FEDERAL BRUCELLOSIS ERADICATION PROGRAM

The questionnaire was prepared by the Brucellosis Committee and reviewed by the Committee on Evaluation and Development of State-Federal Programs of the United States Animal Health Association in an effort to determine how closely the brucellosis programs of the various States adhered to the provisions of the Uniform Methods and Rules.

It is evident from the tabulation of responses that the provisions of the Uniform Methods and Rules have been selectively adopted and only a few States accept the entire Uniform Methods and Rules as the minimum standard for certification and eradication. The lack of uniformity in the conduct of local brucellosis programs is detrimental to an early achievement of a brucellosis eradication goal. Not only do these inconsistencies allow the movement of animals of questionable status but also interfere with evaluations on national progress. Furthermore, these inconsistencies are confusing to the livestock industry when interstate interests are involved. It is particularly important that the minimum standards accepted by USAHA and adopted for the Cooperative State-Federal Brucellosis Eradication Program be uniformly implemented. It is obvious that a number of changes are needed such as control on movement of exposed cattle, quarantine authorities, length of quarantine periods, testing frequency in infected herds, tracing of MCT reactors, and immediate slaughter of all reactors.

Standardization of all provisions in the Uniform Methods and Rules is desirable even for relatively minor factors because of the impact on the total program. For example, disagreement on factors such as the minimum age for testing non-vaccinated animals could result in violation of Federal regulations governing interstate movement of cattle. Other examples of inconsistencies by the States in the interpretation of the Uniform Methods and Rules are abundant. In response to the questionnaire, 24 States indicated that their quarantine feedlots were in agreement with the Uniform Methods and Rules. However, 11 of these States then qualified the answer by stating that they did not comply with one or more conditions in the definition. In another situation, only 18 of 39 States routinely classified plate suspect-positive card as reactor animals in an infected herd. Five States would routinely classify these animals as suspects. The other 16 States indicated flexibility in classification.

Answers on the other provisions of the Uniform Methods and Rules covered by this questionnaire indicated similar inconsistencies. The net effect of these variations can only delay the elimination of brucella infection and consequently delay the attainment of brucellosis eradication. The progress of a national program depends in large measure upon the acceptance and implementation of uniform procedures in all areas. The responses to this questionnaire indicated that there is a general lack of agreement of minimum standards for a uniform program.

Tabulations of the responses have been furnished to each State for review. At the request of the Committee on Evaluation and Development Programs, each State will be advised of its deviations from the Uniform Methods and Rules and present policy.
AN IN VITRO GROWTH INHIBITION TEST FOR LEPTOSPIRAL NEUTRALIZATION

D. N. Tripathy, L. E. Hanson and W. A. Krumrey
University of Illinois at Urbana, Illinois

Leptospirosis has been recognized as one of the major diseases of cattle and swine in recent years. *Leptospira pomona*, *hardjo* and *grippotyphosa* are the principal serotypes encountered in the United States although *canicola* and *icterohaemorrhagiae* have also been isolated.

In order to minimize the rate of infection especially in areas where foci of infection exist, vaccination of animals is being practiced on experimental basis. Commercial bacterins of several serotypes have been used to a limited scale. The reactor rate of *pomona* has declined in recent years (Hanson *et al.*, 1965; 1969) because of vaccination. There has been difficulty in serologic evaluation of bacterins due to variable antibody responses of animals. Although several tests, e.g., microscopic agglutination (MA), macroscopic agglutination, direct and indirect fluorescent antibody and agar gel precipitation tests are commonly used to detect antibody response, they have not satisfactorily evaluated antibody responses in vaccinated animals. The microscopic agglutination test is most commonly used because of its serotype specificity and higher sensitivity compared to other tests. However, in contrast to a natural infection by a pathogenic serotype or in an experimental infection with live leptospires where the animals develop appreciable MA titer, vaccinated animals usually do not develop concentrations of antibodies measurable by the MA test. Killinger *et al.*, 1970 observed that only two fo 135 animals had a MA titer of 1:100 or higher two months post vaccination with *pomona* bacterins. Under these circumstances it is difficult to evaluate immune status of vaccinated animals except by direct challenge of the animals or by hamster protection test. Direct challenge is often unsatisfactory as laboratory cultures cause inconsistent clinical responses. Hamster protection test can be used only for those serotypes, e.g., *grippotyphosa* and *pomona*, which are pathogenic for this species but not for *hardjo* which infects them without causing consistent death patterns. The present investigation was conducted to evaluate the neutralizing antibody response of vaccinated animals with a growth inhibition test.

MATERIALS AND METHODS
(a) *Bacterins.* Coded commercial *pomona* bacterins supplied by Veterinary Biologics Division of ARS-USDA were used in vaccination of cattle. An

The authors would like to thank Mrs. Rachel Marlowe for performing the MA test and Dr. A. H. Killinger through whose courtesy adjuvants were obtained from the Ft. Dodge Laboratories.

This study was supported in part by the Veterinary Biologics Division of the Agricultural Research Service, USDA Contract Nos. 12-14-140-2096-94 and 12-14-140-1918-94 and by the University of Illinois Agricultural Experiment Station Federal Hatch Grant 70-302.
TEST FOR LEPSOTPIRAL NEUTRALIZATION

Experimental hardjo bacterin used in vaccination of cattle was supplied by Affiliated Laboratories, a Division of Rohm and Hass Company, White Hall, Illinois. Bacterins used in rabbit experiments were prepared in our laboratory using liquid bovine albumin polysorbate 80 medium (Ellinghausen and McCullough, 1965). The cultures were grown for 8-12 days and then killed with formaldehyde.

(b) Serums. Cattle serums were obtained from vaccinated calves maintained at Dixon Springs Agricultural Research Center. Rabbit sera were obtained at weekly intervals after vaccination. All sera were tested for MA antibody response. For growth inhibition (GI) test all sera were inactivated at 56º C for 30 minutes.

(c) Growth Inhibition Test. In preliminary studies to each of the five tubes containing 5 ml of liquid bovine albumin polysorbate 80 medium 0.25 ml of inactivated serum was added. For each serum sample five tubes of medium were used. To each tube was then added 0.1 ml of diluted culture of actively growing culture of leptospires, usually 7 to 10 days old. Leptospires were counted in a Petroff-Hausser bacteria counter (Arthur H. Thomas Company, Philadelphia, Pa.) and appropriate dilutions were made in liquid medium. Various concentrations of organisms were tried in earlier experiments. However, later each of the five tubes contained 2.5 ml of liquid medium to which 0.1 ml of inactivated serum and 0.1 ml of liquid culture containing approximately 1 million organisms were added. For control serums obtained before inoculation and liquid medium without serum were included. The inoculated tubes were incubated at 30º C and were examined for presence or absence of growth under direct light for turbidity and also under the darkfield microscope at weekly intervals for two weeks. If at two weeks tubes negative for turbidity showed less than 10 organisms per field under darkfield at magnification of 120X, the tubes were considered positive for antibody while tubes showing more than 10 organisms at this magnification were considered negative for antibody.

Experiment I. In this study sera from 50 cattle were examined for growth inhibition activity. All animals had been vaccinated with pomona and 25 were vaccinated with hardjo bacterin twice at one month’s interval. To each of the five tubes of liquid medium 0.25 ml of inactivated serum, and 0.1 ml of hardjo culture containing approximately 1.2 X 10^5 organisms were added.

Experiment II. In this study sera obtained at various periods from eight cattle were used. Six of these animals were selected from groups of animals that had been vaccinated with pomona bacterin and two from control group. Two animals were vaccinated once with pomona bacterin; two animals were vaccinated twice at one month’s interval with pomona bacterin and two animals were vaccinated at two month’s interval with pomona bacterin. Five tubes of media were used for each serum and 0.1 ml of inactivated serum and 0.1 ml of pomona culture containing one million organisms were added.

Experiment III. A total of sixteen rabbits was used in this experiment. Their sera were tested prior to vaccination by the MA test against serotypes grippotyphosa, pomona, autumnalis, hardjo, icterohaemorrhagiae, canicola and illini strain 3055. None of the rabbits showed any serological evidence against these serotypes tested. These rabbits were divided in four groups as follows: (a) Four rabbits were inoculated subcutaneously with 0.5 ml of bivalent hardjo and grippotyphosa bacterin with no adjuvant. (b) Four rabbits were inoculated similarly with bivalent hardjo and grippotyphosa
bacterin with "Gel 21" adjuvant.* (c) Four rabbits were inoculated with 
grippotyphosa and hardjo bacterin with O/W3* adjuvant. (d) Four rabbits
were inoculated with a trivalent 
grippotyphosa, hardjo and autumnalis
bacterin with O/W3 adjuvant. Serums collected before and at weekly intervals
after vaccination were tested for MA antibody against the antigens used for
vaccination. However, for GI test only hardjo culture was used. For the GI
test 0.1 ml of liquid culture of hardjo containing one million organisms and
0.1 ml of inactivated serum were added to each of the five tubes of 2.5 ml of
liquid medium.

RESULTS

In Experiment I serums from 21 out of the 25 hardjo vaccinates showed growth
inhibition when tests were read after 2 weeks incubation of the tubes (Table I).
Growth inhibition was also observed in 11 of 25 sera from non-vaccinated controls.
None of these sera from either vaccinated or control animals had detectable MA
antibody.

In Experiment II five of six vaccinated animals had evidence of complete or
partial growth inhibition one month after vaccination with pomona bacterin (Table
II) while only three animals had complete or incomplete MA antibody titer at this
time. At two months after primary vaccination, sera from all animals completely or
partially inhibited growth of pomona but only two animals had complete or
imcomplete MA titers. Growth inhibition was detectable at four months in the sera
of five of six animals while only one animal had incomplete MA titer. None of the
sera oof the vaccinates had a detectable MA titer at six months while three animal
sera inhibited growth. At eight months after primary vaccination the sera from two
animals completely or partially inhibited growth of pomona culture.

The results of Experiment III showed only two of 16 vaccinated rabbits
inoculated with experimental bacterins (Table III) had MA titers of 1:100 against
hardjo at one week after vaccination while sera from 13 vaccinated animals had
evidence of complete or partial growth inhibition. At two weeks after vaccination,
hardjo growth was inhibited completely or partially by sera from all vaccinated
animals. Growth inhibiting activity was detectable in sera of all animals at three,
four and five weeks after vaccination. Complete or partial growth inhibition was
detectable in nine of 13 sera at six weeks after vaccination and in three of seven
sera tested at seven weeks after vaccination. The maximum number of animals that
showed an incomplete or complete MA titer at 1:100 or higher at any time during
this observation period was three.

DISCUSSION

It is apparent that animals vaccinated with leptospiral bacterins usually develop
poor MA antibody response because of lack of multiplying antigen to provide an
adequate antigenic stimulus for the production of IgM antibody which is primarily
responsible for the MA reaction. Low levels of IgM antibody produced following
vaccination may not be detectable by MA test or only for short intervals. Further
with the high concentration of antigen used in the MA test, concentration of

* Supplied by Difco Laboratories.
antibody molecules may not be enough to cause the agglutination of antigen at a 50% level. We have not yet determined in this study the class of antibody which is reacting in growth inhibition test. Studies of Negi et al. (1971) indicate that IgG antibody is present in sera of some cattle for a period of one year after vaccination with leptospiral bacterins. In the present study, sera of some cattle collected as long as eight months after primary vaccination produced growth inhibition when MA titers were negative. It may be the dilution of the antigen in the GI test provides a more appropriate concentration to combine with a low dilution of antibody in the serum. Also a longer incubation period allows a greater opportunity for the antibody to react with antigen resulting in the detection of low levels of antibody in sera which are not detected by the MA test.

The GI test is a relatively simple, economical procedure which can be used for all serotypes tested. This study indicates it can be used with the hardjo serotype for which protection test cannot be applied.

Growth inhibition activity by some control animal sera in Experiment I may either have been due to presence of natural antibody or low levels of antibody resulting from inapparent infection which were not detected by the MA test.

SUMMARY

A growth inhibition (GI) test was conducted to evaluate the antibody response of cattle and rabbits vaccinated with pomona and hardjo bacterins. A known amount of inactivated serum and a known concentration of live antigen added to bovine albumin polysorbate 80 medium was incubated and growth inhibiting activity was determined at two weeks after incubation. GI antibody was detectable in sera of vaccinated animals for a longer period than MA antibody and also in some sera which were negative in the MA test.

REFERENCES


TABLE I
GROWTH INHIBITION RESPONSE OF CATTLE SERA AGAINST HARDJO CULTURE.
THE CATTLE WERE VACCINATED WITH POMONA AND HARDJO BACTERIN* AND
SERA COLLECTED 5 MONTHS AFTER PRIMARY VACCINATION

<table>
<thead>
<tr>
<th>TIME WHEN TESTS,</th>
<th>VACCINATED</th>
<th>POSITIVE</th>
<th>CONTROL</th>
<th>POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WERE READ FOR GI</td>
<td>POSITIVE FOR</td>
<td>FOR MA</td>
<td>GI ANTIBODY</td>
<td>TITER</td>
</tr>
<tr>
<td>AFTER INCUBATION</td>
<td>MA</td>
<td>TITER</td>
<td>TITER</td>
<td>TITER</td>
</tr>
<tr>
<td>Two weeks</td>
<td>21/25</td>
<td>0/25</td>
<td>11/25</td>
<td>0/25</td>
</tr>
</tbody>
</table>

Numerator shows serums showing positive GI antibody response; denominator shows serums tested.

*POMONA BACTERIN SINGLE INOCULATION. HARDJO BACTERIN - 2 INOCULATIONS AT ONE MONTH'S INTERVAL.

TABLE II
GROWTH INHIBITION RESPONSE OF POMONA CULTURE WITH
SERUMS FROM POMONA VACCINATED CATTLE

<table>
<thead>
<tr>
<th>ANIMAL NO.</th>
<th>TREATMENT</th>
<th>BEFORE VACCINATION</th>
<th>1 MONTH</th>
<th>2 MONTHS</th>
<th>4 MONTHS</th>
<th>5 MONTHS</th>
<th>8 MONTHS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GI</td>
<td>MA</td>
<td>GI</td>
<td>MA</td>
<td>GI</td>
<td>MA</td>
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</tr>
<tr>
<td>508</td>
<td>Vaccinated once</td>
<td>N N 100 + I + N + N + N ±</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>573</td>
<td></td>
<td>N N N + N + N N N N N N</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>510</td>
<td>Vaccinated twice at monthly intervals</td>
<td>N N N ± N + N + N N N N N</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>588</td>
<td></td>
<td>N N 100 + 100 + N + N + N +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>524</td>
<td>Vaccinated twice at two months interval</td>
<td>N N I N N + I ± N N N N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800</td>
<td></td>
<td>N N N ± N ± N + N + N N</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>512</td>
<td>Control</td>
<td>N N N H N N N N N N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>523</td>
<td></td>
<td>N N N N N N N N N N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GI Test: + = Positive for growth inhibition; ± = Partial growth inhibition where some tubes showed evidence of growth inhibition while some did not; N = Negative for growth inhibition. Growth inhibition results were read at 2 weeks after incubation of medium tubes.

MA Test: Number under MA refers to reciprocal of MA titer; I = Incomplete for MA at a titer of 1:100; N = Negative for MA at a titer of 1:100.
### TABLE III
GROWTH INHIBITION AND MA ANTIBODY RESPONSE AGAINST HARDJO ANTIGEN OF RABBITS FOLLOWING VACCINATION

**ANTIBODY RESPONSE**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. OF ANIMALS</th>
<th>VACCINATION</th>
<th>BEFORE VACCINATION</th>
<th>AFTER VACCINATION AT 1 WEEK</th>
<th>2 WEEKS</th>
<th>3 WEEKS</th>
<th>4 WEEKS</th>
<th>5 WEEKS</th>
<th>6 WEEKS</th>
<th>7 WEEKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4</td>
<td>HARDJO AND GRIPEPO-TYPHOID BACTERIA WITH OIL ADJUVANT</td>
<td>4  4  4  4 N 3+ 4 N 2+ 4 N 4+ 4 N 2+ 3 N 2+ 3 N 2+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>HARDJO AND GRIPEPO-TYPHOID BACTERIA WITH NO ADJUVANT</td>
<td>4  4  4  3 N 1 100 3+ 1 1 2+ 3 N 1 3 3+ 1 1 4+ 1 3+ 3 N 3 3±</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>HARDJO AND GRIPEPO-TYPHOID BACTERIA WITH OIL ADJUVANT</td>
<td>4  4  4  2± 1 3 1± 3 N 1± 1 1 3+ 1 1 3+ 2 N 1 1 D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>HARDJO, GRIPEPO-TYPHOID AND ADJUVANT BACTERIA WITH OIL ADJUVANT</td>
<td>4  4  3 N 1± 1 100 4+ 1 3 3 N 3 N 4+ 4 N 4+ 1 1 4+ 4 N 2+ 4 N 3 N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*OIL = OIL ADJUVANT, MA TST - N = NEGATIVE AT 1:100 DILUTION, I = INCOMPLETE FOR MA AT A TITER OF 1:100, D = DIED.

*GIF TST - + = POSITIVE FOR GROWTH INHIBITION; ± = WHERE GROWTH WAS PARTIALLY INHIBITED, I.E., SOME TUBES SHOWED GROWTH AND SOME DID NOT.

*N = NEGATIVE FOR GROWTH INHIBITION, D = DIED.
LEPTOSPIROSIS IN CATTLE AND SWINE IN MINNESOTA

J. W. Glosser¹, S. L. Diesch², J. M. Higbee³ and D. Hasz⁴

INTRODUCTION

Cattle leptospirosis in North America was first diagnosed based on histologic findings by Jungherr in 1943¹. In 1948, a spirochete subsequently isolated as serotype pomona was isolated from sick cattle². The disease in swine was first recognized in Alabama in 1950, and the first isolation from swine was achieved in 1952³, ⁴.

In the past, the medical disciplines generally regarded leptospirosis as being caused by one of three serotypes: pomona, canicola, and icterohaemorrhagiae. It was believed that pomona was a problem of cattle and swine, canicola of dogs, and icterohaemorrhagiae of rats. Evidence gathered over the last 10 years has shown this concept of species specific serotypes to be a gross oversimplification of the problem. In the United States, epidemiologic studies have shown at least 11 different serotypes have caused clinical disease in man. Approximately 25 serotypes have been isolated from 31 animal species⁵. These studies have established that serotype specificity does not exist, but that one species may be a reservoir or source of infection for two or more serotypes in the same geographic region at the same time.

The 1969 and 1970 reports of the Leptospirosis Committee of the U.S. Animal Health Association (AHA) noted that the principal serotypes posing a problem in domestic animals were: canicola, grippotyphosa, hardjo, icterohaemorrhagiae, and pomona. The order of importance varies with the species. Their findings were based on extensive serologic evidence and repeated or sporadic isolations of these serotypes. The reports state that the incidence of pomona has decreased in many areas; however, the relative incidence of the other serotypes has increased. The committee believed this could be explained in part by the widespread use of the pomona bacterin. The 1969 report recommended that surveys to establish the prevalence of these serotypes should be initiated by the state diagnostic laboratories.

The purposes of this report are to: (1) present the Leptospiral serology data for fiscal year 1971 (July 1, 1970, through June 30, 1971) in Minnesota and (2) discuss the clinical, diagnostic, and epidemiologic features of four outbreaks in
which laboratory confirmation of leptospirosis was achieved.

SEROLOGIC STUDIES

Serologic evidence has indicated that leptospirosis due to serotype *pomona* existed in Minnesota’s cattle and swine populations for many years. The State Veterinary Diagnostic Laboratories have offered serodiagnostic services since 1956. Initially, the service consisted of screening cattle and swine serum with a *pomona* plate antigen. The serum of any animal with a history of abortion was also screened against *canicola* and *icterohaemorrhagiae* with the microscopic agglutination (MA) test. Since 1968, in addition to the *pomona* plate test, a pooled plate test antigen containing *autumnalis*, *pomona*, and *sejroe* came into use.

Early in 1970, the MA test replaced the above procedures as the routine screening test. Test antigens routinely employed were live cultures of the five serotypes as recommended by the AHA Committee on Leptospirosis. Stock cultures were obtained from the Leptospirosis Unit, Center for Disease Control, Atlanta, Georgia. These stock cultures were maintained on semisolid albumin polysorbate media and transferred every two to four months. Antigen production was accomplished by use of liquid albumin polysorbate medium, with transfer every 5 days. Five-to-6-day-old cultures were standardized to yield a 30 nephelometric reading (approximately $2.0 \times 10^8$ cells/ml); they were then tested against fourfold dilutions of homologous rabbit antiserum. Any antigen varying more than one tube in dilution from the established end-point titer of the antiserum was discarded. Known negative and positive serum samples were run with each day’s tests as controls. Dilutions were made to represent 10-fold dilutions after antigen addition. The sera were screened at the 1:100 dilution. Serial 10-fold dilutions were made on any serum reacting at the 1:100 dilution to determine the end-point reaction. The end-point titer was regarded as that dilution of serum in which 50 percent or more of the leptospires were agglutinated.

The annual prevalence of leptospiral antibodies for cattle and swine in Minnesota during the 11-year period (Fiscal years 1961-1971 inclusive) are shown in Figure 1. The median prevalence for cattle was 7.7 percent with a range of 2.7-14.2 percent. During this same period, the median prevalence noted for swine was 6.3 percent with a range of 1.1 to 11.2 percent. The rates prior to fiscal year 1971 do not include any positive reactions to serotypes *grippotyphosa* or *hardjo* as these were not included in the test battery. In addition, the number of sera reacting to *canicola* and *icterohaemorrhagiae* were not available because seropositivity rates were not reported by serotype. However, the majority of positives probably reacted to *pomona* since this was the only serotype reported to be a problem.

During fiscal year 1971, 5,404 cattle sera were submitted for testing; 679 (12.6 percent) were positive at a titer of 1:100 or greater. Of the 1,522 swine sera, 96 (6.3 percent) were positive. It should be recognized that these sera do not reflect the true prevalence of leptospiral antibodies in cattle and swine since they were submitted for a specific purpose, namely: testing for sales, interstate shipment, exhibition, and more importantly because the referring veterinarian observed or suspected a disease condition. Therefore, these sera represent a selected population.

A more meaningful interpretation of the data can be made if cattle and swine sera which were submitted to the laboratory for routine testing are regarded as controls. “Routine testing” is done on animals destined for interstate shipment,
sale, exhibition, etc. The balance can be classified as diagnostic sera from diseased animals. The diagnostic sera were subdivided into groups with known and unknown or unstated herd history on the submission records to compare the seropositivity rates. The rates obtained subsequent to these adjustments are shown in Tables I and II. For cattle, the seropositivity rate for *pomona* was significantly higher (p > 0.001) in both subgroups of diagnostic sera than in the control sera (Table I). Differences in seropositivity rates for the other serotypes were not significant. Significant differences for *pomona*, *icterohaemorrhagiae*, *grippotyphosa*, and the total rates were also noted for swine (p > 0.001). Positives for *hardjo* and *canicola* were not observed (Table II).

The prevalence of leptospiral infection by herd was determined from the test charts where herd history of abortion was given. In cattle, 133 of 591 (22.5 percent) herds had positive leptospiral serologic tests. In swine, 16 of 63 (19 percent) herds were serologically positive.

**REPORT OF FOUR OUTBREAKS**

Positivity in a single serum sample is only presumptive evidence of infection. Leptospirosis is considered confirmed when one or more of the following criteria are met: isolation of the leptospire, demonstration of a fourfold rise or fall in titer, or demonstration of seroconversion. Confirmation of leptospirosis due to serotype *pomona* was achieved on four premises, once serologically and three times by isolation of the leptospire. Cultures of the three isolates reacted with homologous *pomona* antiserum at high dilutions, thus provisionally placing them in the *pomona* serogroup. These isolates have been definitively identified at CDC as belonging to this serogroup, and final serotyping is now in progress. To the best of our knowledge, these isolations represent the first leptospirae to be recovered from domestic animals in Minnesota. *Hardjo* and *grippotyphosa* infections in cattle were also confirmed by serologic evidence in paired sera. Four outbreaks were recognized as a result of these confirmations.

**OUTBREAK I:**

During the summer of 1970, five dairy cows from a herd of approximately 30 adults aborted in their third trimester. No other clinical signs were observed. On the day of the fifth abortion, a 7-month fetus and serum from the aborting cows were submitted to the laboratory.

The aborting cows were seropositive for *pomona*. Two cows had titers of 1:10,000; the other three, including the dam of the submitted fetus, had titers of 1:100,000. *Pomona* was isolated from the fetal kidney despite the fact that leptospirae were not seen in Levaditi's stained slides of fetal kidney. The isolation methodology employed serves as an example of the difficulties and complexities encountered with isolation techniques. One ml of a 10 percent kidney suspension was inoculated into each of the three young guinea pigs each weighing approximately 250 grams. In addition, one drop of 10-fold serial dilutions of the kidney suspension was inoculated into four tubes of media. Two tubes of media were Fletcher's semisolid medium, one of which contained approximately 200 micrograms of 5-fluorouracil to minimize bacterial contamination. The remaining tubes were bovine albumin polysorbate semisolid medium, one containing the same concentration of 5-fluorouracil as stated previously. A small amount of each
LEPTOSPIROSIS IN CATTLE AND SWINE

Culture was checked by darkfield microscopy at weekly intervals for 10 weeks for the presence of leptospires, with negative results. Blood was drawn by cardiac puncture from the guinea pigs 3 days post-inoculation. One drop of blood was inoculated into each of the four tubes of medium previously described. Leptospiremia was proven in two of the guinea pigs when leptospires were seen in all of the cultures 42 days post-inoculation. Leptospires were also isolated from the kidneys of both guinea pigs when they were sacrificed on the 30th day post-inoculation. It took 45 days from the occurrence of abortion to isolate leptospires, whereas the serologic results were available in 1 day.

Upon receipt of the serologic results, the entire herd was vaccinated with a pomona bacterin. No abortions occurred after vaccination. After a leptospirosis outbreak about 10 years before, the owner had vaccinated his herd for 4-5 years, but he then discontinued this practice.

OUTBREAK II:

An explosive and rather extensive epizootic of cattle leptospirosis due to pomona occurred in the fall of 1970. Within a 3-week period, signs compatible with leptospirosis were observed on 11 premises in an approximately 36-square-mile area. The clinical signs included hematuria, hemoglobinuria, flaccid mastitis, and numerous abortions. Retained placentas were noted in a few instances. Calves and heifers tended to be more severely affected. Some of the involved premises were dairy farms, whereas others had both dairy and beef cattle. Although the clinical history was most accurate for dairy cattle, abortions were reported in one beef herd.

The index case in this epizootic was a 2-year-old Holstein heifer that aborted at 8 months. An acute serum specimen from this animal had a 1:1000 titer for pomona. Convalescent serum drawn 15 days later had a titer of 1:100,000. Investigations of this case resulted in the detection of leptospiral infections in four other herds in the same general area.

Whole herds were tested on two premises. In the first, 25 of 59 (41 percent) cattle had positive titers; of these, 21 (84 percent) had titers of 1:100,000. An acute and a convalescent serum was collected from three cows. Serologic confirmation resulted in each case. Shedding of leptospires in urine was demonstrated on the premises. Leptospires were isolated from urine of two of 15 (13.3 percent) cows sampled. Serum from these two animals contained antibodies at titers of 1:1000 and 1:100,000. The inoculation of cultures with urine was performed by the procedure described earlier. Leptospires in the cultures were first seen on the 21st day of incubation.

On the second farm, 22 of the 46 (48 percent) cows had positive titers; 7 of 22 (32 percent) seropositives had titers of 1:100,000. Paired sera taken from one of the cows in this herd drawn 6 days apart had a stable titer of 1:100,000. Three horses and the farm dog were also tested. The horses were negative, and the dog had a titer of 1:100 against pomona. Urine was collected from five cows and cultured, with negative results.

There were two important epidemiologic features of the outbreak: (1) abortions started approximately 3 to 4 weeks after the end of a rainy period, and (2) abortions occurred until 1 to 2 weeks after the herds had been vaccinated with pomona bacterin. Leptospirosis had been a problem on some of these premises
before. The owners stated they had given pomona vaccination annually for a few years and then stopped. The true extent of the outbreak was not defined, however, clinical signs and abortions were reported in neighboring areas, and these may have been due to leptospirosis.

OUTBREAK III:

Cattle leptospirosis due to serotype hardjo was diagnosed in the winter of 1971. The index case was a cow that had aborted with a titer of 1:1000 in late December 1970. Abortions, stillbirths, and weak newborn calves were observed in this herd for the next 2 months. In late February, an additional 20 cows were tested, and four had significant titers to hardjo. The histories of these 20 animals were as follows: 4 calves died shortly after birth, 4 abortions, 4 repeat breeders, 3 stillbirths, and 5 anestrus. The problem continued, and therefore additional animals were bled in April and June. As of July 1, 104 of 242 (43 percent) have had positive titers, of these 23 (22 percent) had titers of 1:10,000 or greater. Serologic confirmation was obtained in six animals where paired sera were available. Attempted isolation of hardjo by culture of the urine from 13 animals was unsuccessful. Significant titers to hardjo were obtained in 3 of 15 (20 percent) on another premise with a history of abortion and from single serum submissions from three different premises within this same county.

OUTBREAK IV:

In February 1971, an outbreak of leptospirosis in swine was discovered. The outbreak manifested itself primarily as an abortion problem as 22 of 40 (55 percent) sows aborted. No other clinical signs were noted. Eleven serum samples and two fetuses were submitted to the laboratory. All sera reacted against pomona; seven of 11 (64 percent) had titers of 1:100,000. Hamsters and media were inoculated with kidney suspensions from the fetuses. No leptospires were isolated; however, a hamster died 25 days post-inoculation. The same fetal kidney suspension produced seroconversion to pomona in the surviving hamsters, which were sacrificed at day 30. Leptospires were isolated from the kidneys of all hamsters inoculated with this specimen.

DISCUSSION

Figure 1 indicates that the prevalence of leptospiral antibodies in cattle and swine declined steadily between 1962 and 1969 and then rose in the last 2 years. It is impossible to know whether this increase is real or the result of increased surveillance in 1971, because the serologic methodology used in these periods was not comparable. Statistically significant differences were noted between diagnostic sera and routine sera in both cattle and swine. It is also significant that approximately 20 percent of the cows which were described by veterinarians as having a history of abortion or stillbirth had antibodies to leptospira. Another interesting aspect of these data is the incidence of antibodies against icterohaemorrhagiae in swine herds with a history of abortions, stillbirths, or weak newborn pigs. Isolation of this serotype from swine was reported recently in Illinois10.

Some of the pitfalls and limitations encountered in diagnosing leptospirosis were mentioned. The length of time required to isolate leptospires demonstrates that
routine isolation attempts with the present techniques have little practical value in rendering a rapid diagnosis to the veterinarian. Despite these limitations, the MA test when coupled with clinical history can result in a reliable diagnosis. In Minnesota, it is desirable to substantiate the MA test results with clinical disease for serotypes other than *pomona* by isolation of the infecting serotype. The most efficient way to obtain this information would be to select animals in which the chances of achieving an isolation are optimal. The method of choice is random urine collection from animals in a herd where positive serology is detected. Random collection is desirable because leptospires may be intermittently shed. Also, the shedding of leptospires from animals not having a titer has been reported. Another practical approach is the collection of kidneys from seropositive animals shipped for slaughter. If an animal is showing clinical signs other than abortion, such as hematuria and icterus, a citrated sterile blood sample should be taken and forwarded to the laboratory for attempted isolation.

In Minnesota, the MA test should prove to be a reliable tool. While substantiation of positive serology by leptospiral isolation for serotypes other than *pomona* has yet to be achieved within the state, this has been demonstrated repeatedly wherever the test is used routinely. Moreover, it is a technique where good presumptive evidence of leptospirosis can be reported promptly to the practicing veterinarian. The MA test is considered by many to be a cumbersome and time-consuming test to perform, but it was our experience that under most circumstances when a serum sample was received at the laboratory in the morning, the results were reported that afternoon.

The data obtained in fiscal year 1971 indicate that serotypes other than *pomona* occurred in Minnesota. Serotypes *grippotyphosa* and *hardjo* have not yet been isolated in Minnesota, although their presence is known based on serologic confirmation. Solid evidence exists for the presence of these serotypes in the midwestern states. In Illinois, Hanson and others reported that the seropositivity rates for *pomona* had declined from 20.0 in 1955 to 5.3 in 1965; however, in 1965, rates of 17.6 and 7.0 were observed for *hardjo* and *grippotyphosa*, respectively. Wisconsin reported seropositivity rates in the period 1965-1967 to be, in order of importance for cattle, *hardjo*, *icterohaemorrhagiae*, *pomona*, and *grippotyphosa*, and for swine, *icterohaemorrhagiae, pomona*, and *grippotyphosa*. In Iowa, Diesch and associates demonstrated that *hardjo* and *grippotyphosa* are prevalent in cattle. Although *pomona* had a seropositivity rate of 13.2 in one township, rates of 9.9 and 4.2 were also demonstrated for *hardjo* and *grippotyphosa*, respectively. The most impressive aspect of these data is that through intensive surveillance, the overall rate of 30.5 was observed. This reinforces the observation that the majority of leptosporal infections of cattle and swine are subclinical and would go unnoticed without adequate surveillance. The relative importance of individual leptospiral serotypes as causes of disease and economic loss in domestic animal populations can be determined only by such a sustained surveillance system.

Surveillance encompasses three vital functions: systematic collection of clinical and diagnostic data, comprehensive analysis of the collected data, and prompt feedback to those who need to know the results—the practicing veterinarians, and veterinarians in disease control agencies for utilization.

Lastly, efforts should be directed by all responsible at the state and national level toward an improved case reporting system of animal leptospirosis. Cases
reported to the Animal Health Division, United States Department of Agriculture (ANH, USDA), by the states should be listed wherever possible in two categories: first, with diagnosis based on clinical criteria only, and second, those with laboratory confirmation. For example, the ANH animal leptospirosis report for calendar year 1970 indicates that there were 13,102 cattle cases and 3,124 swine cases in the United States. The report shows that Minnesota reported 17 cattle cases and three swine cases, despite the fact that serologic evidence of infection was present in at least 63 cattle from herds where laboratory confirmation of cases was established. There were no laboratory confirmed swine cases in 1970. Accurate reporting would prove invaluable in prompting better national surveillance from which estimates as to the magnitude of leptospirosis in domestic animals could be made.

With the cooperation of all concerned, leptospirosis, although not amenable to eradication, can be controlled and its affect on the domestic animal population reduced.

SUMMARY

In fiscal year 1971, all cattle and swine sera submitted to the Minnesota State Veterinary Diagnostic Laboratories for leptospiral serology were tested with the microscopic agglutination (MA) test for the detection of antibodies against serotypes canicola, grippotyphosa, hardjo, icterohaemorrhagiae, and pomona. The seropositivity rate for all serotypes was 12.6 percent for cattle and 6.3 percent for swine. However, rates of 19.0 percent for cattle and 21.6 percent for swine resulted when only the sera submitted because of a herd health problem (abortions, stillbirths, etc.) were considered. These rates were significantly different when compared with cattle and swine sera routinely before interstate shipment, sale, exhibition, etc. (p > 0.001).

Confirmation of leptospirosis due to serotype pomona in cattle was achieved on three premises, once serologically and twice by isolation of the leptospire. Hardjo and grippotyphosa infections in cattle were also confirmed by serologic evidence in paired sera. Pomona was also isolated once in swine. Three outbreaks in cattle and one in swine were recognized because of these confirmations. The clinical history for pomona infections in cattle consisted of abortions, retained placentas, mastitis, hematuria, and hemoglobinurina. The hardjo outbreak in cattle presented a clinical picture of only abortions, stillbirths, and deaths of newborn calves. In the swine, the outbreak consisted on only an abortion problem.

Serotypes other than pomona occur in Minnesota. Whether they pose a serious threat to cattle and swine can be determined only by continuing surveillance.
TABLE I

Prevalence of Leptospiral Antibodies in Cattle by Presumptive Infecting Serotype - Minnesota - Fiscal Year 1971

<table>
<thead>
<tr>
<th>Serotype*</th>
<th>Diagnostic Sera$^1$</th>
<th>Routine Sera$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abortion &amp; Stillbirth</td>
<td>Unknown History</td>
</tr>
<tr>
<td><em>pomona</em></td>
<td>148/1243 (11.9)+</td>
<td>231/2793 (8.3)+</td>
</tr>
<tr>
<td><em>hardjo</em></td>
<td>65/1243  (5.2)-</td>
<td>98/2793  (3.5)-</td>
</tr>
<tr>
<td><em>grippotyphosa</em></td>
<td>19/1243  (1.5)-</td>
<td>38/2793  (1.4)-</td>
</tr>
<tr>
<td><em>icterohaemorrhagiae</em></td>
<td>3/1243  (0.3)-</td>
<td>10/2793  (0.4)-</td>
</tr>
<tr>
<td><em>canicola</em></td>
<td>1/1243  (0.1)-</td>
<td>5/2793  (0.2)-</td>
</tr>
<tr>
<td>Total</td>
<td>236/1243 (19.0)+</td>
<td>382/2793 (13.8)+</td>
</tr>
</tbody>
</table>

$^*$Only serotypes tested with Microscopic Agglutination Test

$^+$Number positive sera with a titer of 1:100/total sera submitted in this category (percent)

1Sera submitted due to a herd health problem and unstated history

2Sera submitted from healthy animals to meet requirements for interstate

+Statistically significant difference (p>.001)

- No statistically significant difference
TABLE II

Prevalence of Leptospiral Antibodies in Swine by Presumptive Infecting Serotype - Minnesota - July 1, 1970 - June 20, 1971

<table>
<thead>
<tr>
<th>Serotype*</th>
<th>Diagnostic Sera&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Routine Sera&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abortion &amp; Abortion &amp;</td>
<td>Abortion &amp; Abortion &amp;</td>
</tr>
<tr>
<td></td>
<td>Stillbirth</td>
<td>Stillbirth</td>
</tr>
<tr>
<td><em>pomona</em></td>
<td>27/176**(15.3)+</td>
<td>32/931 (3.4)+</td>
</tr>
<tr>
<td>icterus-</td>
<td>10/176 (5.7)+</td>
<td>18/931 (1.9)+</td>
</tr>
<tr>
<td><em>haemorrhagiae</em></td>
<td></td>
<td>1/415 (0.2)</td>
</tr>
<tr>
<td><em>grippotyphosa</em></td>
<td>1/176 (0.6)+</td>
<td>7/931 (0.8)+</td>
</tr>
<tr>
<td>Total</td>
<td>38/176 (21.6)+</td>
<td>57/931 (6.1)+</td>
</tr>
</tbody>
</table>

<sup>*Serotypes canicola and hardjo were also tested with the MA test</sup>  
<sup>**Number positive sera at a titer of 1:100 or greater/sera submitted (percent)</sup>  
<sup>1Sera submitted due to a herd health problem or unknown history</sup>  
<sup>2Sera submitted from healthy animals to meet requirements for interstate shipment, sales, exhibition, etc.</sup>  
<sup>+Statistically significant difference (p>.001)</sup>  
<sup>-No statistically significant difference</sup>
Figure 1 LEPTOSPIRA SERO-POSITIVITY RATES IN CATTLE AND SWINE, MINNESOTA, FISCAL YEAR 1961-1971

- CATTLE 101,162
- SWINE 32,334
REFERENCES

Chemotherapy of Renal Leptospirosis:  
*Leptospira grippotyphosa* and *Leptospira hardjo*  
in Hamsters and Swine  

O. H. V. Stalheim, D.V.M., Ph.D.  

INTRODUCTION  
Urinary transmission is the principal means for disseminating leptospires among animals and to man. A search for a cheap, safe, and effective treatment for renal leptospirosis, led to recommendations for the use of a single dose of dihydrostreptomycin (DSM), 25 mg/kg of body weight. This recommendation was developed for cattle and swine infected with *Leptospira pomona*, the first leptospire to be recognized in this country as an important cause of economic loss in cattle and swine. However, additional leptospires are now recognized as being economically important in cattle and swine. Although differences in sensitivity to DSM were not found during *in vitro* studies with 12 leptospiral serotypes, the possibility existed that *in vivo* studies might reveal unsuspected difficulties in eliminating renal infections with other leptospires. The apprehension was not supported by the results of the present studies using *Leptospira grippotyphosa* and *Leptospira hardjo* in hamsters and swine.

MATERIALS AND METHODS  
Cultures of *L. grippotyphosa* and *L. hardjo*, grown in medium supplemented with bovine albumin, were given to hamsters and swine as previously described. Infected animals were treated with DSM (25 mg./kg. of body weight) 18 to 22 days after exposure to leptospires. Swine were given a single injection; hamsters were treated for 3 consecutive days. Urine samples were collected from swine and tested for leptospires by microbiological cultural procedures and hamster-inoculation techniques for 18 to 24 days after chemotherapy, when the animals were killed and examined for evidence of renal leptospirosis by cultural and hamster-inoculation techniques. Serum samples were tested for agglutinins by the microscopic agglutination test.
After 15 swine were exposed to *L. hardjo*, leptospiruria was not detected and none of the pigs had renal leptospirosis at the time of necropsy. Therefore, another culture of *L. hardjo* was obtained† and passed twice through hamsters. Cultures grown from the infected kidneys of hamsters were given to 5 swine. Each pig received approximately $1 \times 10^9$ viable, renaltropic *L. hardjo* on each of 2 consecutive days.

RESULTS

After exposure to *L. grippotyphosa*, the clinical signs in hamsters and swine were slight. These signs included mild fevers of up to 40.3°C for 1 day in 8 of 14 swine with moderate inappetence and lassitude.

All pigs exposed to *L. grippotyphosa* were leptospiruric by post-exposure day 20. After treatment with a single dose of DSM, leptospiruria ceased and *L. grippotyphosa* was not isolated from renal tissues at the time of necropsy. Serum samples prepared at the time of necropsy agglutinated *L. grippotyphosa*. The mean titer was 1,810 and 1,000 for treated and nontreated (control) pigs, respectively. After treatment with 3 doses of DSM, leptospires were not isolated from the kidneys of 10 hamsters (Table 1).

Dihydrostreptomycin was also effective for the elimination of renal leptospirosis due to *L. hardjo*, when given to hamsters for 3 consecutive days. Leptospires were not isolated from the kidneys of treated hamsters whereas nontreated (control) hamsters harbored *L. hardjo* (Table 1).

Although 2 different strains of *L. hardjo* were given to 20 swine, leptospiruria was not detected and none of the pigs had renal leptospirosis at the time of necropsy.

DISCUSSION

These limited studies indicate that DSM was as effective against renal leptospirosis due to *L. grippotyphosa* and *L. hardjo* as it was against *L. pomona* infections. *One treatment eradicated* *L. grippotyphosa* *from swine and 3 doses cured hamsters infected with* *L. grippotyphosa* and *L. hardjo*. Similar studies in cattle are needed because they harbor both *L. grippotyphosa* and *L. hardjo*, and because chemotherapy can be useful in not just individual cattle but also in herds and populations.

Additional studies should also be done with those leptospires which might become established in swine in this country. For example, *Leptospira icterohaemorrhagiae* and *Leptospira canicola* have been isolated from swine; *Leptospira pyrogenes*, *Leptospira australis*, and *Leptospira bataviae* might infect swine since they are maintained among wild animals in this country; and *Leptospira tarassovi* (formerly *Leptospira hyos*) is widespread in South America and is the most common cause of swine leptospirosis in Argentina. Dobson eliminated *L. pomona* from 2 large, commercial swine herds in Australia by injecting DSM (25 mg./kg.) and draining the water holes. He proposed

† Supplied by Dr. H.G. Stoennner, Rocky Mountain Laboratory, Hamilton, Montana.

TABLE I

EXPERIMENTAL DESIGN AND RESULTS OF DIHYDROSTREPTOMYCIN TREATMENT* OF HAMSTERS AND SWINE INFECTED WITH LEPTOSPIRES

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Species</th>
<th>No.</th>
<th>Treated animals</th>
<th>Nontreated animals (controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of doses given</td>
<td>No. of shedders</td>
</tr>
<tr>
<td>L. grippotyphosa</td>
<td>Hamsters</td>
<td>10</td>
<td>3</td>
<td>N.D.</td>
</tr>
<tr>
<td>L. grippotyphosa</td>
<td>Swine</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>L. hardjo</td>
<td>Hamsters</td>
<td>10</td>
<td>3</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

*Dihydrostreptomycin was given at the dose level of 25 mg./kg. of body weight. Eighteen to 24 days later, the animals were killed and the kidneys were tested for the presence of leptospires by cultural and hamster-inoculation techniques.
an “Accreditation system” based on these procedures together with serologic tests on serums from breeding stock.

For purebred swine herds in this country, the following items would be essential to an accreditation system: (1) Blood drawn for the brucellosis validation test should also be tested for leptospirosis using the microscopic agglutination test. Subject to recommendations by the U.S. Animal Health Association, or other agency, tests should be done with 4 leptospiral serotypes as antigen (L. pomona, L. grippotyphosa, L. canicola, and L. icterohaemorrhagiae). (2) If serologic evidence of leptospirosis is found in any breeding stock, (to be defined by U.S. Animal Health Association or other agency) all swine should be treated with DSM except those to be marketed within 30 days. Treated swine should not be sold for breeding stock or exposed to susceptible animals for a minimum of 14 days nor sold for slaughter within 30 days. (3) Swine that are purchased for breeding purposes or return to the herd after possible exposure at sales, fairs, and shows should be treated with DSM. (4) For certain herds, vaccination of breeding stock with L. pomona bacterin may be advisable. (5) Health certificates for swine to be sold should contain adequate information on leptospirosis.

Uniform regulations for the control of leptospirosis in swine have not been developed and little guidance is available to the modern swine breeder who wishes to maintain a leptospirosis-free herd and sell only leptospirosis-free swine. The U.S. Animal Health Association Committee on Leptospirosis stated that, in their opinion, leptospirosis is not amendable to eradication because of the numerous serotypes encountered, the difficulty of detecting carrier animals, and the wide range of domestic and wild animal hosts. This opinion does not mean that leptospirosis should be tolerated in circumstances or herds where eradication is possible, or where eradication might be the most satisfactory means of control.

For many well-managed swine herds, the proposed accreditation system seems to hold considerable promise for the eradication and prevention of ren-entry of leptospirosis. It could thereby contribute to the economy of the industry and reduce the risks of leptospirosis to swine growers and the public.

SUMMARY

Dihydrostreptomycin was given to hamsters and swine for the treatment of renal infections with L. grippotyphosa and L. hardjo at a dose level of 25 mg./kg. of body weight. Eighteen days after 3 daily treatments, L. grippotyphosa was not isolated from the kidneys of 10 artificially infected hamsters; nontreated hamsters (5), however, harbored L. grippotyphosa. After a single dose of DSM was given to 10 carrier/shedder swine, L. grippotyphosa was not recovered from the kidneys of treated swine but was isolated from 3 of 4 nontreated, control swine.

Dihydrostreptomucin eliminated the carrier condition in 10 hamsters artificially infected with L. hardjo but 7 nontreated control hamsters harbored L. hardjo. Renal leptospirosis was not established in swine using 2 strains of L. hardjo.

A proposal for the control of swine leptospirosis by serology, chemotherapy, and sanitation is discussed.
REFERENCES

REPORT OF THE COMMITTEE ON LEPTOSPIROSIS

L. E. Hanson, Co-chairman

The Committee reviewed reports presented which involved serologic surveillance of leptospiral serotype reports, chemotherpay studies, serologic test procedure, vaccination studies, and recommendation for control of leptospirosis.

Serologic surveillance reports available from diagnostic laboratories from various areas of the United States indicate the incidence of serotypes of pomona reactor rates continue to decline wherever pomona bacterins are utilized annually in swine or cattle. Several states indicated pomona infections had reoccurred in herds following discontinuation of vaccination. Reports of serologic tests for other leptospiral serotypes indicated hardjo and grippotyphosa antibodies in cattle sera and grippotyphosa antibodies were detected with less frequency in both cattle and swine. Limited serologic studies in horses indicate leptospiral serotypes are frequently observed, but their significance is not apparent. On the basis of the new information it is suggested that some laboratories investigate the incidence of hyos and autumnales serotype antibodies in cattle sera. Preliminary serologic tests involving moose sera from Minnesota indicated the presence of grippotyphosa and pomona antibodies.

Licensed diagnostic plate antigens for hardjo, grippotyphosa, ictero-haemorrhagiae, canicola, and pomona serotypes are now available. The Committee is grateful to the Biologics Industry and the Biologics Division of Animal and Plant Health Services for the development and licensing of the new antigens. As the result of this development, the Committee recommends that veterinary diagnostic laboratories restrict their leptospiral serologic testing to the use of microscopic agglutination (MA) test or to the use of plate antigens licensed by the Veterinary Biologics Division of the Animal and Plant Health Services to assure adequate uniformity in leptospiral testing. Establishment of designated regional reference laboratories from among current laboratories utilizing the MA test to provide check testing would assure greater uniformity in the testing programs. Authorization for licensing of diagnostic reagents is contained in Title 9 of the Code of Federal regulations.

Chemotherapeutic studies indicated grippotyphosa and hardjo serotypes in hamsters and swine were effectively controlled by streptomycin therapy. The studies indicate dosages of 25 mgm. of streptomycin per kilogram of body weight have wide application in the control of leptospirosis in domestic animals for a variety of serotypes.

Field studies involving experimental hardjo and grippotyphosa bacterins indicate protecting antibodies can be induced which are detectable with hamster protection tests or growth inhibition tests. Also, the bacterins induced protection which prevented clinical signs associated with leptospirosis in cattle and swine and prevented extension of the disease within the vaccinated herds. The experimental vaccine, similar to the commercially available bacterins, did not induce appropriate
levels of agglutinins detectable with the MA test. In view of the widespread distribution of *hardjo* and *grippotyphosa* in cattle and *grippotyphosa* in swine, the Committee urgently recommends that the biologics industry and the Veterinary Biologics Division give high priority to the development and licensing of monovalent and polyvalent leptospiral bacterins which include the five prevalent serotypes.

The Committee reviewed the available knowledge concerning prevention and control of leptospirosis in domestic animals. The application of this information in the development of control procedures is recommended. This includes annual serologic surveillance, treatment of infected animals with antibiotics subject to the limitations of FDA requirements (such as 25 mgm. of streptomycin per kilogram of body weight), annual vaccination with bacterins of the prevalent serotypes, utilization of closed herds or serologic testing of all herd additions and the institution of adequate management control measures to reduce introduction of leptospirosis through contaminated surface waters, or by contact with wildlife and rodents.

The Committee will study leptospiral control programs aimed at the possible certification of herds free of leptospirosis. The goal is the development of a certification program which is initially dependent upon the availability of adequate testing in each state.
OVERVIEW OF THE 1971 TEXAS VENEZUELAN EQUINE ENCEPHALOMYELITIS EPIZOOTIC

Richard O. Spertzel

The etiologic agent of Venezuelan equine encephalomyelitis (VEE) is a member of Casal’s group A arboviruses. There are currently 4 major antigenic subtypes, of which the first is subdivided into 5 minor antigenic variants. The nonepizootic sylvatic subtypes occur endemically throughout major portions of Central and South America and in parts of North America, while major epizootics with variants 1A, 1B, and 1C have occurred in South America. The present epidemic of highly virulent subtype 1B probably had its origin in Ecuador, and was introduced into Guatemala in 1969. From there, the disease spread rapidly through El Salvador and portions of Honduras and Nicaragua. In 1970, VEE reoccurred in Honduras and spread into Costa Rica and Mexico. Although slowed in 1970, VEE continued its inexorable spread toward the United States, and by April, 1971, was occurring near Tampico, Mexico. By early June, equine cases appeared within 35 miles of Brownsville, Texas. The first recognized encephalitic horse in South Texas was sick on 23 June. Vaccination was begun on a voluntary basis on 25 June in a 13-county area of South Texas. After confirmation of VEE by virus isolation on 9 July, vaccination was extended statewide in Texas on 13 July, and to New Mexico, Oklahoma, Arkansas, and Louisiana on 17 July. A fee-basis, Federal supported vaccination program was initiated in these 5 states. On 25 July, the vaccine area was extended to California, Arizona, Mississippi, Alabama, Georgia, and Florida, to create an ocean-to-ocean barrier. More recently, on the recommendations of a tri-agency task force, the vaccine area was extended to 8 more states: South Carolina, North Carolina, Tennessee, Kentucky, Virginia, Maryland, Delaware, New Jersey, and the District of Columbia. Missouri was invited to participate, but declined.

A State and Federal quarantine was established for Texas on 13 July; for Oklahoma, New Mexico, Arkansas, and Louisiana on 19 July; and for Mississippi on 2 August. State quarantines were established in Florida, Georgia, and Alabama. Interestingly, embargoes were placed on United States horses by Canada on 14 July. Several European countries also banned import of United States horses.

In addition to vaccination and quarantine, a mosquito-abatement program, consisting of low-volume aerial spraying of Malathion or Dibrom, was maintained along the coastal counties of Texas until 90% of the equine population was vaccinated.

Although statewide vaccination was not allowed until 13 July, suspect encephalitis cases in horses were reported in counties outside the allowable vaccine area by 10 July. Reported cases of equine illness compatible with encephalitis are shown in Fig. 1 (through 24 July) and Fig. 2 (25 July to 28 August). It should be noted that the epizootic apparently reached its maximum extent by 24 July.

From the U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Md. 21701
some of these counties, however, encephalitis in nonvaccinated horses is still occurring. Counties in which VEE virus was isolated, or specific antibody was detected in sera from nonvaccinated horses, are shown in Fig. 3. In addition, 2 border parishes of Louisiana and 2 border counties of Arkansas also reported an unusually high incidence of suspected cases of equine encephalitis. Cessation of such reports within 10 days after VEE-vaccination leads the author to suspect infection with VEE.

After the 1969 Central American epizootic and the extensive use of the live attenuated VEE vaccine in Central America and Mexico, where it appeared to be both safe and effective, the U.S. Department of Agriculture (USDA) was urged to license the vaccine for export, and for contingency use within the United States. At that time, USDA officials expressed serious reservations on the stability of the vaccine virus in this natural host, and would not authorize production of the vaccine in the United States despite authoritative recommendations to do so.

Vaccine administration to Equidae is characterized by a low, irregular viremia with transient fever in approximately 50% of animals. Unlike man, where 35-40% of vaccinated individuals may show some reaction to the vaccine, only 1% of horses show even a transient reaction consisting of anorexia and depression for 12-24 hours. Although no evidence of reversion to virulence was observed during serial passage of the virus by subcutaneous or intraperitoneal (IP) routes in small laboratory animals, several laboratories attempted horse-to-horse passage of the virus. Five serial passages have been attained in our laboratory and by USDA personnel with no evidence of reversion to virulence.

In addition to these back-passage studies, additional safety studies were conducted in the field. Observation of approximately 22,000 Equidae by USDA and/or U.S. Public Health Service personnel in 5 separate states indicated a reaction rate of less than 1%. These results were consistent with those reported by our laboratory and with empirical observations in Central America. They are in conflict with results of the limited study at another laboratory in which 3 of 6 animals showed severe depression and anorexia. In 1969 and 1970, numerous field observations attested to the efficacy of the vaccine; deaths of nonvaccinated animals were documented in herds where all vaccinees survived. These same observations have been made in Texas. A not-uncommon herd report, from an area with active encephalitic cases, follows:

On 20 July, 38 working horses were vaccinated, but the owner considered it too much trouble to round up the remaining horses. In mid-August, encephalitis and death began to occur in these nonvaccinated animals. A field investigation was made on 31 August. Horses were pastured in 3 noncontiguous areas on the ranch. The 38 vaccinated horses remained healthy, while 3 of 5 unbroken geldings on the same pasture died, and one was sick at the time of investigation. In Pasture A, all 16 colts and 11 of 16 mares died; the other 5 mares were noticeably encephalitic. Similar results were seen for Pasture B.

This striking protection with one-dose immunization is consistent with the high degree of serologic conversion observed in field use of this vaccine. Of 157 paired serum samples collected during the Mexican vaccination campaign in 1970, all with preimmunization titers less than 10, 150 (96%) had HI titers greater than or equal to 20 within 30-45 days postvaccination. With this abundance of field and laboratory information, and the pressure of necessity, a provisional license for
commercial production and sale of live attenuated VEE vaccine was granted to a commercial veterinary biologics firm in July, 1971.

FIGURE 1 — Encephalitis Cases Reported On or Before 24 July 1971.

FIGURE 2 — Encephalitis Cases Reported After 24 July 1971.
OVERVIEW – 1971 TEXAS VEE EPIZOOTIC

FIGURE 3 – Confirmed VEE Cases Reported, 1971

REFERENCES

ISOLATION OF MYCOPLASMA MYCOIDES VAR. CAPRI
FROM GOATS IN THE UNITED STATES

R. J. Yedloutschnig, W. D. Taylor, and A. H. Dardiri

SUMMARY

This report describes four highly pathogenic mycoplasma isolates recovered from goats in different areas of the United States. Serological and biochemical evidence shows they are indistinguishable from Mycoplasma mycoides var. capri isolates from Nigeria, Mexico, and Connecticut. Mycoplasma were cultured from oesophageal pharyngeal (OP) fluids, milk, and urine samples from inoculated goats and sheep as well as from dead foetuses of inoculated dams. Leukopenia was routinely demonstrated prior to death.

INTRODUCTION

Contagious caprine pleuropneumonia (CCPP), a serious economic threat to goat raisers throughout the world, was first identified in North America in Pueblo, Mexico in 1959. The etiological agent, Mycoplasma mycoides var. capri, had been regarded as foreign to the United States until 1966 when it was cultured from a goat in Connecticut. The role of mycoplasmas in infections of goats is not clearly defined. Several other mycoplasmas have been isolated from goats and sheep in the United States and other countries. Five of the several isolates submitted to the Plum Island Animal Disease Laboratory (PIADL), seemed closely related to M. mycoides var. capri. Results of serological, biochemical and infectivity studies involving four of these isolates are reported here.

MATERIALS AND METHODS

Mycoplasma isolates

Each of the 4 isolates came from relatively small goat herds on widely separated premises. Each mycoplasma culture received at the PIADL was assigned an accession (Acc.) number and propagated in a medium which supported vigorous growth. Organisms were then serially subpassaged during initial investigations or aliquots were frozen at -70°C.

The history of each isolate (Table 1) is as follows:

From the Plum Island Animal Disease Laboratory, Veterinary Sciences Research Service, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 848, Greenport, Long Island, 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.

166
TABLE 1. Geographical Distribution of Four Isolates of Mycoplasma Mycoides var. Capri in the United States

<table>
<thead>
<tr>
<th>Acc. No.</th>
<th>Date of isolation</th>
<th>Premise location</th>
<th>Goats infected</th>
<th>Sheep and goats in herd</th>
<th>Isolation site</th>
</tr>
</thead>
<tbody>
<tr>
<td>169</td>
<td>Aug. 1967</td>
<td>Laurel, Maryland</td>
<td>1</td>
<td>35</td>
<td>Cervical abcess</td>
</tr>
<tr>
<td>208</td>
<td>Aug. 1968</td>
<td>Fallston, Maryland</td>
<td>1</td>
<td>12</td>
<td>Synovia</td>
</tr>
<tr>
<td>212</td>
<td>Oct. 1968</td>
<td>Mifflinburg, Pennsylvania</td>
<td>2</td>
<td>9</td>
<td>Synovia and lung</td>
</tr>
<tr>
<td>222</td>
<td>May, 1969</td>
<td>Otisville, New York</td>
<td>5-10</td>
<td>80</td>
<td>Keratitis</td>
</tr>
</tbody>
</table>

Acc. 212. – Mifflinburg, Pennsylvania.

Two young goats, purchased about 3 months previously at a local auction, were added to a herd of 5 adults and 2 kids. The 2 new kids showed lameness, a stiff gait, and a temperature of about 102.8° F. for 10 days. Broad spectrum antibiotic therapy produced no favorable response. Necropsy revealed consolidation of the apical, cardiac, and diaphragmatic lobes of the lung. The Bureau of Animal Industry Laboratory in Harrisburg, Pennsylvania isolated mycoplasma from lung and heart sections as well as from swabs of pericardial and synovial fluid.

Acc. 222. – Otisville, New York.

This isolate was recovered from eye swabs of several goats, all from one pen, with a keratitis infection. These goats were only a few of many goats and sheep used in a research project by National Institute of Health. The keratitis responded favorably with parenteral therapy with tetracycline. The culture was submitted by the Public Health Research Institute of New York.

Acc. 169. – Laurel, Maryland.

One recently purchased goat, added to a milking herd of 35, suddenly showed signs of illness and a large swelling in the cervical area was observed. Broad spectrum antibiotics given systematically did not correct the condition. Necropsy by the Livestock Sanitary Service Laboratory of College Park, Maryland, revealed a hard, grey and red, mottled mass in the intermandibular space extending from the third cervical vertebrae to the base of the tongue (5-6 inches long). Actinobacillosis was suspected; however, mycoplasma was isolated.

Acc. 208. – Fallston, Maryland.

One goat, about 14 months old, from a herd of 12, developed a painful, swollen hock joint. The Livestock Sanitary Service of Frederick, Maryland, recovered mycoplasma from the synovial fluid of the infected joint.
Media

Either of 2 serum-enriched growth media proved suitable for these isolates: (1) brain-heart infusion broth (Difco) with 20% rabbit serum (BHIR) or (2) buffered viande foie-ox serum (BVF-OS). Sera were heated at 56°C for 30 minutes. Bacterial inhibitors in the media were 1,000 units potassium penicillin G per ml and 1:2,000 thallium acetate. Agar plates were prepared from BVF-OS.

Animal infectivity

In this study, sheep and goats were inoculated by the intramuscular (IM) or intrathoracic (IT) routes. Inoculums, 5.0 ml of broth culture in the logarithmic growth phase, contained $1 \times 10^8$ to $10^9$ colony-forming units (CFU) of mycoplasma per ml. In some studies, 5 ml of the same culture was inoculated IT into steers or calves and IM into pigs. Inoculations into the joint cavity (1.0 ml), the conjunctiva (0.1 ml) or the nostrils (2.5 ml) of goats were also done in some studies. Six-to 9-day-old chick embryos were inoculated with 0.2 ml of undiluted culture via the yolk sac.

Tissue samples

During infectivity studies, 1 ml of venous blood was withdrawn aseptically from each experimental animal and cultured in BHIR broth. Oesophageal pharyngeal samples, taken with a small probang, were immediately added to 20-40 ml of BHIR broth and incubated. At necropsy, heart blood and 1 cm$^3$ blocks of tonsil, lung, trachea, spleen, kidney, and inoculated muscle were rinsed in sterile saline and immediately put into 10 ml of BHIR broth. Following 2-3 days' incubation at 37°C, these tissue samples were streaked on BHIR agar plates and examined daily during 6 days' incubation for mycoplasma.

Serology and hematology

Mycoplasma antigen for complement fixation (CF) and agar gel diffusion precipitation (AGDP) tests was grown in BHIR broth for 2-4 days at 37°C. Organisms were sedimented at 48,200 G for 2 hours. The pellet was resuspended to 1/100 of the original broth volume following 2 washings in merthiolated saline. Hyperimmune rabbit serum was prepared against each of the M. mycoides var. capri strains: Connecticut, Vom (Nigeria), Mexico (Pueblo) and PIADL Acc. 169 and 208, plus M. mycoides var. mycoides strain V-5, a bovine strain. Blood from inoculated goats was collected in Unopette disposable blood diluting pipette and leukocyte counts made.

The growth inhibition procedure was modified from those of Edward and Clyde by using holes in BHIR agar for serum wells.

Biochemical tests

Fermentation studies used carbohydrates fermented by Mycoplasma mycoides var. capri, i.e. glucose, levulose, maltose, trehalose, mannose, mannitol, and sorbitol.

RESULTS

Biological and serological characteristics

Properties of the 4 isolates shown in Table 2 were almost identical to those of M. mycoides var. capri strains. All fermented the same carbohydrates. Each of the 4 isolates reacted in CF and AGDP tests against the 3 M. mycoides var. capri strains and cross reacted in CF tests with M. mycoides var. mycoides, V-5 strain. Slight
differences which occurred in growth inhibition studies were considered insignificant. All the isolates killed chick embryos.

TABLE 2. Biological Properties and Serological Cross Reactions of Goat Mycoplasma Isolates

<table>
<thead>
<tr>
<th>Tests</th>
<th>Antiserums</th>
<th>PIADL Accessions</th>
<th>Mycoplasma isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>169</td>
<td>208</td>
</tr>
<tr>
<td>Carbohydrate fermentation</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chick embryo mortality</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Complement fixation (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comm.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mexico</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Vom</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>V-5</td>
<td>+</td>
<td>N.D.</td>
</tr>
<tr>
<td>Agar gel diffusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comm.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mexico</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Vom</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth inhibition (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comm.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mexico</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Vom</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

(1) + = 50% hemolytic units of complement fixed by antigen dilutions greater than 1:1600 in the presence of indicated sera.

(2) + = Precipitin bands present with indicated sera.

(3) + = Serum inhibited growth of organism. - = Serum did not inhibit growth of organism.

N.D. = Not done.

Animal infectivity

Intramuscular inoculation of each of the 4 isolates into goats and sheep produced fever within 2 days, lameness, depression and extensive edema in the affected limbs. Necropsy disclosed a severe myositis throughout the inoculated thigh muscle mass and an overlying subcutaneous edema which extended toward the distal portion of the leg. Intrathoracic inoculation was equally dramatic and temperatures of 104.5° to 106.5° F. immediately preceded deaths of these goats or sheep. Severe pleuropneumonia, usually with fibrinous adhesions, developed in the thorax on the inoculated side. Death usually occurred within 3-5 days after inoculation (DPI).

Acc. 212: This isolate killed goats and sheep within 5-10 days when inoculated IT or IM. Inoculated pigs and steers did not develop clinical signs of illness or antibody response to the inoculated organism, nor was mycoplasma recovered from tissues samples upon necropsy 14 DPI.

ACC. 169: This isolate caused high fevers within 2 DPI, severe depression and death when inoculated into the thoracic cavity or thigh musculature of 3 goats and 2 sheep. When inoculated into the stifle joint of a goat, this mycoplasma incited swelling with severe lameness. This goat developed severe arthritis, periarthrthritis, periart break, and synovitis.

Goat 3681, an uninoculated control, gave birth to a kid the day after the other goats in the room were inoculated with Acc. 169. Although the nanny developed
no fever or clinically detectable illness, its kid's temperature gradually increased to 106.2°F, and it was found dead 10 days after exposure.

Mycoplasma recovered from the spleen, tonsil, blood, lung, and mesenteric lymph nodes of the kid reacted in the CF test with rabbit serum against Acc. 169. A broth culture of the organisms from the blood killed other sheep and goats within 4 DPI by IM or IT route, or 10 days following intranasal instillation.

In one of two calves inoculated with Acc. 169, mycoplasemia persisted until the calf died 34 DPI. Mycoplasma was recovered from small pneumatic lesions in the right diaphragmatic lobe of the lung. Both calves developed antibodies against the organism inoculated, but mycoplasma was not recovered from the other calf during a 60-DPI observation period or from tissues collected at necropsy.

Acc. 222: Two goats developed the signs and lesions described earlier and died 4 DPI by IM or IT inoculation. Mycoplasma was cultured from blood sampled at 2 and 3 DPI.

An un inoculated goat in contact with these 2 sick goats remained clinically normal throughout 10 days before he was inoculated subconjunctivally with Acc. 222. The inoculated eye swelled and was severely congested. Mycoplasma developed 4 DPI and persisted until the goat was found dead 7 DPI. Severe edema around the inoculation site involved the eye and temporal and masseter muscles.

None of 160 serums from sheep and goats in the herd from which Acc. 222 was isolated had CF or precipitating antibody against Acc. 222 or strains Vom, Mexico, or Connecticut. Approximately 25 sheep and goats were surveyed by repeated bleedings. Some serums were from animals with keratitis, while others came from clinically normal animals which had been in direct contact with infected goats and sheep held in nearby pens. Two groups of animals had keratitis; mycoplasmas were recovered from lesions of some, but not from eyes of the others.

Acc. 208: Inoculation by IM or IT routes with this isolate into goats and sheep usually caused the typical pattern of illness and death at 5 DPI. However, the IM-inoculated sheep (ET 449) remained ill, became moribund, and was killed 16 DPI. Severe swelling, edema and myositis involved the entire muscle mass of the inoculated limb. Mycoplasma was detected throughout the 16 DPI period.

An uninoculated control goat, ET 4159, remained clinically normal for 32 days after contact exposure (DPC) but developed CF antibody against M. mycoides var. capri. An OP sample taken 15 DPC contained mycoplasma. This OP isolate, inoculated IT into a goat, caused a severe pleuropneumonia and death 5 DPI. Necropsy of ET 4159, 32 DPC, revealed a small nodular lung lesion from which a mycoplasma was isolated. This isolate was inoculated by the IT route into a pregnant goat which subsequently died 8 DPI. Severe pleuropneumonia and arthritis were found at necropsy and mycoplasma were recovered from all sampled tissues including those aseptically taken from her dead foetuses.

Periodic leukocyte counts (Table 3) show the dramatic leukopenia which preceded death in sheep and goats infected with Acc. 208. Leukocyte counts returned to normal in sheep ET 449 prior to euthanasia. Leukocyte numbers in the uninoculate goat remained normal.

Another trial with Acc. 208 suggested that the 50% lethal dose for goats was approximately 1 x 10⁷ CFU. Lower dosages produced no clinical illness but did stimulate antibody response detectable by CF (Table 4). Only 2 uninoculated goats (both nursing kids) developed CF antibodies against Acc. 208. One kid (ET 491)
nursed a dam (ET 110) which was inoculated with $1 \times 10^7$ CFR and both developed CF antibody against Acc. 208. The inoculated dam (ET 120) died from pleuropneumonia while her kid (ET 493) was still nursing. A mycoplasma isolated from a drinking trough used by infected goats killed a goat within 5 DPI when inoculated by the IM route.

### TABLE 3. Leukocyte Counts of Goats and Sheep Following Inoculation

with *Mycoplasma Mycoides var. Capri* (Accession 208)

<table>
<thead>
<tr>
<th>DPI</th>
<th>Sheep IM Rt.</th>
<th>Sheep IT Rt.</th>
<th>Goats IM Rt.</th>
<th>Goats IT Rt.</th>
<th>Uninoculated control goat ET #4159</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10,450</td>
<td>8,800</td>
<td>12,200</td>
<td>17,000</td>
<td>15,290</td>
</tr>
<tr>
<td>1</td>
<td>11,200</td>
<td>10,100</td>
<td>14,700</td>
<td>19,400</td>
<td>13,200</td>
</tr>
<tr>
<td>2</td>
<td>13,900</td>
<td>9,900</td>
<td>10,670</td>
<td>10,000</td>
<td>12,100</td>
</tr>
<tr>
<td>3</td>
<td>9,570</td>
<td>4,500</td>
<td>6,330</td>
<td>8,470</td>
<td>10,230</td>
</tr>
<tr>
<td>4</td>
<td>7,700</td>
<td>2,600</td>
<td>4,600</td>
<td>7,040</td>
<td>10,700</td>
</tr>
<tr>
<td>7</td>
<td>5,700</td>
<td>Dead</td>
<td>Dead</td>
<td>Dead</td>
<td>14,000</td>
</tr>
<tr>
<td>8</td>
<td>5,700</td>
<td></td>
<td></td>
<td></td>
<td>16,000</td>
</tr>
<tr>
<td>9</td>
<td>3,300</td>
<td></td>
<td></td>
<td></td>
<td>11,440</td>
</tr>
<tr>
<td>10</td>
<td>3,410</td>
<td></td>
<td></td>
<td></td>
<td>12,320</td>
</tr>
<tr>
<td>11</td>
<td>2,310</td>
<td></td>
<td></td>
<td></td>
<td>11,660</td>
</tr>
<tr>
<td>14</td>
<td>4,730</td>
<td></td>
<td></td>
<td></td>
<td>13,190</td>
</tr>
<tr>
<td>15</td>
<td>7,150</td>
<td></td>
<td></td>
<td></td>
<td>11,000</td>
</tr>
<tr>
<td>16</td>
<td>11,000</td>
<td></td>
<td></td>
<td></td>
<td>10,340</td>
</tr>
</tbody>
</table>

**IM** = Intramuscular route of inoculation.

**IT** = Intrathoracic route of inoculation.
TABLE 4. Complement Fixation Antibody Titers in Goat Serums Following Inoculation with Accession 208 Mycoplasma

<table>
<thead>
<tr>
<th>Inoculated Ear tag</th>
<th>Uninoculated Contact Controls Nursing kids</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI</td>
<td>110 &amp; 116^a</td>
</tr>
<tr>
<td>0</td>
<td>10^f</td>
</tr>
<tr>
<td>16</td>
<td>320</td>
</tr>
<tr>
<td>34</td>
<td>320</td>
</tr>
<tr>
<td>41</td>
<td>480</td>
</tr>
<tr>
<td>67</td>
<td>480</td>
</tr>
<tr>
<td>76</td>
<td>640</td>
</tr>
<tr>
<td>87</td>
<td>640</td>
</tr>
<tr>
<td>94</td>
<td>240</td>
</tr>
<tr>
<td>109</td>
<td>160</td>
</tr>
<tr>
<td>124</td>
<td>160</td>
</tr>
<tr>
<td>172</td>
<td>120</td>
</tr>
<tr>
<td>202</td>
<td>80</td>
</tr>
</tbody>
</table>

DPI = days postinoculation.

a = Ear tag (ET) 110: 1 x 10^7 colony-forming units (CFU); ET 116: 1 x 10^5 CFU
b = ET 121: 1 x 10^4 CFU; ET 125: 1 x 10^4 CFU
c = Uninoculated dam.
d = Inoculated dam, ET 110.
e = Inoculated dam, ET 120 (died)
f = Titers expressed as reciprocal of serum dilution.
*18 DPI of dam, ET 120

Oesophageal pharyngeal smearline was used to detect mycoplasmas and to determine the duration of throat infections. Found only in inoculated goats (Table 5), mycoplasmas could consistently be recovered from 2 goats during 3 to 81 DPI. Each of 3 isolates (one collected from goat ET 116 35 DPI and 2 collected from
goat ET110, 17 and 35 DPI) was inoculated into a previously uninoculated control goat which had remained healthy and developed no Cf antibody during 40 days in contact with goats ET 110 and 116, and 14 others, some of which died following inoculation. All three goats died within 7 days after IT inoculation with the OP isolates.

**TABLE 5. Recovery of Mycoplasma from Oesophageal Pharyngeal Fluids Following Inoculation of Goats with Accession 208 Mycoplasma**

<table>
<thead>
<tr>
<th>DPI</th>
<th>Inoculated</th>
<th>ET #110 &amp; #116 Inoculated</th>
<th>ET #121 &amp; #125 Inoculated</th>
<th>Uninoculated contact controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Dead</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>46</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>81</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>92-210</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CFU = Colony-forming units.

- = No mycoplasma recovered.

+ = Mycoplasma recovered.

Surviving goats were killed 210 days after initial inoculations. During the approximately 6 months preceding the project’s termination, no goat in the room had evidenced clinical illness. No goat had pneumonia nor was a mycoplasma recovered from any of the tissues collected at necropsy.

**DISCUSSION**

*Mycoplasma mycoides* var. *capri*, until recently regarded as foreign to the United States, now seems established in this country.

Peculiarly, the disease and lesion pattern described repeatedly from outbreaks in the Near East and Mexico have not resembled those seen in 5 “outbreaks” in the United States. The expected pattern of acute disease, rapid spread, high mortality,
and severe pleuropneumonia are clearly absent in the United States. Incidence of infection associated with American isolates has been rather low. Though we consistently reproduce the classical pleuropneumonia by inoculating the organism into the nostrils, trachea, or thorax, other goats in intimate contact usually remain clinically normal and don’t develop detectable antibodies against the inoculated organism. Only one goat, a nursing kid used in the infectivity study of Acc. 169, apparently became infected through contact and died. The *M. mycoides* var. *capri* strains sent from abroad as reference cultures seemed of equally low contact-transmissibility in our infectivity studies.

A review of the literature provided no hint of this type epidemiology. Have only the costly, spectacular manifestations of the infection been reported?

We speculate that many mycoplasma-associated infections may have gone undetected simply because the relatively low value of an individual goat discourages thorough diagnostic study of sporadic infections. Or, mycoplasma in samples submitted to laboratories may escape notice because of the special isolation techniques required.

Frequent demonstrations of mycoplasmas in the oesophageal-pharyngeal fluids of goats suggest that localized infection may perpetuate the disease within a herd. Recovery of mycoplasmas from dead foetuses in the uterus of a nanny which died after experimental inoculation suggests transplacental infection as a mycoplasma-perpetuating mechanism. This mechanism has been reported in contagious bovine pleuropneumonia.s,17 The pronounced leukopenias recorded seem significant in differentiating between mycoplasmal and bacterial infections.

ACKNOWLEDGMENTS

The authors acknowledge Drs. R. Shildinger, J. D. Beck, and Harvey Fischman for their alertness and submission of samples and thank Messrs. Peter Mikiciuk and Chester Wisowaty for their technical assistance.

REFERENCES

AFRICAN SWINE FEVER
Dr. E. C. Sharman
Agricultural Research Service
U. S. Department of Agriculture

African swine fever has been described as a new disease caused by an old parasite. The virus of African swine fever has established what appears to be the perfect host-parasite relationship in the African wart hog which serves as a reservoir and inapparent carrier of the virus.

The disease was first recognized by the early colonists in Kenya, Africa, in 1909. Domestic swine imported from Europe and released to freely range in Africa contracted the disease, experiencing an extremely high morbidity and mortality. The colonists recognized that outbreaks of the disease were associated with the presence of the wart hog and domestic swine could be raised only when protected from contact with the wart hog. The disease occurs in African wart hogs in an area lying between the Equator and the Tropic of Capricorn.

The disease remained confined to this area until it began to move in 1933 with an outbreak in Cape Province. In 1939 Algeria experienced an outbreak. The disease remained static for almost two decades before moving into Portugal in 1957, which was rapidly followed by movements into Spain in 1960, France in 1964, the Island of Madeira in 1966, and Italy in 1967. African swine fever for the first time invaded the Western Hemisphere in the Republic of Cuba in May 1971.

France was very successful in eradicating each outbreak of African swine fever which occurred within her boundaries. Italy has conducted an extensive campaign and may have successfully eradicated the disease; however, sufficient time has not elapsed to reach a final conclusion. Spain and Portugal were not so fortunate. Table I indicates the reported annual disease incidence for these countries.

Table I

<table>
<thead>
<tr>
<th>Year</th>
<th>Spain</th>
<th>Portugal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1967</td>
<td>1,419</td>
<td>966</td>
</tr>
<tr>
<td>1968</td>
<td>1,033</td>
<td>1,201</td>
</tr>
<tr>
<td>1969</td>
<td>668</td>
<td>714</td>
</tr>
<tr>
<td>1970</td>
<td>911</td>
<td>671</td>
</tr>
<tr>
<td>1971 (6 months)</td>
<td>457</td>
<td>69</td>
</tr>
</tbody>
</table>

1/ As reported by O.I.E. (Office of International Epizootics)
To further evaluate the impact of the disease on Spain and Portugal (Table II), the outbreaks reported by O.I.E. by month for 1970 and 1971 indicate a very consistent outbreak rate per month with occasional flare-ups.

### Table II

#### AFRICAN SWINE FEVER OUTBREAKS

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Spain</th>
<th>Portugal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>January</td>
<td>71</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>February</td>
<td>75</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>March</td>
<td>79</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>April</td>
<td>106</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>75</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>68</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>53</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>48</td>
<td>255</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>77</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>November</td>
<td>85</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>102</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Total Outbreaks</td>
<td>911</td>
<td>671</td>
</tr>
<tr>
<td>1971</td>
<td>January</td>
<td>78</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>February</td>
<td>109</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>March</td>
<td>92</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>April</td>
<td>71</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>56</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>51</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Total Outbreaks</td>
<td>457</td>
<td>69</td>
</tr>
</tbody>
</table>

Total Swine Population
- Spain: 6,139,000
- Portugal: 1,660,000

1/ As reported by O.I.E.
It appears the disease is well entrenched in these two countries now and that eradication will not be accomplished in the near future. In the meantime, the virus has adapted itself to the domestic swine population with some loss of virulence and a decrease in mortality rates. The presence of the virus in these two countries poses an additional threat to all countries of the world.

The appearance of African swine fever in Cuba established another point source or threat to the free areas of the world. However, the vigorous program of eradication by the Cubans may have eliminated this as a future threat.

The Republic of Cuba is divided into six provinces: Pinar del Rio, Havana, Matanzas, Los Villas, Camaguey, and Oriente, besides the Isle of Pines. The territory consists of 44,218 square miles (for comparison purposes, about the size of the State of Pennsylvania — 45,333 square miles), with a human population of 8,280,000. Their livestock population consists of 7,100,000 cattle, 84,000 goats, 280,000 sheep, 1,490,000 swine, and 730,000 horses.

The swine industry is divided into a private sector and a state sector. The private sector rears swine primarily for home consumption and consists of small operations while the state sector consists of large breeding and finishing operations.

The Veterinary Service is totally run by the State, organized and directed by the National Institute of Veterinary Medicine which functions through a Regional, a Provincial, and a National Organization.

The first outbreak of African swine fever (ASF) was detected in Havana province on May 6, 1971, in a hog-finishing farm containing 11,425 animals. The source of the outbreak has not been precisely determined. This farm received animals from state hog producing units and private sectors. Possibly routes of introduction of African swine fever virus were pigs from private farms, personnel, vehicles, or garbage from the city of Havana. The garbage was obtained from dining rooms and cafeterias and sterilized before being fed. The incubation period for pigs exposed by contact was 5 to 12 days with an average of 7 days. The clinical forms of the disease varied and included fulminating, acute and subacute cases.

Clinical signs recorded for the three forms were of the classical description with few signs and death in the fulminating forms, cyanotic areas of the skin, diarrhea, abortions, incoordination and marked depression prior to death in the acute form and a chronic type infection in the subacute form. The mortality rate varied between 95 and 100 percent of the animals attacked.

The first diagnosis was obtained simultaneously in the laboratories of Cuba and the Soviet Union on the 17th of June 1971, a delay of 42 days from the first detected outbreak on May 6.

Up to July 25, 1971, 298 specimens were submitted from suspect herds from all provinces. Of these, 33 were positive for African swine fever, all in Havana Province.

In addition to the initial outbreak in a state hog-finishing unit, three other finishing units with close association with the initial unit became infected, involving some 9,000 animals. The 33 outbreaks recorded involved 32,524 animals.

The control and eradication measures consisted of the following actions:
1. Stop order on the movement of swine for the entire country.
2. A swine census in the Province of Havana which revealed a population of 463,322 swine.
3. Slaughter and incineration or burial of all infected swine and their contacts.
AFRICAN SWINE FEVER

Of the 32,524 animals in the 33 outbreaks, 12,173 died and 20,351 were slaughtered.

4. Slaughter and burning or burial of all presumptive outbreaks of African swine fever or hog cholera.

5. Sacrifice of all swine in the border region 5 km wide between Havana and Pinar del Rio and Havana - Matanzas.

6. Sacrifice of all privately-owned swine in the Province of Havana. Each owner was allowed to slaughter for consumption 3 pigs in the urban area and 5 in the rural area. The remaining animals were sent to slaughter houses for processing into canned and sterilized meat products. This operation involved 383,322 animals.

7. Slaughter of state-owned hogs in the Province.

8. Conventional quarantine and disinfection procedures and organization of a detection system.

The eradication program was carried out by a Commission made up of various branches of the government which was responsible for carrying out all the measures for eradication of the disease in the National Territory and prevent African swine fever from spreading to other countries of the world.

The plans for rebuilding the swine industry in Havana Province are very exacting and if followed as outlined should prevent another epizootic of African swine fever from developing either from residual virus or newly introduced African swine fever virus. In addition, steps have been taken to bolster the program to prevent the entry of foreign animal diseases at the various ports of entry.

REFERENCES


Laboratory record keeping at the Plum Island Animal Disease Laboratory (PIADL) has problems common to other biological laboratories. The variety of inter-related records cover virus stocks, sample log books, specialized equipment log books, clinical data of a variety of animals, test results, and other factors. Investigators ordinarily make chronological entries in notebooks and in sample or equipment logs. Chronological entries, however, seldom give a clear overall picture of what was done. The situation is aggravated when there is a separation between the laboratory where the data are generated and the place where data are further processed and prepared. Notebook records of an individual investigator are often clear only to him and then usually for a limited period. What was perfectly clear at the time of writing has a tendency to become more vague with time.

Problems arise in collaborative research where the members of a team must have access to the notes made by other team members. Both active and lengthy research projects have need for a good record system. In the former, numerous results from many collaborators in a short time can be overwhelming if exchanged informally in raw form. In the latter, the length of time between results permits data to be misplaced or associated details to be forgotten.

A computer-based record system does not necessarily resolve all the problems mentioned above and will cause problems of its own. Some of these are: 1) a duplication of effort if the system does not replace an existing one, 2) the need to introduce a new method of record keeping, 3) a tendency for the system to become an end in itself rather than a means to an end, 4) a possibly employment of additional personnel, 5) a possible confusion because of too great an escalation in complexity, 6) a declining motivation if the person who enters the data is too far removed in space or time from the person who uses the retrieval data, 7) a requirement to learn how to communicate with the computer, 8) a delay because of computer turn-around time or because of keypunching or other data entry procedures, 9) a tendency to become irrelevant because of the continuous change in emphasis in the research of most investigators, especially if forms of rigid format are used, and 10) a possible need for too many compromises involving size, complexity, cost, and time.

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“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.”

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance and support of Drs. Graves and McVicar and thank Mr. Walter Harris for his technical assistance.
The purpose of this report is to show 1) how we designed our record system to avoid some of the pitfalls, 2) what the computer can do for our research program with regard to storage, retrieval and ordering of meaningful records and 3) some applications that would have been very difficult without computer assistance. We will not give details of the computer program or of its operational procedures since it was written specifically for the Control Data Corporation 6600 computer at Brookhaven National Laboratory.*

STRUCTURE OF BASIC RECORD

Each "basic" record is 80 characters in length and physically is represented by one 80 column punch card or by its image on magnetic tape. The records originate in the laboratory where research data are hand-recorded on standard 80 column 44-row ruled sheets. These sheets are divided in vertical columns so that only one numeric or alphabetical character can be written in each square. The information contained on each line is punched into a standard computer card. A deck of such cards forms the input for the computer. Fig. 1 shows how the information from the first three lines of an input sheet appears as computer cards. Some structuring of the basic records has been exceedingly helpful in organizing animal disease research data for computer retrieval systems. The 80-character record length is divided into 2 parts: the first 20 characters have a "fixed" format while the last 60 characters can be used in any format of the user's choosing. The information in the fixed format portion of the basic record is: experiment number, subject number, date, form number and continuation. This is carried through on all the input sheets of Fig. 1-3, but in Fig. 3 the fields are so named. The usefulness of this hierarchical structure will be illustrated with a hypothetical experiment described chronologically.

In this particular example, an experiment is recorded on 4 cattle inoculated with foot-and-mouth disease virus. Fig. 1 shows the early records. The first 5 characters, PS101, are the number assigned to the experiment, followed by 5 zeros in the spaces used in other lines to write an animal ear tag number as the subject number. The next characters are the date of the record, written as month, day, year (July 28, 1970) followed by 3 blank spaces. Column 20 of this and subsequent lines contains a continuation card number to permit these records to remain in order. This is needed since these lines are used for the heading of the experiment. The next day, on July 29, it was observed that the steer 701 was very nervous and this information is written on line 4. On August 1 the cattle were exposed to foot-and-mouth disease virus, 2 steers intranasally (IN) and 2 steers by tongue inoculation (IDL). Here the use of columns 17, 18 and 19 is indicated. These columns identify the type of information recorded i.e. the format of the record (CLN stands for clinical information; IDT for information related to the animal identification; VIR for virus exposure). One further detail on this figure: the vertical undulating lines indicate that a repetition of the information is required on subsequent lines. For example, the keypunch operator will duplicate PS101 as the

* Copies of the FORTRAN program listing and user's guide can be obtained from the authors. Changes of the program will probably be needed for other computers.
first characters in each card.

In Fig. 2 it can be observed that the first 20 columns are used for the same purpose as in Fig. 1. The records of the upper part of this figure cover clinical data (CLN). Follow for instance steer 701: it had tongue vesicles one day after inoculation (DPI) while the feet had starting lesions; the next day (line 5) there were foot lesions and a large vesicle in the nostril; and on August 9, this animal died. Three lines are needed to describe the necropsy. The same input sheet (lower half of Fig. 2) was used to record some conclusions and to summarize some observations: temperature (TMP) (101.4 F as 14 etc.), fever (FEV) and viremia (VIR) (+ or -), and blood titers (BLD) (1.7 PFU/ML as 17 etc.).

During the experiment, several samples (SPL) were taken and which were recorded on a separate sheet (Fig. 3). For easy reference, this sheet has been given self-explanatory headings. It might be noted that, on August 9, a sample of heart tissue (HRT) was taken from steer 701. This sample was given the log number 1016 and stored as 10% suspension in box GY. Test results (RLT) were recorded as they became available (lower half of Fig. 3.) It can be seen that sample 1016 from steer 701 was tested on September 15 with the infectivity result expressed as plaque-forming units per ml (PFU/ML).

The keypunch operator transfers the information of each line from the input sheets illustrated in Fig. 1-3 to a punch card as indicated in Fig. 1. These punch cards are included with other cards containing the research data of the same investigator to form his permanent master card file. Alternatively, the information on the punch cards can be added to a magnetic tape containing card image of previous research data. However, these is no basic difference: both the card and the tape store a coded representation of each line of the input sheet.

RETRIEVAL OF RESEARCH INFORMATION

The computer program has been written to select the card image that meet a chosen set of criteria and sort those images in the memory of the computer according to any particular sequence of columns. For these retrieval and sorting operations the user supplies simple control cards. The example just described shows how ordinary research information was entered. The same particular example of 4 cattle used above will now be considered in terms of retrieval.

First, we have the computer recall from the master file the records with certain specified characters in a given position, for instance, all records with PS101 in position 1-5. The computer searches the file and stores in its memory all records that fulfill this requirement. Before the “print-out” of the information, we request that the collected records be ordered in some useful manner. This ordering uses the information stored in the memory of the computer and is not by physically sorting of cards. Fig. 4 a, b is a computer print-out of the records that were ordered by eartag number, chronologically and then by format code.

The SORT ORDER given in the third line indicates the sequence of columns that are to be used. Start with left-most figure “1” and read to the right until all the “1’s” are finished, then go to the left-most “2”. The date has been sorted by year, month, day before the format code in columns 17-19 and continuation column 20 are used.

Two points can be noted: 1) If we want to recall records on certain characters, these characters must always be in a defined place (e.g. PS101 must be in the first 5
positions). In deciding on a format, the investigator should always ask himself "Do I ever want to select or sort on this category?" On the other hand when the investigator is convinced that he will never use information in this way, there is no need to adhere to a rigid format (e.g. the narrative clinical reports). 2) The computer program must produce a readable print-out. With our computer program all repetitive information in the first 19 columns is deleted from the print-out to give greatly increased readability. The program also provides vertical spacing, headings, etc. and prints titles supplied by the user on control cards.

Second, we demonstrate in Fig. 5 how the new information of Fig. 1-3 can be integrated with other research records. In this case the request was for all records with IDT in positions 17-19. In the illustration chosen, two experiments are recovered: PS100 and PS101. In the upper part of Fig. 5, we requested these records be ordered by age (111 in positions 28-30), weight (2222 in positions 35-38) and eartag (33333 in position 6-10). Another “sort” on the same retrieved records is illustrated in the lower part of Fig. 5 where the request was for breed (11111), weight (2222) and then eartagg (33333). These sorts may not be particularly useful but they illustrate the potential for other applications.

Third, we construct from all our records the sample log book. The basic retrieval calls for a chronological listing by sample number of all records with SPL or RLT in positions 17-19. This output (Fig. 6) lists all samples and their test results. Narrative information such as the status of the sample, can be included. For example it can be noted that in the last entry for sample 1017 that it was used up.

A fourth example is shown in Fig. 7. This is a print-out of the sample log without test results but with records sorted by storage location. Another print-out that we found extremely useful is a sort by eartag and by sample number, which groups together all samples obtained from the same animals.

An input sheet with summary data is shown in Fig. 2. For these records we chose a rather rigid format because in that form data can be used for the comparison of larger groups of records. For instance, if we want to study the differences in viremia after different routes of exposure to the virus, we: 1) Have the computer search the master file for all records of intranasally exposed animals from the desired experiments. For this there must be VIR in position 17-19 IN (intranasally) in position 35 and 36 and the required experiment number in columns 1-5. 2) Search the master file for all related records of these animals. 3) Select from these records the ones with SUM and VIR in positions 17, 18, 19 and 21, 22, 23, respectively. After the appropriate sort (given in line 3) the upper part of Fig. 8 would be produced. The same could be done for IDL (intradermal lingual) inoculated cattle resulting in a print-out as shown in the bottom part of Fig. 8. Inspection of the print-out immediately calls to our attention that both the onset and the duration of the viremia probably are influenced by the route of exposure to the virus. In both of these print-outs the connecting lines have been drawn by hand. Once the data are tabulated, additional computer programs could be used for analysis of the information. However, we have found that after ordering the data, further calculation often can be done readily with a desk-top computer.

It should be pointed out that the examples given all have a relatively small number of records. In practice, we use from 1,000 to 20,000 records in a file. The “all-in-memory” sort program used can select and sort as many as 10,000 records in a matter of seconds and print them in a matter of minutes.
DISCUSSION

The basic record system described here has been in operation for 2 years and has proven its usefulness. It is designed to be used during a research project, for summarizing it after completion, for updating it if new data become available and for integrating the total research effort. If relies on the careful entry of information, not on any editing routine in the computer. It uses standard input forms to avoid cost of pre-printed forms and to allow for flexibility. We have not hired any computer technicians per se. The investigators and the technicians who need the data are those who enter them. We use the original input sheets as the primary record as much as is feasible, and so the new system replaces the old, rather than be an additional burden.

The use of computer input sheets with defined fields has improved the recording of data. Formerly, certain items were frequently omitted that were invaluable later when reports or progress are written. A balance has to be made, however, so that a hard core of real information is recorded without the hindrance of minutia. We have avoided coding data as much as possible by using continuation cards or additional forms where more recording space is needed. Free use is made of narrative headings, provided by the user, to improve legibility and make unnecessary the consulting of dictionaries or vocabularies.

Overall, we have not found that the computer saves us time. For instance, it takes a certain amount of time to verify, and if necessary, to correct punch cards. However, once records are correct they can be manipulated and printed over and over without being subject to typing errors. Also, periodically, someone from the laboratory has to go to the computer center forty miles away to submit the jobs. Routine updating of current experiments and sample logs can be done by a trained technician, but more involved retrieval and sorting may require a trip by the investigator himself. There has been a considerable saving in time in locating a sample in cold storage for further tests by using the subject listings of all the samples on hand.

The greatest saving, we believe, has been the more efficient use of the very costly animal experimentation. It has been possible to go back to old records and integrate them with recent research data, keep track of large numbers of samples with the results obtained on each sample, and analyze the results much more thoroughly than possible before. For example, we have had cattle in our laboratory that were used for vaccine studies by one group and subsequently for the study of pathogenicity and virus persistence by another group. As many as 30 steers were used and they were followed by different investigators for more than 6 months. Each animal generated approximately 25 samples. Most of the samples were tested both by different methods and by different investigators. Moreover, records were kept on the vaccine, the challenge viruses, and the clinical response of the cattle after the challenge. Thus, it is estimated that this experiment generated at least 3,000 items of information. Management of this number of records becomes unwieldy and inefficient when done in the conventional laboratory manner.

With computerized record keeping, results and samples become more and more valuable when information is added to the master file on the experiment since the combined records are easily available and can be presented in an orderly manner. For instance, a new research idea was recently proposed and appropriate ones of the existing samples were located and retested. This lead to a re-evaluation of some
aspects of the original experiment\(^3\). Also, when a new immunological test became available, all serum samples collected in our laboratory during a 5-year period, including the samples of the just mentioned experiment, were examined. The results of this test and the old records were used in a retrospective study to evaluate the applicability of the new immunological test for epizootiological surveys\(^4\). This would have been extremely difficult if the records had not been easily accessible.

**SUMMARY**

A flexible system of computer storage and retrieval of animal disease research records has been in operation for 2 years, using a Control Data Corporation 6600 computer located 40 miles from the laboratory. The input consists of research data recorded on key punch sheets and does not have to be coded. Clinical and laboratory data on animals, experiments, test results and sample storage locations can be recalled and printed out in any arrangement desired by means of control cards. Each investigator's file is continually updated as new experiments generate additional specimens and data. This system has significantly increased the efficiency of research record handling and has made data available in forms hitherto unobtainable.

**REFERENCES**

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<td>00:04</td>
<td>2 ASPERATED EPL 120</td>
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<td>00:05</td>
<td>3 FEET LESIONS, LARGE VESICLE IN LEFT NOSTRIL</td>
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<td>00:06</td>
<td>4 NORMAL, MARRIED ALL FEET</td>
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<td>NASAL DISCHARGE SWAB TAKEN ON 16 ML VIN</td>
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<td>00:08</td>
<td>5 VESICLES BOTH NOSTRILS, EATING, FEET</td>
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<td>00:09</td>
<td>6 NOSTRILS, HEALING, EATING, FEET</td>
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<td>00:15</td>
<td>2- CATTLE INOCULATED WITH THE EPL-VARIANT</td>
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**Fig. 1**

**Fig. 2**
COMPUTER RETRIEVAL OF RESEARCH RECORDS

Fig. 3

---

SA~PLE USEO

UP

F#P

ndusE TEST

PPu/nL

6. 3

LP-VARIANT

PPu/nr.

4. I sP-vARx~(rr 7.3

SER

?:

GRXS

Fel

CF TYPINt

5CR

B0V

AGbP

CLEARLY iP

PATTERN

pRT

BeV

AGDP

CLEARLT LP PATTERN

TYPE

okf6

---

187
COMPUTER RETRIEVAL OF RESEARCH RECORDS

**Fig. 4b**

**EXPERIMENT NUMBER:** PS01

**PS101 00701 072970 CLN**

- ET 76: VERY NERVOUS
- RID 4VY: 3.5 YR 0940 LBS CAST.
- MB 00: 
- VIR FFL: 2-1 IDL 10000 PFU SP-VAR.
- SPL 073
- 08270 CLN DPI 1 TONGUEVESICLES, FEET MAY BE STARTING
- 063970 CLN DPI 2 FEET LESIONS, LARGE VESICLE IN LEFT NOSEIL
- 06970 CLN 1 DPI 6 FOUND DEAD IN MORNING
- 2 AUTOXY TONGUE SCARS, NOSEIL, NEUROGENIC MEMBRANES,
- 3 FEET HEALING LESIONS, HEART TYPICAL PFU LES
- 4 NO OTHER LESIONS, HEART TISSUE SPL 1016
- SPL 01010: MRT BOV DPI 00 1/10 SUSP.
- GY 091570 RLT C0116: MRT BOV PFU/ML 6.3 LP-VARIANT
- 091870 RLT C0116: MRT BOV AGDP CLEANLY LP PATTERN
- SUM 1 IMF 31 22 40 56 90 24 21 11 10 9 10 14 14
- 2 FEV 
- 3 LLC 00 00 00 00 7 4 10 00 00 00
- 4 VIK

**LAYS POST EXPSUSE:**

- 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

**EXPERIMENT NUMBER:** PS101

**06270 072970 IDT**

- 4.0 YR 1020 LBS CAST, MRF BRAND 9371FM
- 06170 SPL C01032: SER BOV DPI 00
- VIR FFL: 0-1 IDL 10000 PFU SP-VAR.
- SPL 073
- 063970 CLN DPI 2 NORMAL, FINISHED ALL FEED
- 064070 CLN DPI 3 NASAL DISCHARGE, SWAB TAKEN IN 10 ML MLW
- SPL 01131: NASAL BOV DPI 03 SWAB
- GY
- 06570 CLN DPI 4 NORMAL, NO LESIONS
- 06170 SPL C01122: OPF BOV 0-1 DPI 14
- 01135: SER BOV 0-1 DPI 14
- GY
- 062770 RLT C01035: SER BOV MPI 3,3
- 06170 RLT C0115: SER BOV PD50 2.5
- 091870 RLT 031915: SER BOV AGDP CLEANLY LP PATTERN
- SUM 1 IMF 12 18 20 22 22 18 18 14 14
- 2 FEV 
- 3 LLC 00 00 00 00 12 22 10 00 00
- 4 VIK

**LAYS POST EXPSUSE:**

- 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

**EXPERIMENT NUMBER:** PS01

**092570**

1 CONCLUSIONS 1-ICL PRODUCED FAM MOME SEVERE DISEASE
2 THAN INTRANASAL INOCULATION
3 2-CATTLE INOCULATED WITH THE SP-VARIANT
4 PRODUCE ALL 3 VARIANTS
### SUTMOLLER AND TRAUTMAN

#### Final Information Retrieval Program IR6

**09/21/71**

**Fig. 5**

**SCRT CPUCH**

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### Identification Records Sorted by Age, Weight and EarTag Number

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### Fig. 6

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**Fig. 7**

**SUTMOLLER AND TRAUTMAN**
INTRAABALLY EXPOSED CATTLE SORTED BY ONSET OF VIREMIA

LAYS POST EXPOSURE

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NUMBER OF RECORDS PRINTED: 13

INKLAGE INOCULATED CATTLE SORTED BY ONSET OF VIREMIA

LAYS POST EXPOSURE

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NUMBER OF RECORDS PRINTED: 11

END OF JOB
Foot-and-Mouth Disease in Sheep and Goats: Early Virus Growth in the Pharynx and Udder

by


INTRODUCTION

We have previously reported on the early growth of foot-and-mouth disease (FMD) virus in the pharyngeal region of cattle. That report and other studies suggest that the bovine pharynx is the initial site of virus multiplication after natural exposure. Burrows has also recovered FMD virus from pharyngeal samples up to 5 days before the appearance of clinical disease in cattle exposed by contact with infected animals. One cow in his study had virus in the milk 4 days before lesions were observed and 2 days before virus was detected in the blood. We have reported that FMD virus readily infected both sheep exposed intranasally or by contact and that a high percentage of these animals became virus carriers. This report describes studies on the early growth of FMD virus in the pharyngeal region of sheep and goats and in the udder of goats after intranasal exposure or contact with infected cattle.

MATERIALS AND METHODS

Virus — All viruses were of bovine origin with not more than 6 passages in primary bovine kidney (BK) cell cultures before use. Viruses used were: type O, subtype 1, strain CANEFA*-2 (01); type A, subtype 10, strain CANEFA-1 (A10); and type C, subtype 4, strain Tierra del Fuego (C4).

Experimental Animals — One- to 3-year old sheep and goats of mixed breed were used. Several does were nursing kids at the time of virus exposure.

Virus Exposure — Intranasal inoculation was accomplished by the instillation of 0.2 ml of virus suspension into one nostril. The virus contact exposure was done by placing sheep and goats in contact with a steer infected by injection of the appropriate virus into the tongue epithelium.

Collection, Treatment and Assay of Samples

Pharyngeal Samples — The sheep and goats used to study the growth of FMD virus in the pharyngeal region were sampled every 20 minutes for the first 2 hours after inoculation, every 2 hours for a day, and then daily as indicated. Oesophago-pharyngeal (OP) fluid was obtained with a probang as previously described. Samples were tested for the presence of virus in BK cultures under Hanks’ balanced salt solution with 0.5 percent lactalbumin hydrolysate (HLH).
Those samples not producing cytopathic effect (CPE) after 72 hours at 37°C were considered negative. Positive samples were plaque assayed under agar1 with titers expressed as plaque forming units per sample (PFU/sample). Trace amounts of virus were ascribed to those samples producing CPE but no plaques.

**Blood and Milk** — Blood samples were obtained every 6 hours for the first day and then daily. One ml of freshly drawn blood was mixed with 9 ml of HLH containing heparin. Milk samples were taken daily and always contained approximately equal amounts from each side of the udder. All samples were tested for virus as described above with titers expressed as PFU/ml.

**EXPERIMENTAL AND RESULTS**

*Growth of FMD Virus in Sheep after Intranasal Inoculation* — The initial growth of FMD virus in the pharyngeal region was studied in a group of 4 sheep inoculated intranasally. Each animal was given $10^3$ PFU of FMD virus type O. Virus titers of pharyngeal samples and onset of viremia are shown in Figure 1. Sheep 1 and 2 reacted somewhat the same. Virus was not detected until 8 hours after inoculation. Titers increased for the next 18 hours to a level of about $10^5$ PFU/sample. Peak titers occurred 2-3 days after inoculation followed by a gradual decline. Virus was first detected in the blood 24 hours after inoculation. Both animals had fever on day 2. Oral lesions were also first observed on day 2 for sheep 1 while no lesions of any kind were observed on sheep 2.

**FIGURE 1.**

![Graph showing virus growth and viremia](image-url)
Virus was not detected until 16 hours after inoculation in pharyngeal samples from sheep 3. Titers then increased again reaching a peak on days 2 and 3. This animal was febrile on day 2 and had oral lesions at that time.

Pharyngeal samples from sheep 4 collected 28 hours and 4 days after inoculation contained small amounts of virus but blood samples were negative throughout that period. Samples were not obtained on days 5, 6, or 7 but virus was recovered from both OP fluid and blood on day 8. Fever and extensive oral lesions were also observed. This animal had a normal temperature and was not viremic the following day (day 9). Since single day viremias and fevers have not been the rule with FMD in sheep, it is likely that infection started a day or two earlier.

**Growth of FMD Virus in Goats After Intranasal Inoculation** — The curve in Figure 2 represents the growth of FMD virus type 01 in the pharyngeal region of goats. Four goats were inoculated intranasally each receiving $10^4$ PFU of virus. The virus titers of the OP fluid samples were so similar that the data were pooled and the figure represents a composite curve. Pharyngeal samples contained traces of virus 20 and 40 minutes after inoculation most probably representing residual inoculum virus. No virus was then detectable until 4 hours after inoculation. Slightly greater amounts of virus were present at 6 hours with a transitory peak at 8 hours followed by a gradual rise over the next 10 hours. Viremia was not detected until 22 hours after inoculation and fever was present at 48 hours. Three of the goats developed oral lesions starting 2 days after inoculation.

**FIGURE 2.**
Growth of Virus in the Udder — Comparative virus titers of blood and milk of nursing goats are presented in Table 1. Goats 1, 2, and 3 were inoculated intranasally with 01 virus while the remaining 10 goats were exposed by contact with a steer infected with either this same virus or virus types A10 or C4. Usually virus appeared in the milk on the first or second day of viremia. Goat 8, on the other hand, had virus in the milk a day before viremia was detected and goat 3 had virus in the milk on day 5 while virus was never detected in the blood.

It is apparent that, once the udder becomes infected, milk titers usually exceed blood titers often by a considerable amount. It also can be seen that virus can persist in the udder for several days after the cessation of viremia.

### Table 1 - Virus Titers of Blood and Milk of Nursing Goats Exposed to FMD Virus Types A, 0 or C.

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a) Blood  
b) PFU/ml  
c) Sample negative  
d) Milk  
e) Sample positive, no plaques

Blank = No sample obtained or tested
DISCUSSION

In sheep and goats, the steady rise in FMD virus titers of pharyngeal samples which occurred after intranasal inoculation and before the appearance of detectable viremia or clinical signs is evidence that the initial site of virus multiplication is in the pharyngeal region. Burrows\textsuperscript{2} supports this contention with a report of the recovery of FMD virus from the pharyngeal samples of sheep exposed by contact with infected donor animals. Virus was detected as early as 5 days before the appearance of clinical signs.

The varied response of the 4 sheep may be due to the relatively low dose of virus with which they were inoculated. Sheep 1 and 2 developed curves quite like those seen for goats except for the absence of the transitory peak at 8 hours. Sheep 3, on the other hand, had only traces of virus in the OP fluid as late as 16 hours after inoculation. From that point on, the multiplication in the pharynx progressed with viremia starting at 30 hours. However, lesions developed at the same time as in sheep 1 which had earlier virus multiplication.

The response of sheep 4 was particularly interesting. The isolation of virus from the OP fluid samples collected 28 hours postinoculation raises the possibility of a very low level pharyngeal infection which resulted in clinical disease 3 or 4 days later. All 4 animals were housed in the same isolation unit, however, and it is also possible that initially it resisted the 1,000 PFU of inoculated virus and only later became overwhelmed as a result of exposure to its infected roommates.

The goats inoculated intranasally with a 10-fold higher dose of the same virus responded quite uniformly. Perhaps the 8-hour peak represented early rapid virus multiplication followed by suppression of growth perhaps by local body defenses. Once multiplication again resumed, the shape of the curve was not unlike that of sheep 1 and 2.

When compared with the growth curves developed earlier for cattle\textsuperscript{5}, the pharyngeal virus titers of sheep and goats appeared to be slower in reaching a plateau. Virus titers of bovine pharyngeal samples peaked 10-12 hours after inoculation whereas titers in the goats and in sheep 1 and 2 were still rising slightly at 24 hours. Whether this slower rise is a function of the lower virus doses used or a species difference is not known at this time.

The usual isolation of virus from the milk early in the viremic period might indicate that the udder became infected via the blood stream. The observations that, in one instance, the milk contained virus before viremia and, in another, that the udder became infected in the absence of viremia suggest another route, however. All of the kids became infected soon after virus exposure and it is possible that nursing caused an ascending infection through the teat canal.

Burrows\textsuperscript{3} found that in cows the virus titer of an inoculated quarter was much higher than that of quarters not inoculated. We found virus titers in goat milk approaching the values which Burrows reports for inoculated quarters. Whether these high titers are characteristic for goats or mean an “inoculation infection” by the nursing kids cannot be answered by these studies. Burrows\textsuperscript{3} also found that virus could be recovered from the milk of infected cows as long as 51 days after virus exposure. We did not test the milk for more than 12 days after infection since lactation ceased in most of the goats either as a direct result of the disease or because the nursing kid died.
SUMMARY
Virus titers of oesophageal-pharyngeal (OP) fluid obtained from sheep and goats after intranasal inoculation of foot-and-mouth disease (FMD) virus are evidence that the primary site of virus multiplication is in the pharyngeal area. Virus titers of the milk of nursing does suggest that the udder may be infected through the teat canal as well as the blood stream.

ACKNOWLEDGMENTS
The authors gratefully acknowledge the technical assistance of Mr. William Parrish and the clerical assistance of Mrs. J. R. Faller.

REFERENCES
Dermatropic Bovine Herpesviruses, Bovine Herpes Virus, Bovine Herpes Mammillitis and Allerton Viruses.
I. Serologic Relationships as Indicated by Cross-Reaction Tests

D. C. Gigstad*, S. S. Stone, and A. H. Dardiri

SUMMARY

Three dermatropic bovine herpes viruses (DBHV), bovine herpes and Allerton viruses, and bovine herpes mammillitis, all producing a similar "lumpy-skin"-like lesion in cattle are serologically related. This relationship was determined by reciprocal fluorescent antibody tests using DBHV-infected bovine kidney monolayers and in the skin lesions of infected cattle. In addition, a reciprocal serological relationship among the soluble antigens of these viruses was shown using complement fixation and agar gel diffusion precipitin tests.

A serological relationship could not be shown between DBHV and the Neethling pox virus historically associated with the more severe form of "lumpy skin" disease.

A bovine herpes virus (BHV) has recently been isolated from cattle in Minnesota.¹ Serologic tests, virus neutralization (VN) and agar gel diffusion precipitation (AGDP) tests³ show a strong relationship between this virus and the bovine herpes mammillitis (BHM) virus isolated and studied in the United Kingdom⁴,⁵,⁷,⁸,¹². The BHM virus in the United Kingdom causes painful lesions on the teats and udder which interfere with milking or nursing. The BHV from cattle in Minnesota causes generalized skin lesions in both naturally and experimentally infected animals.

Another bovine herpes virus producing generalized skin lesions is the Allerton virus found in some regions of Africa¹,⁶,¹¹.

In addition to these, there is another dermatropic virus that may produce a similar clinical disease in cattle, i.e., Neethling pox virus.

Since these four viruses produce similar skin lesions in cattle, study of their relationships is essential to facilitate differential diagnosis and may help clarify their epizootiology.

This report compares the serologic relationships of the three herpes viruses and, to a very limited extent, the Neethling pox virus as determined by fluorescent antibody (FA), AGDP, and complement-fixation (CF) tests.

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MATERIALS AND METHODS

Viruses

Bovine herpes virus. This virus had been passed twice in bovine kidney (BK) cells after being isolated from a steer inoculated with the original virus from Minnesota.\(^1\)

Bovine herpes mammilitis. The TC strain\(^1\) was received as an 8th passage in calf kidney (CK) cells from Dr. E. P. J. Gibbs, University of Bristol, England.

Allerton virus. This virus, a third BK passage received from Dr. Plowright, East African Veterinary Research Organization, Kenya, has been described.\(^1\)

Neethling pox virus. Received from Kabete Veterinary Laboratory, Kenya, East Africa, as the 14th passage in lamb testes cells of the 2490 strain.

Cell Culture.

Secondary BK cell cultures were prepared on 1 x 5 cm coverslips in Leighton tubes (16 mm x 185 mm) using approximately 100,000 cells per tube in 2 ml of Hanks' balanced salt solution containing 0.5% lactalbumin hydrolysate (HLH) and 2% fetal calf serum. Cultures were incubated for 72 hours at 37° C before the fluid was replaced. Monolayers were inoculated with virus when confluent, usually 24 to 48 hours later. Uninoculated control cultures were handled similarly. Infected and control monolayer culture tubes were removed at intervals, fixed and stained with FA.

Cattle

Grade Hereford steers, approximately 19 months old and weighing between 300 and 350 kg, were inoculated to reproduce each of the 4 viral diseases. Each steer was inoculated by the intravenous route, with 10-20 ml of BK culture fluid containing \(10^5\) to \(10^6\) TCID\(_{50}\)/ml of a single virus type. At various intervals, lesions\(^3\) were excised under local anesthetic and blood samples taken at the same time. Sera stored at -20° C were heated to 56° C for 30 minutes before use. Eight to 10\(\mu\)L thick sections, cut on a cryostat microtome, were transferred to glass slides, fixed in acetone at room temperature, and reacted with FA.

Fluorescent antibody preparation

The sera for fractionation were collected 20 to 35 days after the cattle were inoculated. Each serum was adjusted to pH 6.8 (\(\text{NH}_4\))\(_2\)SO\(_4\) was added to achieve 40% of saturation at 0-5° C and held overnight at 5° C. The resulting precipitate was separated by centrifugation and washed once with the original volume of 40% of saturation (\(\text{NH}_4\))\(_2\)SO\(_4\) dissolved in water equivalent to half the original serum volume, dialyzed against saline to remove the excess (\(\text{NH}_4\))\(_2\)SO\(_4\), and then reprecipitated as before. In some instances, the globulin was isolated by the batch chromatographic procedure, using DEAE G-50 Sephadex as described.\(^9\)

In all cases, the globulin was conjugated at pH 9.5 with a protein: fluorescein isothiocyanate ratio of 25:1 for 18 hours at 5°C. Excess fluorescein was removed by gel filtration and the globulin conjugate concentrated to half the original serum volume by lyophilization. Bovine serum which had no antibody to herpes virus by the VN test was similarly conjugated for use as a control. All fluorescent conjugates were adsorbed with 30 mg/ml of acetone extracted powder of CK. The control and antiherpes convalescent conjugated globulins were adjusted to the same protein concentration by spectrophotometric analysis according to a method previously...
Coverslips were removed at various intervals, washed in phosphate buffered saline (PBS) and fixed in cold acetone for 30 minutes at -20°C. Monolayers stored 24 to 48 hours in cold acetone revealed no significant loss of fluorescence in the FA reaction.

Fluorescent antibody was left on the monolayers for 30 minutes at 37°C, washed for 5-7 minutes in 3 changes of PBS, rinsed in distilled water, and air-dried. Coverslips were mounted with 50% PBS in glycerol. The slides were examined on an American Optical Microstar microscope using an AO fluorolume Model 634 illuminator, an Osram HBO-200 mercury vapor arc lamp, and a No. 702 exciter filter. The microscope had a No. 724 barrier filter and an oil immersion dark field condenser.

Pictures were taken on GAF T/100 35-mm film using 0.5 to 2-min. exposures. Black and white prints were made from the color transparencies.

**Agar-gel diffusion precipitation tests**

Besides the cell suspension extract used as antigen in the AGDP test, a good precipitating antigen obtained by concentrating the cell-free supernatant fraction of the infected cells with half-saturated (NH₄)₂SO₄ was used. A concentration of 200 X was required to produce a clear line of precipitate.

Agar plates prepared using a borate pH 8.6 buffer contained 0.1 mg protamine sulfate/ml. Wells, 2 mm in diameter, were 2 mm apart.

**Complement fixation tests**

Viral antigen, prepared and assayed as previously described, was an extract of infected BK cells harvested as CPE neared completion. Viral antigen and serum in volumes of 0.025 ml each were combined with 0.05 ml of guinea pig serum containing five 50% hemolytic units of complement using overnight fixation at 5°C. The final dilution of serum having 50% hemolysis as estimated by visual comparison to known 50% hemolysis wells was considered to be the end point.

**RESULTS**

Cattle inoculated with BHM, BHV, Allerton and Neethling viruses developed the lumpy skin lesions characteristic of these diseases. The clinical disease will be described.*

In BK cultures infected with undiluted BHV, specific fluorescence could be detected as early as 7 hours postinoculation but not at 4 hours. At 12 hours, 2 or 3 cells in each field (100 X magnification) fluoresced. Cultures inoculated with 10⁻¹ or 10⁻² dilutions of virus showed few FA reaction sites before 24 hours after which a few individual cells and a few small clusters of cells fluoresced (Fig. 1).

Cytopathogenic effect (CPE) was usually first visible after 24 hours and was manifested by the formation of large, strongly fluorescing multinuclear cells having brilliant intranuclear inclusion bodies. At 36 and 48 hours postinoculation, CPE appeared as clear acellular areas bordered by large multinucleated fluorescing cells (Fig. 2) or as foci of rounded, shrunken cells with bright, diffuse fluorescence. By 72 hours, CPE was almost complete, and only isolated linear clusters of rounded shrunken cells emitting diffuse fluorescence remained.

Bovine kidney cell cultures, infected with BHM or with Allerton viruses,
fluoresced similarly to those infected with BHV. However, CPE occurred earlier.

Table I shows the cross reactions among the three herpes virus-infected BK cells by the FA method. There was no clearly defined difference in the intensity of fluorescence caused by use of heterologous anti-herpes serum conjugate.

Results of cross testing by CF are shown in Table II. No significant difference was detected among the BHM, BHV, and Allerton viruses.

Relationships among the soluble antigens are shown in Fig. 3. The contiguity of precipitin lines produced between the three antigens and sera can readily be seen.

Sections of skin lesions from cattle infected with each of four viruses were reacted with the three anti-herpes FA (BHM, BHV, or Allerton) (Table III, Fig. 4). Fluorescence could be seen only in tissues of the herpes-infected animals. No detectable fluorescence identified the Neethling virus after exposure to the anti-herpes FA. Fluorescence could be detected in lesions during 6 to 8 days after they appeared (5 to 6 days postinoculation) but not in older lesions. Virus could be isolated from the lesions produced with all four viruses during this period.

DISCUSSION

Our data support previous work \(^3\) which established the relationship between BHM and BHV indicated by VN and VGDP tests. Other work \(^7\) also established a relationship between BHM and Allerton virus. This was confirmed in our laboratory and suggests that BHM and Allerton virus are similar, if not identical. Therefore, cumulative evidence indicates that the three dermatropic herpes viruses, BHM, BHV and Allerton are serologically identical. Each virus is neutralized by antibodies against the other two \(^3\)*. Furthermore, the AGDP test (Fig. 3) shows that the three soluble antigens are identical. This was confirmed by the FA and CF tests. Slight quantitative differences in fluorescence may be due to minor antigenic variations or to differences in the virus invasiveness. We agree with previous work \(^11\) that suggests that there is little justification for the three names.

Historically, the Neethling pox virus has been associated with the severe form of "lumpy skin disease". Unlike this virus, the skin lesions produced by the dermatropic bovine herpes virus (DBHV) is usually milder but may be mistaken for those of the Neethling virus. The DBHV studied here did not cross react with the Neethling virus and our limited studies with this virus against anti-BHV antibody on the AGDP test were negative. This gives additional evidence of the serological differences between the DBHV and the Neethling pox virus.

Results of FA tests of frozen skin sections and of herpes-virus-infected BK cells indicate that the techniques may be useful for identifying the bovine herpes dermatropic virus.

When fluorescence was observed in skin lesions, herpes virus could be isolated. Fluorescence of herpes-virus-infected BK cells precedes detectable CPE and may be useful for the rapid identification.

We emphasize that identification by FA staining of herpes virus in a skin lesions depends to a very large degree upon selection of a recently developed lesion.

ACKNOWLEDGEMENT

The authors acknowledge the excellent technical assistance of Mrs. Grohoski and

REFERENCES

TABLE III

Cross Reactions Using Bovine Skin Lesions Caused by Bovine Herpes Mammillitis, Bovine Herpes, Allerton, and Neethling Viruses and Herpes Fluorescent Antibodies

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Fluorescent Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BHM</td>
</tr>
<tr>
<td>Bovine herpes mammillitis (BHM)</td>
<td>+</td>
</tr>
<tr>
<td>Bovine herpes virus (BHV)</td>
<td>+</td>
</tr>
<tr>
<td>Allerton</td>
<td>+</td>
</tr>
<tr>
<td>Neethling</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Fluorescence in cell.
- = No fluorescence in cell.

Figure 1 – Bovine herpes virus (BHV)-infected bovine kidney cells. Twenty-four hours postinoculation. X450. Cells stained with BHV fluorescent antibody. Three fluorescent cells in a field of normal-appearing non-fluorescing cells.
TABLE I
Cross Reactions Using Various Herpes Viruses Infected Bovine Kidney Cell Cultures and Fluorescent Antibodies

<table>
<thead>
<tr>
<th>Viruses</th>
<th>BHM</th>
<th>BHV</th>
<th>Allerton</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine herpes mammillitis (BHM)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bovine herpes virus (BHV)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Allerton</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Fluorescence in cell

TABLE II
Cross Complement Fixation (CF) Tests Using Bovine Herpes Mammillitis (BHM), Bovine Herpes (BHV) and Allerton Viruses Convalescent Bovine Sera and Cell Culture Antigen

<table>
<thead>
<tr>
<th>Convalescent Bovine Sera</th>
<th>Cell Culture Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPI</td>
</tr>
<tr>
<td>BHM</td>
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</tr>
<tr>
<td>BHV</td>
<td>23</td>
</tr>
<tr>
<td>Allerton</td>
<td>33</td>
</tr>
</tbody>
</table>

DPI = days postinoculation sera collected.
Figure 2 – Bovine herpes virus (BHV)-infected bovine kidney cells 36 hours postinoculation. X100. Cells stained with BHV fluorescent antibody. Clear acellular areas bordered by large multinucleated fluorescing cell resulting from cytopathogenic effect of the BHV.

Figure 3 – Agar gel diffusion precipitin cross reactions with Allerton, bovine herpes virus and bovine herpes mammillitis antigens and sera.
Figure 4 – Section of skin lesion from bovine herpes virus (BHV)-infected steer. Five days postinoculation. X450. Stained with BHV fluorescent antibody. Epithelial cells in epidermis around hair follicles strongly fluorescent. Numerous infiltrating cells (neutrophils, etc.) exhibit non-specific fluorescence.
REPORT OF THE COMMITTEE ON FOREIGN ANIMAL DISEASES

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


Sub-Committee on Vesicular Diseases:

In this past year we experienced in the United States an epizootic of Venezuelan Equine Encephalomyelitis (VEE), a disease heretofore regarded as an exotic animal disease, although it was recognized that Type II VEE virus existed in endemic foci in Florida where clinical infection in man was diagnosed in 1968. The outbreak in the United States was an extension of the epizootic which began in Central America in 1969 and spread to this country via Mexico. In June of 1971 the Internal Office of Epizootics received an official report from the Government of Cuba that an outbreak of African Swine Fever was occurring on that island, a land mass somewhat analogous to Plum Island in its geographical relationship to the North American Continent but lacking of course the security measures for confining the virus of ASF to the island.

Apparently the VEE epizootic in horses has been confined to Texas, and information obtained on the Cuban outbreak would indicate that ASF has been restricted to the province of Havana by virtue of the aggressive measures of the Cuban Government and cooperation of the people. Despite our political and philosophical differences, we must admire the effectiveness of the Cuban control measures once their officials recognized and accepted the threat which this disease constituted to their swine and the country's economy.

As a consequence of our own program within the United States to control the epizootic of VEE in Texas and the measures taken by Cuba aided by the Soviets and Canadians to control ASF on that island, the immediate threat of these highly communicable and fatal diseases of animals appears to have been contained. Nevertheless the proximity of the occurrence of the most serious of swine disease on a land mass controlled by a government with which we have no exchange of medical and veterinary intelligence poses a serious problem for our swine and other livestock industries and those of Latin America and Canada.

Our recent experiences with foreign animal diseases to include the FMD
REPORT OF THE COMMITTEE

outbreaks in Mexico in the 1940's and 50's would suggest that we need to re-examine our policies regarding the threat of foreign animal diseases to the Western Hemisphere and develop a Monroe Doctrine, if you will, to prevent the establishment of extension within this hemisphere of exotic animal diseases which would threaten the livestock and pleasure animals of this country.

Your Committee on Foreign Animal Diseases will evaluate the feasibility of developing guidelines for such a policy during the coming year and report to you on our findings. Specifically, we shall examine the VEE epizootic using our experience as a model for study of our prevailing policies to prevent the entry of foreign animal diseases, the effect of legislation on exercising control measures, isolationism vs. confrontation of disease problems outside the continental limits of the United States, the extent of current research applicable to animal diseases foreign to the United States, and the application of research findings to policy and preventative measures.

The purpose of the Committee in undertaking this evaluation is to provide further guidelines for preventing the entry of new animal diseases into the United States. We believe if surveillance reports of a foreign animal disease indicate the possibility of their encroachment and entry into the United States, we should strike a cooperative blow with our research, technical and logistical resources to eradicate the threat as far from our borders as possible.

Foreign Animal Disease Priorities and Consultants

The Committee recognizes that the total bank of knowledge in veterinary medicine is now doubling every few years. The ever-increasing number of specialty groups attest the fact that no man can now be fully competent in all of the disciplines of veterinary medicine.

The updating of available information essential to the control of a major infectious domestic disease now requires the assembly of a team of experts from the various disciplines if all aspects of epidemiology, pathogenesis, etiology, diagnosis, treatment, biologics development, field management, control and eradication are to be accomplished most efficiently.

Since this is true for a major domestic disease, it is obvious that when a serious foreign animal disease appears no organization or federal agency has the inhouse competence to make all of the decisions necessary for control and eradication without a consultation with the most knowledgeable and experienced team the nation can assemble.

During the last forty years, the United States has acquired and must now live with a number of animal diseases which now handicap livestock production. Consistent with these and the views previously expressed, the Committee on Foreign Animal Diseases will:

a. Prepare a priority list of selected major exotic animal diseases which pose a serious threat to United States livestock production.

b. Assemble a list of United States and foreign veterinarians deemed most knowledgeable and experienced with each of these diseases.

Men from these lists will be utilized to periodically update the Committee’s Foreign Animal Disease Handbook and other plans and recommendations for control of exotic diseases. These lists of diseases and experts will be made available to United States regulatory agencies.
Potential Sources for Introduction of Foreign Animal Diseases

The Committee recognizes the danger of international garbage being a prime vehicle for the introduction of foreign animal disease, and has information that international garbage is not handled and disposed of in all cases in a manner that would preclude the introduction of foreign animal diseases. The Committee has been informed that the Agriculture Quarantine Inspections Division and the Animal Health Division of Agriculture Research Service are strengthening protective measures in garbage handling. The Committee urges that the additional protective measures be implemented without delay.

The Committee on Foreign Animal Diseases has reviewed and unanimously endorsed the Resolution, Mandatory Agricultural Clearance of International Carriers, to be presented to the USAHA for approval.

Status of Foreign Animal Diseases

**Venezuelan Equine Encephalomyelitis**

VEE spread from Mexico into the United States in the past year. An account of the epizootic in the United States is given in a detailed report elsewhere in these proceedings. While confined to Texas this year, it is the opinion of many experts that the virus will continue to spread even in the presence of a 90% immune equine population either through the non-vaccinates or some other virus host system. In Mexico, a massive vaccination campaign which was initiated with the Department of Defense vaccine is being pursued with vaccine produced in Mexico. The Committee on Foreign Animal Diseases regards the uncontrolled movement of horses as an initial contributory factor in the early spread of the disease prior to the establishment of quarantine and controlled movement of horses. The Committee prepared and submitted a Resolution which would seek legislative authority for state and federal livestock officials to institute quarantine and control measures when a foreign animal disease appears as an imminent threat to the livestock and before the disease actually exists in this country.

**Rinderpest**

The rinderpest situation remains substantially unchanged since the last report. The control program in Eastern Africa continues to progress effectively, but the maintenance activities in previously controlled areas lags, and there have been a number of outbreaks which pose serious threats to the ultimate goal of eradication. New rinderpest foci have been identified in India, Lebanon, Sudan, Afghanistan and several countries of West Africa.

**Contagious Bovine Pleuropneumonia**

C.B.P.P. continues to be a major epizootic disease in Africa. Vaccination continues to be the major control thrust and controlled movement of animals or elimination of affected animals from enzootic areas continues to be neglected. Further research is needed on more effective diagnostic systems and development of improved vaccines.

Australia is approaching the final phases of its eradication program and the residual infected areas are well isolated.

**Hemoproteozal Diseases and Trypanosomiasis**

This group of diseases continues to be the major constraint to livestock development in the tropics. Trypanosomiasis and East Coast Fever are endemics in large areas of Africa. Progress is being made in development of immunizing agents
against these diseases and new systems for vector control are being organized by the U.S. Agency for International Development, the United Nations specialized agencies and a number of foundations.

Babesiosis and other protozoan diseases are being studied in South America and improved control systems for these diseases are developed.

_African Swine Fever_

African Swine Fever continues to be endemic in Spain, Portugal, and Africa with the activity in the Iberian Peninsula assuming a predominantly chronic nature. However, periodic acute outbreaks do occur in Spain.

The recent outbreak in Cuba posed a serious threat to the swine industry in the Western Hemisphere. Fortunately, Cuban authorities recognized early the significance of the disease and aided by Soviet and Canadian technical personnel quickly took the necessary steps to confine it to the Havana Province and completely eliminate the swine population in that affected area. The details of the Cuban Program are presented in a separate committee paper elsewhere in these proceedings.

**VESICULAR DISEASE SUBCOMMITTEE 1971 REPORT**

_Foot and Mouth Disease_

1970 has marked a further step forward in the control of foot-and-mouth disease in Europe. Twenty countries enjoyed complete freedom from the disease throughout the year which is a new record for the European Continent.

The 1970 incidence of FMD in the Danube Region and other important animal production and trade areas of Europe was the lowest so far recorded.

Sporadic outbreaks occurred in Italy, mostly during the period January to March 1970, with young stock and especially imported animals affected. There was a persistence of the disease throughout the year in several provinces of Spain with an extension of Portugal and part of Turkey was free for the third consecutive year. However, Anatolia has reported outbreaks every month, and in May 1970 type A$_{22}$ reappeared in several provinces of this region after nearly a year of absence.

In October 1969, Greece had an outbreak due to a sub-type of A virus markedly different from A$_{5}$ and A$_{22}$. A single vaccination against A$_{22}$ would not protect against the Greek strain (A Greece 1969). It was subsequently found that a second vaccination 3 weeks later with a trivalent vaccine containing A$_{5}$, would reinforce immunity to a satisfactory level against "A Greece 1969." Fortunately, this virus has not appeared again since January 1970. However, in January 1970 a strain of Type C virus appeared in Greece. It corresponded to the C strain found in Belgium (December 1969) and to one of the Latin American subtypes. In November and December 1970 other sporadic outbreaks were caused by Type O, most linked with imports of meat and live animals.

In many of the western European countries (The Netherlands, Belgium, Luxemburg, France, the Federal Republic of Germany, Italy, and Switzerland) the annual systematic trivalent vaccination of the entire cattle population has continued; the cattle population of large areas of Spain, Hungary, and frontier areas in Czechoslovakia, Greece, Turkey and some other countries have received with bivalent or monovalent vaccine according to local needs.
In the Near East, Type SAT 1 was confirmed in a sample from the Riyad district in Saudi Arabia in January 1970. This finding was the result of an epizootiological investigation following an occurrence of that type in Kuwait in November 1969 which had been the first diagnosis of Type SAT 1 in the Near East since the regional epizootic which started in 1962 and ended in 1963. During 1970, Kuwait had 3 outbreaks and Saudi Arabia had 5 outbreaks of Type SAT 1. In March 1970, one outbreak of Type SAT 1 occurred in Samaria territory controlled by Israel, affecting cattle, sheep, and goats. This outbreak was immediately suppressed and no spread occurred. General vaccination of all cattle and some sheep in Israel and in the controlled areas was subsequently carried out with inactivated vaccine.

In Africa, Type SAT 1 was identified in a localized outbreak in the frontier areas of Tanzania and Malawi. This type had not been recorded in either of the two countries previously. Foot-and-mouth disease remains prevalent throughout Africa with virus types SAT 1, SAT 2, A, O, and C being reported.

Foot-and-mouth disease continues to be enzootic in South America, with Types A, O, and C being reported. Control measures through comprehensive vaccination campaigns were continued. (See Table 1 for FMD outbreaks per country.)

The Central American Republics and Panama have been historically free of foot-and-mouth disease. One reason for remaining free of FMD has been their isolation from the infection in South America by the Darien Rain Forest on the Colombian-Panamanian border. A highway is presently being constructed through this 240-mile jungle barrier. This highway is scheduled for completion by 1976. When it is completed, it will eliminate one of the last remaining physical barriers to FMD spreading northward from South America. Should foot-and-mouth disease spread into Panama and Central America, detection with vigorous control and eradication measures would be required to prevent progress northward through Mexico and pose a real threat to the livestock industry in the United States.

At present, we have two U.S. veterinarians working as advisers with the Central America countries and Panama. Additional veterinarians are needed to adequately cooperate with and advise the animal health officials of each of the Central American countries. It is anticipated that two additional veterinarians and one program specialist will be added to the Central American force in the coming year. These advisers will cooperate with the veterinarians of each of these countries to develop effective programs for prevention, detection, and eradication of exotic animal diseases.

It should be noted that the Interamerican Development Bank (I.D.B.) has or is in the process of loaning millions of dollars to South American countries to control and ultimately to eradicate FMD. These loan agreements are intended to support stepped-up vaccine production and the establishment of expanded field vaccination programs. However, the loan agreements do not guarantee quality control of vaccines nor specifically provide systematic vaccination procedures. Although the loan activity has stimulated some inter-country planning as regards coordination of programs, overall sub-continent FMD control planning remains weak. Consideration must be given to establishing a strong organization perhaps patterned after the European Commission for FMD Control to assure maximum coordinated program activities.

The Mexico-U.S. Commission for the Prevention of Foot-and-Mouth Disease is staffed by three U.S. veterinarians and several Mexican nationals. The Commission
emphasizes the following activities in its disease surveillance and prevention program:

a. Investigation of suspicious cases of vesicular diseases and rinderpest. In 1970, 51 such investigations were made and 10 proved positive for vesicular stomatitis. As of September 27, 1971, 43 investigations have been made and 13 have been positive for vesicular stomatitis.

b. Formation of vigilance committees, composed of ranchers, to improve surveillance for FMD and rinderpest.

c. Visitation of livestock producers, officials of Cattlemen’s Associations, veterinary practitioners, Government veterinarians, experiment station personnel, and other Government officials to remind them of their patriotic duty to report all suspicious cases of vesicular conditions and rinderpest to the commission.

d. Maintenance of diagnostic capability at the Palo Alto Laboratory and constantly updating the antiserum stock so new strains of FMD or VS will not be missed

Legislation presently before Congress, if passed, will authorize the USDA to cooperate with the Governments of Mexico, the Central American countries, and Panama in prevention and eradication of any communicable disease of animals and poultry which the U.S. Secretary of Agriculture deems is a threat to the livestock or poultry industries of the United States.

**Vesicular Stomatitis**

**United States Surveys**

Thirty-four of 212 Louisiana deer serums collected in game management areas during the 1970 hunting season had New Jersey type virus neutralizing antibodies. In Horry County, South Carolina, 11 or 25 swine serums were positive for New Jersey type Vesicular Stomatitis (V.S.). The positive serums came from one to two year old animals bled during the fall of 1970 following the diagnosis of V.S. on five North and South Carolina premises during the summers. On Ossabaw Island, Georgia, the presence of New Jersey type neutralizing antibodies in 80 percent of 73 cattle tested, plus the presence of type specific complement-fixing antibodies in six sera indicated recent vesicular stomatitis activity.

Four-hundred-ninetten Georgia mammal sera collected during 1964 were provided by Dr. Frank A. Hayes, Director, Southeastern Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, Georgia. Of these, 4 of 105 raccoons, 2 od 115 opossums, and 1 of 9 gray squirrels had neutralizing antibodies for New Jersey type virus.

Details of these survey results obtained by Diagnostic Services, Animal Health Division, National Animal Laboratory, will be published separately.

**Investigation**

There have been 85 vesicular investigations in the United States during the period of October 1, 1970, through September 30, 1971. The results are shown in Table 2.

**RESEARCH ON VESICULAR DISEASES**

**Vesicular Exanthema of Swine** – While vesicular exanthema of swine (VES) remains an extinct disease, some additional research has been reported on some
properties of the virus. Oglesby et al.\textsuperscript{1} found that particle to be ether resistant and not stabilized by cations. It had a sedimentation rate of 207 S, consisted of 80 percent protein and 20 percent single-stranded RNA. A heterogeneity density distribution in CsCl from 1.36 to 1.38 was found. The virus differed from most currently recognized groups but was most closely allied with the picornavirus group.

Uskavitch\textsuperscript{2} has brought the bibliography on VES up to date through June 1971.

\textbf{Vesicular Stomatitis} – Bats inoculated with vesicular stomatitis (VS) virus were found to have a relatively long viremia while hibernating. The cocal virus was the one studied and \textit{Aedes aegypti} mosquitoes which fed on viremia hibernating bats were able to transmit the virus to suckling mice.\textsuperscript{3}

Steck et al.\textsuperscript{4} studied two outbreaks of a disease in which vesicles were produced in the mouth. They were able to demonstrate infectious bovine rhinotracheitis (IBR) virus and specific antibody in most of the infected animals.

The microtiter technique was found significantly more sensitive for virus assay and antibody titer determination than the conventional plaque assay.\textsuperscript{5}

Several reports have been made on chemical studies of the coat protein and molecular biology of VS virus. Many involve the use of the temperature-sensitive (TS) mutants of the virus.\textsuperscript{6, 7, 8} Seventy-one spontaneous TS mutants were classified into five major groups by reciprocal complementation by Flamand and Pringle.\textsuperscript{6} Lafay and Berkaloff\textsuperscript{7} showed that such mutation is probably expressed at the level of the virus covering.

Cohen et al.\textsuperscript{9} studied the interaction of VS virus structural proteins with HeLa plasma membranes and Burge and Huang\textsuperscript{10} found the difference between VS virus and Sindbis virus glycoproteins was due mainly to the number of sialic acid residues per glycopeptide. Removal of these residues by mild acid treatment rendered the viral proteins indistinguishable.

\textbf{Foot-and-Mouth}

\textit{Epidemiology and Pathogenesis}. The pathogenesis and spread of foot-and-mouth disease (FMD) occupied an important place in the research literature of the last year.\textsuperscript{11} The epidemiology of the disastrous outbreak in Great Britain in 1967-68 continued to be studied. The weather was concluded to have been an important factor in the initial stage of the outbreak.\textsuperscript{12} Farm rats were eliminated as a major factor in spread from farm to farm.\textsuperscript{13} A field survey of milk samples collected during the outbreak showed that high titers of FMD virus (FMDV) could be found in all types of containers in which milk from infected premises was shipped to market.\textsuperscript{14} It was shown that virus could be shed in the milk as long as 33 hours before the appearance of clinical signs in the infected animal. Dawson\textsuperscript{15} in another study of the same outbreak found a strong correlation of the bulk milk collection system with the disease spread.

The spread of FMD in swine in Northwest Europe in recent years was examined by van Bekkum.\textsuperscript{16} He postulated that the high degree of immunity in vaccinated cattle has led to adaptation of the virus to pigs. Transmission over large distances was believed due to shipment of infected pigs during the preclinical stages of the disease. Gocic\textsuperscript{17} has examined FMD epidemiology in South America.

Calf thyroid cells were found most sensitive for detecting virus during large air volume sampling experiments.\textsuperscript{18} Sellers \textit{et al.}\textsuperscript{19} showed FMDV could survive in the throats of humans that had been exposed to virus aerosols. However, none of these
individuals developed clinical disease.

Graves et al.20 found that transmission of FMD from an intranasally-infected donor animal could occur for 8 days after the inoculation and terminate rather abruptly at that time. The longest incubation period occurred in the contact steer during the first day after inoculation and the shortest on the third day.

Boyadzhyan et al.21 showed that FMDV could survive in ticks for up to 74 days but that the disease could not be transmitted to susceptible mice by the bite of these infected ticks. Menzani et al.22 isolated IBR virus and type C FMDV from the same outbreak in cattle in which FMD was characterized by unusual respiratory lesions.

Sellers23 has done a literature review of the quantitative aspects of the spread of FMD.

Vaccine and Vaccination. Allergic reactions in cattle after FMD vaccination have resulted in several reports.24, 25, 26, 27 Fungus infections follow as a complication of skin allergic reactions.24 One factor causing the reaction has been isolated as a cell lipoprotein25 from BHK-21 cells in which the FMDV vaccine antigen was grown. Another substance under suspicion was found by Marthaler27 to be carboxymethylcellulose used with some Frenkel and tissue culture virus vaccines. Brown Swiss were found more sensitive than Semmental cattle. The incidence of adverse reactions in 7.2 million vaccinated cattle was reported by Lorenz and Straub26 and found to be what is considered to be very low – 620 reactions or 0.0086%.

Immunologic response of lambs and maternal transfer of immunity from ewes vaccinated with oil emulsified vaccine was reported by Cunliffe and Graves.28 Morgan et al.29 found 160 nanograms was the minimum effective antigen concentration for immunizing guinea pigs or swine. In another study, tongue epithelium from FMD-immune cattle, one year after their infection, grew type A22 and type O FMDV as well as tissue from noninfected animals.30

Ethylethylenimine has been satisfactorily used as a FMDV inactivant for vaccine preparation.31 Vaccine prepared with this inactivant and diethylaminoethyl-dextran (DEAE-D) as adjuvant gave good immunity in swine up to 18 weeks after vaccination.32, 33

Two reports showed an alteration in relative proportions of milk proteins occurred after FMD vaccination.34, 35

Attenuated FMDV used as vaccine could be distinguished from the virulent virus by genetic markers.36 More rapid attenuation could be achieved by passage of the virus in chick embryos at 31 C.37

Growth of virus in submerged cultures for use as vaccine antigen and in vitro safety testing of vaccines by using tissue culture systems have been described by British workers.38, 39

Discussion of future prospects for FMD vaccine development, experiences with use of Frenkel vaccines and live virus in FMD control have been published.40, 41, 42

Molecular Biology and Pathogenesis. Campbell43 found that FMDV adsorbed significantly differently to homogenized lung than to homogenized mouse brain. Adsorption to homogenized mouse muscle and adsorption properties of both and is the tissue affected in the intact animal. Urethan at high levels caused reduction in resistance of mice to FMDV infection whereas low doses of the same compound
increased the resistance.\textsuperscript{44} Pyran copolymers also induced resistance in mice.\textsuperscript{45} Martinsen\textsuperscript{46} found that agarose greatly enhanced plaque formation of small-plaque-forming virus compared to conventional agar overlay.

The structure of the virus particle, its protein capsid and mechanism of its breakdown at lower pH have been reported.\textsuperscript{47, 48, 49} Ascione \textit{et al.}\textsuperscript{50} extracted an amino acid incorporation initiator from ribosomes of infected BHK cells by high salt concentration. This factor induced incorporation in the cell-free system in the presence of FMDV-RNA.

Trautman and Sutmoller\textsuperscript{51} demonstrated that the FMDV RNA genome could be masked by taking on the coat of bovine enterovirus in dually-infected tissue cultures.

\textit{Techniques.} Mohanty and Cottral\textsuperscript{52} found a fluorescent antibody technique superior to plaque assay or cytopathic effect in inoculated tissue cultures for early detection of FMDV in cultures.

The bentonite flocculation test was proposed as a useful procedure for screening FMDV antibody.\textsuperscript{53} Microneutralization test in tissue cultures compared favorably with the neutralization test in suckling mice.\textsuperscript{54} The use of agar gel diffusion has increased in importance in the study of FMDV antibody. Cowan and Wagner\textsuperscript{55} developed a precise quantitative procedure for study of antibody from infected or immunized guinea pigs by use of radial immunodiffusion. The screening of large numbers of serums for evidence of past infection by detection of virus infection-association (VIA) antibody was reported by McVicar and Sutmoller.\textsuperscript{56} Patty\textsuperscript{57} found that such factors as stage of trypsinization, cell concentration and fluid used to make virus dilutions all influenced the susceptibility of tissue cultures to virus infection.

REFERENCES


32. Versuche zur Schutzimpfung von Schweinen mit monovalenten Athylathylenimin
FOREIGN ANIMAL DISEASES


**RECOMMENDATIONS AND RESOLUTIONS**

**Foot and Mouth Disease Vaccine**

This Committee has been informed that the technical advisory committee to Plum Island has recommended stockpiling of FMD vaccine for possible use in the United States. The Committee does not have information concerning the technical and economic feasibility of the proposal; however, if vaccine is stockpiled we recommend that virus be produced and stored as a raw virus product rather than production and storage of a finished vaccine.

Authority to Control Movement of Livestock Prior to Confirmation of Disease

VEE erupted explosively in June 1971 in south Texas as predicted. Control procedures including spraying and vaccination were massive and expensive. Because equine traffic control procedures could not be implemented until a laboratory confirmed diagnosis was made, the movement of horses out of Cameron and Hidalgo counties of Texas in late June and early July contributed greatly to the dissemination of the VEE virus in the 1971 Texas outbreak. Additionally, in spite of our awareness of the steady northward progression of the disease through Tamalipas State, Mexico, we could not implement one of the most effective procedures to control the spread of disease. Fortunately an effective vaccine was available for contingency use.

But what of other "exotic" diseases for which no vaccine exists? How can we possibly expect to control a rapidly spreading disease without the authority to institute control procedures prior to the arrival of the disease? It is increasingly apparent that the present speed and mode of livestock transportation increases the threat of introduction and spread of a new disease(s). Therefore a re-evaluation of our concepts of disease prevention and control is required which will provide authority for state and federal livestock disease control officials to act before the fact rather than after the disaster.

Therefore, the Committee on Foreign Animal Disease submits the following resolution for consideration by the U.S. Animal Health Association and recommends its adoption:

Whereas animal disease control procedures cannot be imposed within this country until the disease which threatens the health of U.S. livestock is confirmed in this country by laboratory diagnosis and

Whereas lack of authority to implement disease control procedures results in
unnecessary and undesirable delays in preventing the spread of exotic diseases and

Whereas authority to prohibit livestock movement in the areas immediately threatened may be the primary control measure to contain a disease within a limited area in this country until other control procedures can be implemented:

NOW THEREFORE BE IT RESOLVED, THAT the USDA in cooperation with the appropriate states, take action to obtain the Legislative authority for state and federal livestock officials to institute quarantine and control procedures when a foreign disease is recognized as an imminent threat to the livestock and before the disease actually exists in this country.

BE IT FURTHER RESOLVED, THAT copies of this Resolution be forwarded to the National Association of State Departments of Agriculture and to the Secretary of the United States Department of Agriculture.
Evaluation of the Immunodiffusion Test  
For the Diagnosis of Equine Infectious Anemia*

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INTRODUCTION

Throughout the years many research workers have met with failure in attempts  
to develop a practical, reliable test for the diagnosis of all forms of equine  
infectious anemia (EIA). Practically all conventional laboratory techniques were  
tried. These early efforts are described in the monogram of Dregus and Lombard.  
In recent years, a series of reports by Japanese research workers described the  
replication and serial passage of the virus causing EIA in an equine-leukocyte tissue  
culture system.3-7 The same authors applied Kolmer's complement fixation (CF)  
test and complement dilution variation of the CF test in conjunction with the  
equine-leukocyte culture system to assay for the EIA virus and to demonstrate a CF  
antibody in the serum of horses infected with the virus experimentally. They were  
able to demonstrate CF fixing antibody in 43 of 46 horses that had been inoculated  
with the EIA virus. The CF antibody was generally demonstrable between 17 and  
40 days after inoculation. Duration of the CF antibody was variable. It persisted for  
only 2 or 3 days in some but never persisted beyond 60 days. Recurrent febrile  
attacks did not recall a demonstrable CF antibody response. Since the appearance  
and duration of the CF antibody were variable and transient in nature these  
methods are not suitable for the diagnosis of the chronic and inapparent forms of  
EIA.

Kono8 utilized the equine-leukocyte tissue culture system to demonstrate the  
appearance of a neutralizing antibody in the serum of 14 of 25 horses that had been  
injected experimentally. The variable appearance of the neutralizing antibody  
precluded the use of this method as a reliable practical diagnostic test for EIA. Moore et al.  
reported a tube precipitin test for the diagnosis of EIA, but it has not  
received widespread acceptance due to false positives and negatives.10

More recently, Coggins and Norcross1 reported the development of an  
immunodiffusion test (IDT) for the diagnosis of EIA. They detected an antigen in  
the spleen associated with the infection of EIA that reacts with antibodies  
produced in response to infection with the EIA virus that is demonstrable as a  
precipitin antibody in an agar-gel-immunodiffusion system.
The purpose of this report is to present data which indicates that the immunodiffusion test (IDT) developed by Coggins and Norcross appears to be a reliable diagnostic test for all forms of EIA with the exceptions as follows: (1) Horses in the early stages of infection may give negative results because the precipitin antibody may not appear until 21 or more days after exposure to the virus; (2) Foals nursing positive dams may give false positive results due to colostral antibody.

MATERIALS AND METHODS

The immunodiffusion test was conducted by personnel at the New York State Veterinary College. The methodology employed was essentially as described by Coggins and Norcross. Noble's special agar was prepared in borate buffer at concentrations of 1% and 2%. Five ml. of the 2% agar were placed in an 85 mm. disposable Petri dish and allowed to solidify. It was then overlayed with 15 ml. of the 1% agar. A special agar punch fitted with 7 mm. punches was used to make a 7 well Ouchterlony pattern in the top layer of agar. The 6 peripheral wells were 3 mm. from the central well. Minced spleen collected from a pony during the febrile stage was frozen and thawed 3 to 5 times and used as antigen in the central well. The reference positive serum was placed in the top and bottom peripheral wells, and serums to be tested were placed in the other 4 peripheral wells. A positive reaction was determined by the appearance of a precipitin line that forms a line of identity with the reference serum. The tests were read at 24 and 48 hours and, if necessary, at 72 hours.

Experiment I.

Duplicate numerically coded serum samples from 7 positive and 9 negative equines maintained in screened isolation stalls at the Veterinary Research Farm at Louisiana State University were submitted to the New York State Veterinary College for the immunodiffusion test for equine infectious anemia. Personnel conducting the test were not advised as to the number of positive and negative animals in the group.

With the exception of H-88* which was naturally infected, the 7 positive equines had been inoculated with serum or blood containing EIA virus. Four received inoculum containing the Wyoming strain of EIA. Two developed EIA after receiving whole blood from field cases H-87 and H-88. The remaining positive (H-88) was a field case that had signs of EIA infection and was proven positive by subinoculation of blood to P-78.

The 9 negative ponies were proven negative by subinoculation of 50 ml. of whole blood in a round robin series involving 10 ponies. In addition, 50 ml. of whole blood from each of the 10 ponies was pooled and given intravenously to a horse (H-28-1) previously proven negative by horse inoculation test. The 10 ponies and H-28-1 did not develop a febrile response or hematologic changes indicative of EIA infection. Temperatures were taken twice daily, and hematologic studies were

* Grafar Corp., Detroit, Michigan 48238.
† Throughout this paper H refers to standard horse and P refers to Shetland Pony.
made twice a week. After an observation period of 60 days, H-28-1 was given serum known to contain the Wyoming strain of EIA. He developed signs of acute EIA. He was killed when the signs of the disease became severe, and specimens were utilized for other studies.

In view of the above observations, it was concluded that the 10 ponies in the round robin were free of EIA infection. Since being employed as negative animals for the studies reported in this paper, 1 of the 10 ponies (P-78) developed signs of EIA when given blood from a suspected field case (H-88), and four others developed EIA when given inoculum containing the Wyoming strain of EIA.

The results of the immunodiffusion test for the duplicate coded samples on these 17 horses were in full agreement with the status as determined by the procedures described above. The results along with other pertinent data are summarized in Table 1.

| Table 1—Record of Samples and Results of Experiment I |
|---------------------------------------------|-----------------|-------|-------|
| Animal No. | Strain | Date Inoc. | Sample Code | Results |
| P-5 | W² | 9-25-67 | 001 044 | Pos. Pos. |
| P-11 | W | 2-8-67 | 003 042 | Pos. Pos. |
| P-14 | LSU88 | 4-27-70 | 007 038 | Pos. Pos. |
| P-41 | W | 11-16-67 | 011 034 | Pos. Pos. |
| P-78 | LSU88³ | 5-11-70 | 027 018 | Pos. Pos. |
| H-88³ | Field | Unknown | 037 008 | Pos. Pos. |
| P-70 | Neg. | | 017 028 | Neg. Neg. |
| P-71 | Neg. | | 019 026 | Neg. Neg. |
| P-72 | Neg. | | 021 024 | Neg. Neg. |
| P-73 | Neg. | | 023 022 | Neg. Neg. |
| P-74 | Neg. | | 025 020 | Neg. Neg. |
| P-81 | Neg. | | 029 016 | Neg. Neg. |
| P-83 | Neg. | | 031 014 | Neg. Neg. |
| P-85 | Neg. | | 033 012 | Neg. Neg. |
| P-86 | Neg. | | 035 010 | Neg. Neg. |

¹Throughout this paper P refers to Shetland Pony, and H refers to standard size horse.
²W=Wyoming strain of EIA.
³Positive EIA status confirmed by subinoculation of whole blood to P-78.
⁴Designations given to these Louisiana field strains of EIA.
EVALUATION OF TEST FOR DIAGNOSIS OF EIA

Experiment I.
The horses utilized in this experiment were maintained at the Veterinary Research Farm of Louisiana State University. Single serum samples were submitted from H-19, H-27 and H-28 which were three of the principals of a 5 horse round robin conducted on Nov. 10, 1966. H-19 developed a febrile response following receipt of blood from H-27. H-27 remained normal after receiving blood from H-22. H-22 became febrile after receiving blood from H-28, but made an apparent recovery. She later developed acute EIA after a period of physical stress. H-20 which received blood from H-19 remained normal. H-20 was later given 10 ml. of plasma containing the Texas strain of EIA* but no febrile response occurred. From the results of this round robin it was tentatively concluded that H-20, H-27 and H-28 were naturally infected prior to performing the round robin. In addition, H-19 and H-22, the recipient animals for H-27 and H-28 respectively, became infected during the round robin. The results of the precipitin test being performed at Texas A&M University were negative prior to the round robin and remained negative for all 5 horses following the round robin.10

On May 12, 1969 blood from H-27 was given to P-33, a previously known susceptible pony. The pony developed a febrile response and hematologic changes commensurate with EIA. This reconfirmed the positive status of H-27.

After receiving the results of the IDT from New York State Veterinary College it was decided to determine if blood from H-19 was infective. P-85 was given 100 ml. of blood from H-19 on July 22, 1970 and developed a febrile response 9 days later. P-85 has made an apparent recovery but has experienced several febrile cycles. The results of the immunodiffusion test on serum from P-85 changed from a preinoculation negative to a postinoculation positive.

The animals H-62, H-63, H-64, H-66 and H-69 were purchased in West Texas. Their true status was not known when samples were submitted to New York State Veterinary College for the IDT. All were reported as negative to the IDT. Since receiving the results these horses have all proven susceptible to the Wyoming strain of EIA. They were utilized for other experimental work in progress.

A summary of the data for Experiment II is presented in Table 2. There is 100% agreement with the results of the immunodiffusion test and the status of the horse as determined by horse inoculation or by inoculation with known EIA virus after receiving the results of the IDT.

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* Obtained from Dr. R.W. Moore, Dept. of Veterinary Microbiology, College Station, Texas.
TABLE 2--Record of Samples and Results of Experiment II

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Strain</th>
<th>Date Inc.</th>
<th>Sample No.</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-19(^1)</td>
<td>LSU 27(^5)</td>
<td>11-10-66</td>
<td>045</td>
<td>Pos.</td>
</tr>
<tr>
<td>H-27(^2)</td>
<td>Field Strain</td>
<td>Field Infec.</td>
<td>046</td>
<td>Pos.</td>
</tr>
<tr>
<td>H-28(^3)</td>
<td>Field Strain</td>
<td>Field Infec.</td>
<td>047</td>
<td>Pos.</td>
</tr>
<tr>
<td>H-62(^4)</td>
<td>Status Unknown</td>
<td>Proved Susc.</td>
<td>049</td>
<td>Neg.</td>
</tr>
<tr>
<td>H-63(^4)</td>
<td>Status Unknown</td>
<td>Proved Susc.</td>
<td>050</td>
<td>Neg.</td>
</tr>
<tr>
<td>H-64(^4)</td>
<td>Status Unknown</td>
<td>Proved Susc.</td>
<td>051</td>
<td>Neg.</td>
</tr>
<tr>
<td>H-66(^4)</td>
<td>Status Unknown</td>
<td>Proved Susc.</td>
<td>052</td>
<td>Neg.</td>
</tr>
<tr>
<td>H-69(^4)</td>
<td>Status Unknown</td>
<td>Proved Susc.</td>
<td>053</td>
<td>Neg.</td>
</tr>
</tbody>
</table>

\(^{1}\) H-19 became infected with EIA in a round robin wherein she received blood from H-27. Her positive status was reconfirmed by subinoculation to P-85 on July 22, 1970.

\(^{2}\) EIA positive status determined by subinoculation of blood to H-19 and reconfirmed by subinoculation to P-33 on May 12, 1969.

\(^{3}\) EIA positive status determined by subinoculation to H-22 in a round robin. H-22 developed severe EIA after physical stress and was killed.

\(^{4}\) These horses have all proven susceptible to EIA (Wyoming strain) since the IDT was performed.

\(^{5}\) Designation given to this Louisiana Field Strain of EIA.

Experiment III.

This experiment was designed to determine the effect of temperature treatment of blood and serum on the accuracy of the immunodiffusion test. Nine positive animals, P-5, P-11, P-41, P-78, H-19, H-27, H-28, H-29 and H-88; and 9 negative animals P-70, P-71, P-72, P-73, P-74, P-81, P-83, P-85 and P-86 were used.

Two tubes of blood were collected in vacuatiners between 8:30 A.M. and 1:00 P.M. The samples were divided into 2 sets designated I and II. Set I was placed at room temperature in the laboratory (23-25\(^o\) C) for 4 hours and then placed in the ice box (4\(^o\) C) until 8:30 the following morning. Set II was placed behind the seat of a truck which was used as usual for the remainder of the day. This set remained in the truck until 8:30 the next morning.

Both sets were taken from their respective conditions at 8:30 A.M. the following morning and the serum collected after centrifugation. The serum from each set was divided into 4 aliquots in 1 dram screw cap vials and given subtreatments A, 4\(^o\) C for 22 hrs.; B, 37\(^o\) C for 22 hrs.; C, 56\(^o\) C for 22 hrs.; and D, -20\(^o\) C for 22 hours.

All samples were removed from their respective conditions and assigned a 5-digit code number from a random numbers statistics table. The 2X4 factorial design above resulted in 8 samples per horse. The experimental design is summarized in Table 3.
TABLE 3—A Study of the Effects of Temperature on the Accuracy of the Immunodiffusion Test for EIA

<table>
<thead>
<tr>
<th>July 6, 1970</th>
<th>July 7, 1970</th>
<th>Sub-Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Ideal Handling</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood placed at room temperature for 4 hours—ice box over night.</td>
<td>Both sets were centrifuged to collect serum at 8:30 A.M. Serum from each set was divided into 4 parts and treated as follows.</td>
<td>I-A—4°C for 22 hours.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I-B—37°C for 22 hours.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I-C—56°C for 22 hours.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I-D—Frozen for 22 hours.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>II. Adverse Handling</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood placed behind seat of truck at 10 A.M. on collection day. This set reached a temperature high of 104°F.</td>
<td></td>
<td>II-A—4°C for 22 hours.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II-B—37°C for 22 hours.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II-C—56°C for 22 hours.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II-D—Frozen for 22 hours.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Samples (8 per horse) were shipped airmail special delivery at 12:00 on July 8, 1970. They arrived at Cornell University the morning of July 10, 1970.

1 This same design was used for Experiments IV and V except the times for the main treatments and sub-treatments were changed (See Text).
Of the 144 samples in Experiment III, 24 were lost in transit due to leakage. Six of these samples were from positive and 18 were from negative animals. The IDT results on the remaining 120 samples reported was 100% in accordance with the previously known status of the animals.

**Experiment IV and V.**

Experiments IV and V are replicates of each other and follow the 2X4 factorial design used in Experiment III. Four tubes of blood were taken from 8 positive animals, P-5, P-11, P-41, P-78, H-19, H-27, H-28, H-29 and 8 negative animals, P-71, P-72, P-73, P-74, P-81, P-83, P-85, and P-86. Set I from each experiment was placed at room temperature (23-25°C) for 4 hours and then in the ice box (4°C) for 16 hours. Set II from each experiment was placed at 37°C for 20 hours.

Serum was collected after centrifugation and submitted to subtreatment A, 4°C for 10 hours; B, 37°C for 10 hours; C, 56°C for 10 hours; and D, -20°C for 10 hours.

The serum samples were identified by using 5-digit numbers from a random statistics table. A different set of numbers was employed for each experiment. Samples from Experiment IV were shipped separately in rubber-lines, screw cap 1 dram vials whereas samples for Experiment V were shipped in heat-sealed glass ampules.

The combination of Experiments IV and V resulted in 16 samples per animal. The immunodiffusion test results reported from New York State Veterinary College were 100% in agreement with the known status of the animals.

**Experiment VI.**

Samples from a herd of 55 Quarter Horses were submitted to New York State Veterinary College for IDT. No signs of EIA have been noted in this herd of horses during the last 10 years. Seven adult horses and 4 foals were reported as positive. The data on these horses is presented in Table 4. Besides the 7 positive mares there is only 1 other horse, a 17 year old stallion, that is above 10 years. He was added to this herd 4 years ago. Analysis of the records in this herd indicates that positive mares may produce negative foals. H-1 has a 3 year old and a 7 year old progeny that are IDT negative. H-2 has 2 IDT positive progeny (H-3 and H-4), but also has 2 IDT negative progeny in the herd. One of the negatives is 6 years old and the other is 1 year old. H-3 has 1 IDT positive progeny (H-5) and 2 IDT negative progeny, a 5 year old and a 9 year old in the herd. H-4-1, H-5-1, H-6-1 and H-7-1 are suckling foals less than 6 months of age. All 4 foals are IDT positive. In addition, H-4 has 2 IDT negative progeny (3 year and 9 year old); H-5 has 1 IDT progeny (3 years); H-6 has 3 IDT negative progeny (1, 2 and 4 years old); and H-7 has 2 IDT negative progeny (1 year and 7 years old). For convenience the above data is summarized in Table 5.

One can only theorize as to the mode of transmission in this herd. It is of importance to note that EIA positive mares can apparently produce negative foals. This has been verified under experimental conditions. However, positive mares may also abort as a result of acute EIA infection and mares having the subchronic to inapparent form of EIA may give birth to foals that die of EIA at approximately 60 days of age. The source of infection in this case is thought to be either prenatal or colostrum.
TABLE 4--Data on IDT Positive Horses in a Herd of 55 Quarter Horses

<table>
<thead>
<tr>
<th>Horse Ident.</th>
<th>Age</th>
<th>Sex</th>
<th>Foaled By</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-1</td>
<td>23 yrs.</td>
<td>F</td>
<td>X^4</td>
</tr>
<tr>
<td>H-2^1</td>
<td>21 yrs.</td>
<td>F</td>
<td>X^4</td>
</tr>
<tr>
<td>H-3</td>
<td>15 yrs.</td>
<td>F</td>
<td>2</td>
</tr>
<tr>
<td>H-4</td>
<td>14 yrs.</td>
<td>F</td>
<td>2</td>
</tr>
<tr>
<td>H-5</td>
<td>14 yrs.</td>
<td>F</td>
<td>3</td>
</tr>
<tr>
<td>H-6</td>
<td>13 yrs.</td>
<td>F</td>
<td>X^4</td>
</tr>
<tr>
<td>H-7^2</td>
<td>10 yrs.</td>
<td>F</td>
<td>X^4</td>
</tr>
<tr>
<td>H-4-1</td>
<td>3 1/2 mos.</td>
<td>F</td>
<td>4</td>
</tr>
<tr>
<td>H-5-1^3</td>
<td>5 1/2 mos.</td>
<td>M</td>
<td>5</td>
</tr>
<tr>
<td>H-6-1</td>
<td>3 1/2 mos.</td>
<td>F</td>
<td>6</td>
</tr>
<tr>
<td>H-7-1</td>
<td>3 1/2 mos.</td>
<td>M</td>
<td>7</td>
</tr>
</tbody>
</table>

^1 Intravenous inoculation of 300 ml. of heparinised blood to a pony produced an IDT change from negative to positive by postinoculation day 32 but a febrile response did not occur until postinoculation day 88.

^2 Intravenous inoculation of 300 ml. of heparinized blood produced EIA in a pony after a 16 day incubation period.

^3 Following weaning negative IDT results have been obtained.

^4 These mares are no longer in the herd.
TABLE 5--IDT Results of the Progeny of the IDT Positive Mares in a Herd of 55 Quarter Horses

<table>
<thead>
<tr>
<th>IDT Positive Mare</th>
<th>Progeny</th>
<th>IDT Results of Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ident.</td>
<td>Age</td>
<td>Ident.</td>
</tr>
<tr>
<td>1</td>
<td>23 yrs.</td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>21 yrs.</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>14 yrs.</td>
<td>12</td>
</tr>
<tr>
<td>99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15 yrs.</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>9 yrs.</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>14 yrs.</td>
<td>37</td>
</tr>
<tr>
<td>4-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>14 yrs.</td>
<td>17</td>
</tr>
<tr>
<td>5-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>13 yrs.</td>
<td>26</td>
</tr>
<tr>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>109</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>10 yrs.</td>
<td>10</td>
</tr>
<tr>
<td>119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 This animal was proven positive by horse inoculation. P-80 became febrile after an incubation period of 19 days.
2 Following weaning negative IDT results have been obtained.
3 Subinoculation of 300 ml. of whole blood from H-2 to a negative pony caused a change from IDT negative to IDT positive in 32 days, but a febrile response did not occur until 88 days postinoculation.

DISCUSSION
On the basis of results obtained, the immunodiffusion test conducted at New York State Veterinary College appears to be accurate for the diagnosis of all forms of EIA except in the early stages of infection. Foals nursing infected dams may give false positive results. Animals known to have been infected longer than 3 years and 8 months were detected as positive, and in one case (H-19) the positive status was reconfirmed after receipt of the IDT results. In addition, a false negative has not
been detected up to this point. The 9 ponies proved free of EIA by horse inoculation were detected as negative by the IDT. Furthermore, 4 of the 9 negatives have since proven to be susceptible when given inoculations containing EIA virus. The status of H-62, H-63, H-64, H-66 and H-69 was not known when samples were submitted. The IDT results were reported as negative for the above horses. All of the above horses have developed EIA when given inoculum containing the Wyoming strain of EIA. They were utilized for other experiments in progress. Among the positive horses the Wyoming strain of EIA was represented along with 2 strains from Louisiana. P-14 became infected on April 27 when inoculated with blood from a naturally occurring case. P-78 became infected when inoculated with blood from H-88, a naturally infected horse.

H-5-1, which was weakly positive while nursing its dam (H-5) has reverted to negative since weaning, but H-6-1 still remains positive after weaning. Tests are being conducted at 2 week intervals following weaning.

Although the results of the IDT as conducted at New York State Veterinary College show a high degree of correlation when compared to the status of animals determined experimentally, one should proceed with caution before advocating widespread use of this test in numerous laboratories.

The technique appears to be simple; however, it requires a degree of expertness and experience in order to interpret the results. Adequate experience in reading the results of known positive and negative samples should be obtained before results are reported.

REFERENCES

Immunodiffusion Detection of Antibodies to Equine Infectious Anemia Virus Infection Associated Antigen

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Department of Veterinary Science
University of Kentucky – Lexington, Kentucky 40506

Equine infectious anemia (EIA) is an infectious disease of animals of the genus *Equus* which occurs in intensities varying from peracute to chronic life-long infections lacking clinical signs of disease. The positive diagnosis of which has long been based on transmission of the disease from suspected to susceptible horses by injection of whole blood, plasma or serum from the suspect. This method of diagnosis is limited not only by the expense and time involved, but inconclusiveness resulting both from the lack of a reliable means of selecting susceptible test animals and the near asymptomatic infection induced in some test animals. Recently, Coggins and Norcross1, using spleen material from a horse acutely infected with EIA, and Nakajima and Ushimi2, using EIA virus material purified from EIAV-infected horse leukocyte cultures, demonstrated that serum from horses infected with EIAV would, in agar gel, form a precipitate when diffused against these substances. This finding has been used as the basis of an accurate, rapid, inexpensive diagnostic test for EIAV infections in horses.3, 4

Procedures for the preparation of equine infectious anemia infection associated antigen (EIAA) and techniques for performing the EIA-immunodiffusion test (EIA-IDT) were investigated.

MATERIALS AND METHODS

*Immunodiffusion:* Double diffusion of reagents for the EIA-IDT was carried out in a layer of 0.5% agarose (Gallard-Schlesinger Chemical Mfg. Corp., L.I., NY) in 0.2 M, pH 8.6 borate buffer. The thickness of the agarose gel was adjusted so that the reagent wells had a volume of 0.1 ml. The agarose-gel was poured into plastic petri dishes (100 mm diameter) with high optical quality bottoms. A pattern of seven wells was cut into the gel (four patterns per plate) with the 6 outer wells 3.5 mm from the center well (figure 1.).

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* The investigation reported in this paper (No. 71-4-134) was made in connection with a project of the Kentucky Agricultural Experiment Station, supported in part by a grant made by The Grayson Foundation, Inc., and is published by permission of the Director of the Station.
A thin underlayer of 1% agarose prevented the test reagents from leaking under the 0.5% agarose. The center well was filled with equine infectious anemia associated antigen (EIAA). The first and fourth outer wells were filled with EIAA positive reference serum, and the remaining four wells were filled with sera to be analyzed. After 20 hours' incubation in a moisture chamber at 30°C, the test results were read with the aid of an indirect light and a 4X magnifying glass. The sera being tested were set up in duplicate, thus eight unknown serums were tested in each petri dish preparation.

Preparation of EIAA: Horses, whose serum was negative in the EIA-IDT, were infected with the Wyoming isolate of EIA virus. On the fourth or fifth day of febrile response the infected horses were given an intravenous injection of 2 gm of succinyl choline chloride followed by a saturated solution of chloral hydrate to effect and then exsanguinated. The horse's abdominal cavity was opened and the spleen removed, stripped of fat, cut into small cubes and minced in a sausage grinder. One hundred-gram aliquots of the minced spleen were sealed in plastic bags, frozed and thawed 10 times and stored at -30°C. This processed spleen material was thawed, centrifuged (30,000 x g, 4o C, 2 hours) and the supernate was used as a source of EIAA, and the precipitated spleen pulp was further extracted with 2 volumes of borate buffer 0.2 M pH 8.6. The extraction procedure was repeated until EIAA was no longer detected in the supernate. Supernates of all extraction procedures were polled and the EIAA precipitated by adding an equal volume of saturated (NH₄)₂SO₄. The precipitated EIAA was reconstituted with borate buffer 0.2 M pH 8.6. The (NH₄)₂SO₄ precipitation procedure was repeated and the reconstituted EIAA from the fifth precipitation was used in the immunodiffusion reaction.

EIAA Positive Reference Serum: A horse (clinical history unknown) with a naturally acquired EIAV infection (20 ml of its blood produced EIA in a susceptible horse) and whose serum reacted positively in the EIA-IDT was used as a donor of EIAA positive reference serum. The horse was bled periodically and the serum stored at -20° C or lyophilized.

RESULTS AND DISCUSSION

Agarose-gel (0.5%) was more satisfactory for this immunodiffusion test than the less purified forms or preparations of agar tested. The use of agarose diminished or eliminated the haziness often experienced with other agar preparations during the first 24 hours of the test. Immunodiffusion patterns were composed of reagent wells of a 0.1 ml volume with an interwell distance of 3.5 mm (Figure 1). Larger distances between the reagent wells increased the time required for development of a visible reaction and appeared to decrease the sensitivity of the test as performed with the reagents described in this report.

Serums to be tested were placed adjacent to the positive reference serum. In the case of an EIAA positive serum a precipitin arc, which merges with the positive reference serum precipitin arc, develops between the serum being tested and the antigen (Figure 1). Test serum containing high or low concentrations of antibody are detected by their alteration of the symmetry of the positive reference precipitin arc. Sera which contain high concentrations of antibody are indicated by a lack of visible positive reference precipitin arc in their area of the immunodiffusion pattern. Retesting 2 to 4 fold dilutions of such sera usually results in a visible arc of
precipitation. Sera containing low concentrations of antibody are indicated by a slight deflection of the positive reference precipitin arc toward the antigen source.

Susceptible horses for the production of EIAA were selected on the basis of a negative EIA-IDT. Serum from horses in the third day of the febrile response to infection with the Wyoming isolate of EIAV was used to infect these horses. The EIAV infected horses developed a fever 5 to 8 days post injection of infective serum and were killed 4 to 5 days later at the peak of the febrile response. Horses should be killed in a manner which does not allow the spleen to become engorged with blood. Spleens with the highest concentrations of EIAA were obtained from those horses showing the most severe signs of infection. EIAA could not be detected by immunodiffusion in the spleens of EIA-IDT positive horses undergoing peracute EIAV infection.

The extractable amount of EIAA was increased by freezing and thawing the spleen material several times. This process was facilitated by mincing the spleen in a sausage grinder and sealing small amounts of the minced material in plastic bags. EIAA was extracted from the spleen material and purified with (NH₄)₂SO₄ as described above. Usable amounts of EIAA could be concentrated in this manner from spleens containing low concentrations of EIAA. The EIAA material thus produced was shown to be serologically identical to EIAA material being produced in other laboratories¹, ². Purification of the EIAA reduced the number and magnitude of non-EIAA reactions which often make the EIA-IDT results difficult to interpret. The antigenicity of this EIAA material has remained stable in both lyophilized and fluid preparations stored at -20°C for more than 10 months.

Experimentally infected horses or horses suffering from naturally acquired EIAV infections were used as donors of positive reference serum for the EIA-IDT. In experimentally induced EIAV infections, horses produced EIAA precipitating antibodies 11 to 23 days post injection of infectious material. All EIA-IDT positive horses examined have remained positive as long as observed, some as long as 18 months. However, a foal born of an EIA-IDT positive mare was shown to be negative at birth, positive following the onset of nursing, and again negative at 7 months of age. Other foals nursing EIA-IDT positive mares were positive but became negative at around 6 months of age. It appears that EIA-IDT positive mares can give birth to EIA-IDT negative foals which acquire EIAA precipitating antibodies via colostrum and subsequently become negative. Serum, plasma and whole blood from horses are all satisfactory for the EIA-IDT. The EIAA precipitating antibodies in samples of these fluids from 5 EIA-IDT positive horses were stable for more than 6 months at 26°C and more than 6 weeks at 50°C. Clots taken from the carcass of an EIA-IDT positive horse 72 hours after death were satisfactory for diagnosis by EIA-IDT. Transfusion of blood from EIA-IDT positive horses to EIA-IDT negative horses produced EIAV infections in the 5 cases investigated. The development of antibodies in experimentally induced EIAV infections and the demonstration that EIA-IDT positive horses have an EIAV viremia support the validity of this test.

The sensitivity of the EIA-IDT is determined by the concentration of both antigen and antibody used. Trial and error manipulations of antigen and antibody were used to detect the earliest serum antibody in horses experimentally infected with EIAV. The most sensitive combination of antigen and antibody concentration was confirmed in a similar manner using dilutions of both positive reference serum
and antigen. These data have provided a crude means of standardizing subsequent lots of EIA-IDT reagents. Radial immunodiffusion procedures are being investigated as a more quantitative means of standardization.

SUMMARY

A recently described procedure for the diagnosis of equine infectious anemia (EIA) by immunodiffusion (EIA-IDT) was investigated. Positive reference serum was obtained from a horse with a long standing subclinical EIA-virus infection. EIA-infection associated antigen (EIAA) was derived from the spleens of horses undergoing a primary peracute EIAV infection. The peracute EIA infection was induced by injecting serum from a horse in the third day of febrile response to the Wyoming isolate of EIAV into a susceptible horse. Procedures for the extraction, purification and concentration of EIAA from the spleens by (NH₄)₂SO₄ precipitation were described. Deterioration of EIAA antigenicity was not detected in lyophilized or fluid preparations stored at -20° for more than 10 months. Serum to be tested was set up adjacent to a positive reference serum with the serum wells being 3.5 mm from the antigen well. Using agarose-gel and the reagents described the test results were obtained in approximately 20 hours. Purified preparations of EIAA were shown to facilitate interpretation of the test results. Serum, plasma and whole blood from EIAV infected horses were all satisfactory for diagnosis in the EIA-IDT and were stable for months at both room temperature and 50° C. Whole blood from the EIA-IDT positive horses investigated produced EIAV infections when injected into EIA-IDT negative horses. In experimentally induced EIAV infections the infected horse’s serum became EIA-IDT positive at 11 to 22 days after injection of EIAV material. The EIA-IDT positive pregnant mares investigated gave birth to EIA-IDT negative foals which became EIA-IDT positive with the onset of nursing and thereafter became EIA-IDT negative by approximately 6 months of age.

REFERENCES

Fig. 1. Four immunodiffusion patterns (a, b, c, d) cut into agarose gel and allowed to develop for 24 hours after reagents were placed in the indicated wells.

R = positive reference serum
A = antigen (EIAA)
T = fluid being tested for antibodies to EIAA

Patterns a and b contrast the results of testing serum (pattern a) and plasma (pattern b) from the same horses.
- a1T positively reacting serum from horse #1.
- b1T positively reacting plasma from horse #1.
- a2T negative serum from horse #2.
- b2T negative plasma from horse #2.
- a3T positively reacting serum from horse #3.
- b3T positively reacting plasma from horse #3.
- a4T negative serum from horse #4.
- b4T negative plasma from horse #4.

Patterns c and d are duplicates. These patterns show the results of a positively reacting mare (c4T and d4T); her foal, negative prior to the onset of nursing (c1T and d1T); her foal, positively reacting after nursing for 24 hours (c3T and d3T); her foal, negative at 7 months of age (c2T and d2T).
A Study On The Epizootiology of *Babesia Equi* Infection In A Herd Of Horses Imported Into Florida

John B. Anderson, B.S., D.V.M. and
Walter H. Martin, M.S., Ph.D.

**INTRODUCTION**

*Babesia caballi* was first reported in the U.S. in 1961 and is now enzootic in the pleasure-horse population of southeast Florida. *B. equi* was first identified in the blood of a horse in Florida in 1964. Since then numerous *B. equi* infections have been diagnosed in the U.S. by means of the complement-fixation test and (or) identification of the babesia organism.

The majority of the *B. equi*-infected horses was brought into the U.S. from tropical areas of the western hemisphere or from southern Europe. Histories of a few infected horses are uncertain but only one proven *B. equi* infected horse was known never to have been outside of the continental U.S. (*B. equi* infection was proven serologically and by identification of the organism). With the exception of this one case, there is no confirming evidence that natural infection ever occurred in the U.S.

The U.S.D.A. and Florida Department of Agriculture are engaged in a program to control the spread of *B. caballi* and *B. equi* infections in the U.S. The present study was made to obtain more information on the possibility of congenital *B. equi* infections becoming a source of this disease.

**MATERIALS AND METHODS**

A well managed herd of 156 horses located in northwest Florida was studied. This herd consisted of 61 mares and stallions which had been imported from Colombia between 1965 and 1969 and 95 offspring which had been foaled on the Florida farm. The herd was examined for ticks 4 times during a 14 month period (12-18-69, 3-30-70, 6-10-70, 2-3-71). Occasional *Ixodes scapularis, Amblyomma maculatum,* and *Amblyomma americanum* were found.

All horses were tested for antit-*B. equi* complement-fixing antibodies by a previously described method. Horses whose serum reacted 2+ at a 1 to 5 or higher dilution were considered infected. Blood from 16 of the C.F. positive horses,
including the dams of the 5 C.F. positive foals, was inoculated into test ponies to substantiate the serologic findings.

Inoculations into test ponies were made with 500 ml. of blood from adult horses or 100 ml. of blood from foals. If an inoculation failed to produce B. equi infection in the recipient within 60 days, its susceptibility was proven with known infected blood. Babesia equi infection of the test ponies was determined serologically and by identification of the organism.

Thin blood-films were stained with Giemsa's stain in order to examine them for parasites.

RESULTS AND DISCUSSION

The results of the C.F. tests and animal inoculations are shown in Table 1. Table 2 shows the ages of the 5 C.F. positive Florida-born foals at the times of testing, with their C.F. titers and the titers of their dams.

Table 1

Results of C.F. Tests and Animal Inoculations for B. equi

<table>
<thead>
<tr>
<th>No. Tested</th>
<th>No. C.F. Pos.</th>
<th>% C.F.</th>
<th>No. C.F. Pos.</th>
<th>No. Which Inoculated</th>
<th>Transmitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imported Horses</td>
<td>61</td>
<td>38</td>
<td>62.3</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

Florida-born Foals | 95* | 5 | 5.3 | 5 | 0 |

*Forty-eight of these were from C.F. positive dams.

The value of the C.F. test as a diagnostic tool has been previously reported and is well accepted. The specificity of this test is substantiated by the fact that blood inoculated into susceptible ponies from each of the 16 horses, transmitted B. equi.

Of the horses which had been imported from Colombia, 62.3 percent were positive to the C.F. test and considered to be infected. All of these positive horses were raised in the lowland areas of Colombia near Medellin. Eighteen of the 61 Colombian horses were raised on the plateau near Bogota at an altitude of 8,500 ft. and all were C.F. negative for B. equi. The other 5 C.F. negative imported horses were from several other locations in Colombia.
A STUDY ON BABESIA EQUI

Table 2

<table>
<thead>
<tr>
<th>Foal No.</th>
<th>Dam's Titer**</th>
<th>Foal's Age*</th>
<th>Foal's Titer**</th>
<th>Foal's Age</th>
<th>Titer**</th>
<th>Foal's Age</th>
<th>Titer**</th>
<th>Foal's Age</th>
<th>Titer**</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1+1:40</td>
<td>2</td>
<td>4+1:40</td>
<td>80</td>
<td>neg.</td>
<td>274</td>
<td>neg.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3+1:40</td>
<td>11</td>
<td>1+1:80</td>
<td>89</td>
<td>neg.</td>
<td>283</td>
<td>neg.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2+1:320</td>
<td>27</td>
<td>1+1:80</td>
<td>105</td>
<td>neg.</td>
<td>299</td>
<td>neg.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2+1:1280</td>
<td>10</td>
<td>4+1:1280</td>
<td>88</td>
<td>3+1:80</td>
<td>282</td>
<td>neg.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2+1:40</td>
<td>16</td>
<td>4+1:120</td>
<td>94</td>
<td>neg.</td>
<td>288</td>
<td>neg.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Ages in days.

** At time of initial test.

Blood from the 5 C.F. positive Florida-born foals failed to transmit B. equi. These foals were from 2 days to 27 days of age when tested initially. When they were retested at from 80 to 282 days of age, they had become C.F. negative. Because of the long interval between tests it was impossible to determine the earliest age at which the foals no longer had detectable complement-fixing antibody. The failure of their blood to transmit B. equi is strong evidence that they were not infected and that the complement-fixing antibody was maternally derived.

There are several reports of congenital B. caballi or B. equi infections. Most of these observations were made in areas where tick transmission is common and the resulting infections often severe. The B. equi infections in this herd were clinically inapparent during the time of observation. Forty-eight of the Florida-born foals were from C.F. positive dams. With the exception of the 5 foals mentioned above, all were C.F. negative.

On the basis of serology and animal inoculation, there was no evidence of congenital or tick transmitted B. equi infection of the Florida-born foals. This does not rule out the possibility of B. equi infection of fetuses or foals which did not survive. It appears that under similar conditions of temperate climate, few ticks with no Dermacentor spp., Hyalomma spp. or Rhipicephalus spp., and horses with chronic, asymptomatic B. Equi infections, the likelihood of either vertical or horizontal B. equi transmission is remote.

SUMMARY

A herd of 61 horses originating from Columbia with 95 foals born in Florida was studied to determine if congenital Babesia equi infections had occurred. Of the horses from Columbia, 38 (62.3%) had anti-B. equi complement-fixing antibody.
Blood of 16 of the complement-fixation (C.F.) positive horses was inoculated into susceptible test ponies and all 16 were shown to be infected. Of the Florida-born foals, the 5 youngest were C.F. positive. They were shown by animal inoculation to be uninfected. Their complement-fixing antibody was thought to be maternally derived. It appears that under these conditions the possibility of vertical or horizontal *B. equi* transmission is small.

REFERENCES

During the summer of 1971 a survey of the equine population of Cape May County, New Jersey, was conducted to determine the prevalence of Equine Infectious Anemia (EIA) using the agar gel immunodiffusion (AGID) test described by Coggins and Norcross. The survey was conducted jointly by the New Jersey Department of Agriculture, the Cape May County Extension Service, and the New York State Veterinary College. Six hundred and six animals on 116 premises were included, representing 90% of the estimated total population of 675 equines. Six animals were positive indicating a prevalence of approximately 1%.

As the name implies, EIA is an infectious disease of Equidae. It is world wide in distribution and has been reported in most of the states in the United States and provinces of Canada. Transmission by contact occurs only with difficulty, but transmission is readily accomplished by the inoculation of minute quantities of blood from an infected animal, particularly if the infected animal is clinically ill at the time. Naturally occurring outbreaks usually are in the summer and commonly involve pastured animals rather than stabled animals. Various biting insects, including tabanids, stable flies, and mosquitoes have been shown to be mechanical vectors of the disease. Transmission can be effected by man using contaminated surgical instruments or, more commonly, contaminated hypodermic syringes and needles, and serious outbreaks have occurred following this practice.

Cape May County is the peninsular southernmost county of New Jersey and known insect vectors of EIA occur in great abundance throughout this area. Horse flies and deer flies are a serious pest of horses and Pechuman indicates that 33 different species of tabanids have been collected in this county. Hansens noted unusually large numbers of the stable fly Stomoxys calcitrans, captured in traps during the final weeks of horse fly studies at one of the farms surveyed. Mosquitoes occur in great number and are involved in the transmission of another disease, eastern equine encephalomyelitis, to horses and man in this area. Lafferty reports that 15 different species of mosquitoes, including both salt marsh and fresh water types, were reported from this county during 1971.

EIA was known to have existed at a riding stable in Cape May County during April and May of 1970. The stable consisted of about 100 saddle horses of various breeding and six were positive on AGID tests performed by the New Jersey Department of Agriculture. The reactors included one acute case, one chronic case, and four inapparent carriers, and all were destroyed. The acutely affected animal was immediately suspected of being infected with EIA and isolated pending laboratory confirmation. This action, together with its occurrence before the peak emergence of biting insects, may have prevented a more serious outbreak. The

Research Laboratory for Equine Infectious diseases, Department of Pathology, NYS Veterinary College, Cornell University, Ithica, N.Y. 14850.
This study was supported in part by a research grant from the United States Trotting Assoc.
source of the outbreak could not be traced, but the owner felt it to have been the chronic case. He reported the animal had been purchased from the Pocono section of Pennsylvania two years previously and, retrospectively, had shown suggestive clinical signs since the time of purchase. The reason for the occurrence of acute EIA in an animal before the emergence of large numbers of insect vectors was not determined. It could represent either a new case following other mechanical transmission or the recurrence of clinical signs in an inapparent carrier.

EIA was diagnosed at another farm in this county in May of 1971. This stable consisted of eleven animals and two were positive on AGID tests performed by the New Jersey laboratory; one was an acute case and the other chronic. The acute case had been owned by another individual and had been boarded at this stable for the last three years. The source and previous history of this animal was unknown. The chronic case had been owned by the stable and had been purchased at a sale about five years ago. Information given by the owner suggested that it may have been infected for some time, very probably before the time of purchase. The remaining negative animals had been on the farm for at least a year, and some for several years. The two positive animals were destroyed shortly after their status became known.

The presence of the two infected herds in the area stimulated the interest and concern of many horse owners in the county. The occurrence of EIA in an area where many known insect vectors are present suggested the possibility of a survey to determine the prevalence of EIA in a relatively isolated geographic area, and arrangements were made for a jointly sponsored survey. The county extension service contacted horse owners and arranged for stations where owners of single or a few horses could assemble their animals for sampling. The samples were collected by two veterinarians, one with the New Jersey Department of Agriculture and the other a private practitioner. Bleeding tubes and disposable needles were furnished by the New Jersey Department of Agriculture and samples were forwarded to this laboratory for testing. Original estimates of the horse population were about 500, and the project was undertaken with the understanding that every effort would be made to obtain a sampling from at least 75% of the horses in the county. The original plans were that all samples would be collected on a single day, but the large number of animals presented made this impossible. Five hundred and twelve animals were sampled on the scheduled day and the balance were sampled later.

The survey revealed six positive cases of EIA on four premises, and none of the farms were previously known to have been infected. Each of the positive animals was resampled and results confirmed by the New Jersey Department of Agriculture. The two previously known infected herds were included in the survey with no additional infection revealed, and the survey test was the first test following the removal of the infected animals. A very brief study was made of the four infected herds to determine the source and possible extension of infection. Following are comments on each of the infected premises.

Premise “A” consisted of eleven horses with one being found positive. This animal had been purchased at a sale in another county in New Jersey during March of 1971 and showed no signs of infection. There was no indication of spread of infection within the group or to neighboring farms.

Premise “B” consisted of thirteen horses with two being found positive. One was an eighteen year old horse raised on the farm and the other had been purchased at a
EQUINE INFECTIOUS ANEMIA

sale in New Jersey in March of 1970. Signs of illness had not been observed in
either horse. The owner had purchased horses at a sale in New Jersey the previous
winter and had treated them himself with injectibles, probably using a single
hypodermic syringe and needle. Additional information indicates this farm might
be the source of infection for two other premises. There were no other horses
adjacent to this farm.

Premise “C” was a riding stable with twenty-five horses and one horse was
positive on the survey test. This animal was one of four purchased from premises
“B” in January of 1971. The other three horses had died during the winter and
spring, but a veterinarian had not been called and the cause of death was not
determined. There was no other indication of spread of infection within the group
or to near-by farms.

Premise “D” had twelve horses with two positive cases. Neither horse had any
signs of EIA infection and both had been purchased from premise “B” during the
spring of 1971. There was no evidence of spread of infection within the group and
there were no horses adjacent to this farm.

Personnel from this laboratory has investigated many outbreaks of EIA, several
of which were quite serious with mortality approaching 50% and all or nearly all of
the remaining horses of the group positive on AGID test. In studying the more
serious outbreaks it frequently was found that an animal was seriously ill and often
died while at pasture several weeks before the onset of the outbreak. It has also
been noted that a field or road between pastures may be an adequate barrier to the
spread of infection, provided infected and non-infected horses are not interchanged.

The six positive cases located by the survey were thought to be either inapparent
cases or at least, showing only minimal signs; none had a recent history suggestive
of acute EIA. The cause of the three deaths mentioned at premise “C” were
undetermined, although it is possible that they may have been acute fatal cases of
EIA transmitted by contaminated injectibles or hypodermic equipment. If this
were the case, the animals died before the emergence of biting insects which would
be needed for further transmission of the disease.

The population surveyed was tabulated according to herd size and this
information is presented in Table 1. At the time the survey samples were collected,
information was requested regarding the breed, age, and source of each animal, and
this information is presented in Table 2 and Table 3. The location of the premises
surveyed are shown on a map of the county presented in Figure 1.

The minimum distance between any of the infected farms located by the survey
is 1½ air miles, and the closest any of these farms is to a previously known infected
premise is 2 air miles. Investigation failed to establish any interconnection of
infection in these four places other than previously cited, and no interconnection
with the two infected farms identified before the survey was made. Included in the
negative animals are eighteen which came from the herd known to have been
infected in 1970, twelve of which were present at or near the time of the outbreak.

The equine population of Cape May County is mainly a population of saddle
horses and ponies. Herd sizes are small, the mean being 5.4 and the median 3. The
population is a fluid one, with many horses originating in other states and a few
coming from areas where the disease is considered endemic rather than sporadic.
The recent development of the AGID test has made available a means for
conducting surveys involving large numbers of animals. Prevalences for previous
years are unknown, but there is no reason to believe that it is significantly different than years in the recent past. A control program for EIA does not exist in any of the states. It may well be that the prevalence could remain low in this area, even in the presence of large numbers of known insect vectors, simply because groups are small in size and adequately spaced and acute cases are recognized and isolated.

The author acknowledges and appreciates the assistance of Dr. T. E. Lisowski, New Jersey Department of Agriculture, and Dr. E. W. Zirkle, veterinary practitioner, both from Bridgeton, N.J., for collecting the survey samples, and Mr. John N. MacLeod, Cape May County Extension Service, Cape May Court House, N.J., for organizing and coordinating the survey.

BIBLIOGRAPHY

Map of Cape May County, New Jersey showing locations of premises surveyed.
Table 1. Equine Population Surveyed in Cape May County, New Jersey
Tabulation of Herd Size

<table>
<thead>
<tr>
<th>Number of animals on premise</th>
<th>Number of premises</th>
<th>Number of animals</th>
<th>Percent of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>28</td>
<td>4.6</td>
</tr>
<tr>
<td>2-5</td>
<td>60</td>
<td>178</td>
<td>29.4</td>
</tr>
<tr>
<td>6-10</td>
<td>16</td>
<td>115</td>
<td>19.0</td>
</tr>
<tr>
<td>Over 10</td>
<td>12</td>
<td>285</td>
<td>47.0</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>606</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Parameters of Herd Size

Range  1-109
Mean  5.4
Median  3
Table 2. Equine Population Surveyed in Cape May County, New Jersey, indicating AGID test results, age group, and source of animal.

<table>
<thead>
<tr>
<th>Born on premise</th>
<th>AGID Negative</th>
<th>AGID Positive</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>under 2 years</td>
<td>2 years or more</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>Purchased from</td>
<td></td>
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<tr>
<td>New Jersey</td>
<td>7</td>
<td>247</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>197</td>
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<tr>
<td>Total</td>
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Table 3. Equine Population Surveyed in Cape May County, New Jersey indicating AGID test results, age group, and breed of animal.

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<th>Breed</th>
<th>AGID NEGATIVE</th>
<th>AGID POSITIVE</th>
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<tr>
<td></td>
<td>or more given</td>
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<td>Grade saddle</td>
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<td>Quarter horse</td>
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<td>Thoroughbred</td>
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<tr>
<td>Morgan</td>
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<tr>
<td>Ponies</td>
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<td>Jacks</td>
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<tr>
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A PROSPECTUS ON
EQUINE INFECTIOUS ANEMIA
WITH GUIDELINES – 1971

INDEX

INTRODUCTION
1. Definitions for the purpose of this report
2. Information accepted as fact pertaining to equine infectious anemia
3. Areas of responsibilities and functions
4. Procedure in event of suspected outbreak at track, show, rodeo, or similar
   program or functions
5. Research objectives

Appendix 1 – Immunodiffusion test for equine infectious anemia
Appendix 2 – Control Program
Appendix 3 – Recommendations for handling horses at race tracks and other
   locations to aid in prevention and control of equine infectious anemia
Appendix 4 – Protocol for horse inoculation tests for Equine Infectious Anemia

INTRODUCTION

The original prospectus with guidelines for controlling equine infectious
anemia was formulated in 1966 by representatives of the following organizations:

American Association of Equine Practitioners
American Quarter Horse Association
American Veterinary Medical Association
National Association of State Racing Commissioners
Thoroughbred Owners and Breeders Association
Thoroughbred Racing Association
United States Department of Agriculture
United States Animal Health Association (USLSA)
United States Trotting Association

This prospectus follows the original format with modifications which reflect
developments to this time.

A large investment has been made in basic research and evaluation of the
disease, diagnostic tests, regulatory rules and other factors with the hope that sound
programs may be developed as a basis for national action aimed at the immediate
control and eventual elimination of equine infectious anemia.

Despite the fact that total knowledge is not available and the tools at hand are
less than perfect, a sufficient body of information has been developed which will
permit initial programs to lessen losses while causing minimum disruption to the
horse industry. Inasmuch as a large percentage of the nation’s state veterinarians
have reviewed, approved, and endorsed the materials and methods set forth herein,
it is hoped that the remaining states will also accept and approve them, in order to
establish nation-wide, uniform procedures which will premit the invoking of these
new programs without harassing, stringent regulations. With the adoption of cooperative measures by the states along the line suggested herein, it is felt that spread of the disease can be contained and the incidence of equine infectious anemia reduced with some prospect of eventual eradication.

1. DEFINITIONS FOR THE PURPOSE OF THIS REPORT
   A. Equine Infectious Anemia — A widely spread virus-caused disease of the equine which is infectious in nature and spread by improper use of hypodermic needles, other instruments, and insects. The disease may be acute, subacute, chronic, or inapparent.
   B. State Veterinarian — The Chief livestock regulatory official, usually an employee of the State Department of Agriculture or State Livestock Sanitary Board and responsible for the control and eradication of animal diseases within his state.
   C. Commission Veterinarian — One employed by the State Racing Commission or Board for the purpose of advising and assisting with veterinary matters pertaining to the commission and to direct the veterinary functions and activities of the Commission.
   D. Advisory Committee — A group of consultants composed of practicing, regulatory or other veterinarians who may be appointed by the state veterinarian to assist him in an advisory capacity in dealing with equine infectious anemia.
   E. Suspect Horse — A horse showing evidence of equine infectious anemia without confirmation by tests.
   F. Positive (infected) Horse — A horse that reacts positively to an approved serological test performed by an approved laboratory.
   G. Official Test — The official test for detection of infected horses shall be:
      1. The agar gel immunodiffusion test as described by Coggins and Patten and reprinted in the Proceedings of the 74th Annual Meeting of the U.S. Animal Health Association, 1970. (Appendix I)*; or
      2. The horse inoculation test, a test whereby blood is transferred from a suspect horse to a susceptible test horse under the direction of a state veterinarian following the Protocol in Appendix 4.

2. INFORMATION ACCEPTED AS FACT PERTAINING TO EQUINE INFECTIOUS ANEMIA
   A. Responsibility for Control
      1. The individual State Department of Agriculture or Livestock Sanitary Board is the sole agency legally responsible for the control of equine infectious anemia within the respective states.
      2. The U.S. Department of Agriculture has regulatory jurisdiction over horses entering the United States and over the interstate movement of horses known to be infected entering the United States.
   B. Health Certificates
      1. A health certificate is current and valid only for the period expressed

* Modified by James E. Pearson in collaboration with the Committee on Equine Infectious Anemia of the A.A.V.L.D., October 1971.
by that certificate.
2. A health certificate at no-time assures that a horse is not a carrier of the virus, or has not been exposed to the disease.

C. The Virus
1. The virus has not been definitely classified.
2. The virus causes disease by entering through the skin or mucous membranes.
3. When very high titers exist, there may be difficulty in effecting destruction of the virus. At least 15 minutes of boiling or autoclaving is recommended to destroy the virus.

D. The Disease
1. It is widely spread throughout the country.
2. Clinically, it may resemble other diseases and it is not possible to make a precise differential diagnosis without laboratory tests.

E. Spread of the Disease
1. Injudicious use of hypodermic needles and surgical equipment constitute one major cause of spread.
2. Transmission by biting insects is similarly an important cause of spread.
3. The disease may be passed in utero.
4. An infected horse is of minimal danger to others around it provided:
   a. Biting insects are not present.
   b. Hypodermic or surgical instrument used on the carrier horse is destroyed or sterilized.
   c. Good sanitary practices are followed.

F. Control Measures
1. Control depends upon the identification and eventual elimination of infected horses. This must be done in the most practicable and least costly manner.
2. There is urgent need for a nationally coordinated control effort, along with centralized reporting and analyses of all cases and all tests.
3. The suggested plans and procedures evolved in this report provide the framework for an effective control and research program. (Appendix II).
4. Success of any program will depend upon the cooperation and support extended by individual horsemen as well as all facets of the industry.

3. AREAS OF RESPONSIBILITIES AND FUNCTIONS
A. Responsibility for a central headquarters office for the purpose of collecting and disseminating all pertinent information and coordinating and analyzing all test and control procedures on a nationwide basis has been assigned to:
   Chief Staff Officer, Equine Diseases
   ANH, ARS, USDA
   U.S. Department of Agriculture
   Washington, D.C.
B. State Veterinarian or Chief Livestock Regulatory Official
1. Responsible for the control of contagious disease of livestock within his state including equine diseases.
2. May appoint an “Advisory Committee” from practicing or other regulatory veterinarians including the Commission Veterinarian to assist him in an advisory capacity in the control, prevention and diagnosis of equine infectious anemia.
3. Should have available to him an isolation area, including screened stalls at or near each track, rodeo, show, or other organized gathering of horses suitable for isolating suspect horses (isolation area to be provided and maintained by unit management).
4. Will report the results of testing within his state to the USDA office.
5. Will maintain a list of all suspects and infected horses.
6. Will prescribe and enforce necessary sanitary rules and isolation rules at race tracks, rodeos, shows, and elsewhere as may be indicated.
7. Will declare equine infectious anemia as a required reportable disease in states where such is not already the case so that all cases will be reported and recorded in each state.

C. State Racing Commissions, Commission Veterinarians, Horse Show and Rodeo Officials and Veterinarians.
1. Should see that sanitary and other protective measures prescribed by the State Veterinarian are enforced at race tracks.
2. Should insure that tattoo instruments and saliva collecting equipment are adequately sterilized under supervision of the Commission Veterinarian, (autoclave 15 minutes at 15 pounds pressure) prior to being used on any horse.
3. Should enforce rules preventing the use of hypodermic syringes and needles on horses by other than veterinarians licensed to practice at the track.
4. Should require provision and operation of adequate isolation facilities.
5. The Commission Veterinarian should serve on the advisory committee, will promptly report all cases of reportable disease suspected at the tracks.
6. Should assist and support an accelerated national research program to combat equine infectious anemia.

D. Race Track, Show, Rodeo, and Similar Organized Equine Program Management
1. Should institute and carry out at all times the sanitary and preventive measures outlined in Appendix 3.
2. Should provide and maintain screened isolation facilities adequate to meet the needs of and be acceptable to the state veterinarian.

E. Practicing Veterinarians
1. Will immediately report any horse suspected of being infected with equine infectious anemia to the state veterinarian. If the horse is stabled at a race track, show, rodeo, or similar organized program he will report it to the Commission Veterinarian.
2. Should continue the practice of using disposable hypodermic needles and syringes (one needle – one horse).
3. Off size needles and other surgical and medical equipment which must be reused should be sterilized either by thorough cleaning and boiling for 15 minutes or by autoclaving for 15 minutes at 15 pounds pressure.

4. PROCEDURE IN EVENT OF SUSPECTED OUTBREAK AT TRACK, SHOW, RODEO, OR SIMILAR PROGRAM OR FUNCTION
   A. Any practicing or other veterinarian suspecting a horse at a track, show, rodeo or similar function of being affected with equine infectious anemia because of clinical signs or tests will promptly report the case to the state veterinarian, and the official veterinarian.
   B. Horses at tracks, shows, rodeos or similar functions suspected of being infected because of clinical signs will be moved into a suitable screened isolation area (in case of race tracks, management will provide facilities), subjected to such testing as may be required, and maintained at the owners’ expense under the regulatory supervision of the state veterinarian until released by him.
   C. Horses which are proven to be positive by official tests shall be handled in a manner prescribed by state officials, but under no condition will be allowed to move interstate.

5. RESEARCH OBJECTIVES
   A. To establish the incidence of equine infectious anemia in the United States.
   B. To further knowledge of the epidemiology and pathogenesis of the disease upon which to base improved controls.
   C. To further propagate, purify, and characterize the virus.
   D. To give sustaining support to those institutions conducting research in equine diseases, especially those involved in equine infectious anemia programs.

   It should be noted that much progress has been made concerning equine infectious anemia since the original prospectus was drawn in 1966 and that with continued support by all facets of the equine industry more answers and solutions to this disease should be forthcoming in the near future.
Sufficient laboratory tests and field observations have now been conducted to indicate that the immunodiffusion test for equine infectious anemia (EIA) is a reliable indicator of EIA infection in the horse.\textsuperscript{1} Data was presented at the American Veterinary Medical Convention this year in Las Vegas which showed a direct correlation between positive test results and infectivity as determined by animal inoculation.\textsuperscript{2} It was concluded that the test is at least 95\% accurate for the diagnosis of EIA. Since then twelve additional animal inoculations have been made, four epizootics of EIA have been investigated, and several thousand survey samples have been examined.\textsuperscript{3} The immunodiffusion test is performing satisfactorily in the field. Furthermore I understand that Pearson et al. at the National Animal Disease Laboratory have tested several hundred serums and have obtained equally satisfactory results.\textsuperscript{4}

Since the technique was described only briefly in the original article\textsuperscript{1} and in response to numerous requests for more detailed information on the procedure, the test is described more fully in this paper.

**PREPARATION OF ANTIGEN**

Specific EIA antigen is obtainable from the spleen of a pony or horse acutely infected with any of several virulent isolates of EIA virus. The most satisfactory antigens have been obtained from animals showing a severe febrile reaction after a short incubation period of 3 to 5 days and when the spleen was harvested at 9 to 11 days after inoculation of virulent EIA virus. Antigens have been produced by the inoculation of ponies intravenously with 1 ml. of serum containing Wyoming EIA virus (Serum taken at 9 days postinoculation that has a titer of $10^7$ pony infectious doses per ml.) but larger volumes of infective serum (10 to 100 ml.) appear to more consistently produce short incubation periods. It has been found that a subinoculation of acute stage blood in an amount of about 300 ml. has helped to shorten the incubation period and cause a more severe illness. The spleen from one infected horse became greatly enlarged and engorged with blood so that the antigen was too dilute. Antigens cannot usually be diluted beyond 1:2 or 1:4. It was possible to concentrate the antigen by precipitation with half-staurated ammonium sulfate and reconstitution in a smaller volume.

Spleen is collected sterilely, stored frozen at minus 20 C and is thawed and frozen several times before using. Aging the spleen in the freezer appears to aid in the release of the antigen also. The essential points in antigen preparation are:

1. Select a spleen from a horse showing a very severe acute reaction to EIA
EQUINE INFECTIOUS ANEMIA

255

virus.
2. Harvest the spleen at 9 to 11 days post inoculation.
3. Freeze and thaw the spleen several times before using and avoid dilution in anyway.

PREPARATION OF ANTISERUM

A positive reference antiserum can be chosen from a horse surviving EIA infection. Long term carriers often have been found to have satisfactory serums. The serum should give only one dense, distinct precipitin line when tested with the EIA antigen and the line should form approximately midway between the serum and antigen wells with no tendency to broaden or fade with time. Thprecipitin line must be shown to be specific for EIA by horse infectivity tests or by forming a line of identity with an antiserum which has been shown to specific for EIA by animal inoculation tests. Serums with excess antibody in relation to antigen concentration tend to form broad bands rather than a narrow distinct line which is essential for the accurate determination of line of identity. It is much easier to see the suspect line coalesce with the reference line if the latter is a dense, narrow line. Discrete precipitin lines can be obtained with the higher titered serums but they must be diluted and they seem to be less dense. Avoid serums that give a cloudy ring around the well.

The essential point in antiserum preparation is: Select a serum from a horse surviving EIA infection that give only one dense, distinct precipitin line that is specific for EIA.

PREPARATION OF IMMUNODIFFUSION TEST

Immunodiffusion reactions are carried out in 85-mm plastic petri dishes. Because of occasional seepage of serum underneath the agar, a base layer of 5 ml. of 2% Noble’s special agar in a borate buffer is used. When this layer has hardened a top layer of 15 ml, of 1% Noble’s special agar in the same buffer is added. The borate buffer is prepared by mixing 2 gm. of NaOH and 9 gm. of $H_3BO_3$ in a liter of distilled water. No preservative or sodium chloride is used. The pH of the buffer should be about 8.6. It is suggested that the agar be dissolved and melted by boiling because autoclave temperatures cause some discoloration. The agar is allowed to harden in petri dishes at room temperature with the lids partly open to allow moist air to escape. Excess water in plates can dilute the antigen and should be avoided. Fresh plates are poured daily as old ones tend to become cloudy.

A template with seven circular cutters is used to cut six wells, 7 mm in diameter and spaced 3 mm apart, around a central reservoir of the same diameter. Four of these patterns can be cut in each petri plate. A cork borer can be used to cut the wells as outlined on a paper underneath the petri dish. Care should be taken to lower the cutters through the 1% agar until the resistance of the 2% layer is felt. Agar in the wells is then removed with a short pipette attached to a suction pump.
The 2% agar base layer should be left intact. Splenic pulp is teased from the connective tissue and packed in the central reservoir. Care should be taken not to leave air pockets in the bottom of the well or to over-fill because the tissue tends to swell in some cases. A positive reference serum is placed in two wells on the periphery directly opposite each other and the four remaining wells are filled with suspect serum samples. With this arrangement each suspect serum is next to a reference serum which facilitates easy determination of lines of identity. Plates are incubated at room temperature (20 C) in an inverted beaker containing a wet towel.

READING THE IMMUNODIFFUSION TEST

The immunodiffusion reactions are observed over a strong narrow beam of light and against a black background at 24 and 48 hours. Distinct precipitin reactions are normally visible at 24 hours but weaker reactions sometimes form between 24 and 48 hours. The patterns do not usually change after 48 hours. Doubtful reactions should be retested in duplicate and set up in various patterns to see if they are reproducible. Broad precipitin bands which often occur with carrier serums are not very difficult to recognize because they cause the reference line to stop abruptly about half-way across its normal position and such reactions can be confirmed as specific for EIA by diluting and obtaining a more distinct line. Weakly reacting serums, such as are found in the early stages of EIA infection and in foals with maternal antibody, are more of a serious problem. Such serums usually have to be retested in duplicate and with variations of the patterns. Since the reference precipitin line bends slightly toward the serum well, deviation of this line toward the antigen well as it nears the suspect serum well indicates a weak antibody reaction. It is advisable to recommend that these animals be bled again especially if they are only recently infected. Stronger reactions are commonly seen with serums taken a week or so later. Accurate drawings of the precipitin reactions for future reference is suggested.

INTERPRETATION OF THE TEST

The immunodiffusion reaction has been shown to be an accurate and reliable test for the detection of EIA infection in the horse except for animals in the early stages of infection and foals of infected dams. Since the test measures antibody produced as a result of EIA infection, horses in the first two to three weeks of infection and before antibody is produced will test negatively. Such animals should be bled again in a week or two. In order to make a diagnosis in a young foal it is necessary to determine the antibody status of the dam. If the mare is negative then her positive reacting foal can be declared infected. If the mare has EIA antibody then the foal may or may not be infected. Only a gradual decline and eventual loss of the antibody over a period of several months will indicate absence of infection. A few foals have maintained their maternal antibody for 4 and 5 months.
The EIA immunodiffusion test is not a foolproof technique and requires a degree of expertness in order to interpret the results accurately. Such competence can only be obtained by experience with the test and by observing known positive and negative reactions of varying degrees as well as the non-specific precipitin lines that are occasionally seen. It is felt that initially operators should send duplicate samples to a competent laboratory for an evaluation of their test readings before making routine diagnosis. In addition all reagents should be checked for specificity against known EIA reference antigen and antiserum. Many laboratories will want to do animal inoculation tests to confirm their findings.

Although the presence of precipitating antibody is closely correlated with the presence of EIA virus in the horse, there is no consistent correlation with existing clinical signs of EIA. Many carriers show chronic emaciation, anemia, and periodic febrile illness but some are completely normal clinically. In attempts to demonstrate EIA virus in the inapparent carrier it has become increasingly evident that the virus titer may be very low and that the infective virus may only be found in whole blood and not in the serum. Thus an immediate transfusion of a large volume of whole blood in an experimental test animal may be necessary to transmit the infection and produce a clinical response.

REFERENCES

APPENDIX 2

CONTROL PROGRAM

Most objections to a control program seem to be based on fear of the affect of overly restrictive regulations widely and suddenly applied. The following steps could be implemented with a minimum of disruption to present industry activity while at the same time contributing measurably to the control of the disease. It should be noted that while this appendix recommends national standardization of test methods and laboratory registration, and the prohibition of interstate movement of infected animals, that further restrictions and/or implementation remain with the individual state.

1. The United States Department of Agriculture will be responsible for production, standardization and distribution of antigen.
2. The United States Department of Agriculture will be responsible for establishing standards for laboratories similar to those which exist for other official tests, e.g. brucellosis and anaplasmosis.
3. Currently for administrative purposes, in the absence of clinical signs, a negative test should be considered valid for no longer than six months for movement and assembly.
4. Infected (positive) horses shall be banned from interstate movement.
5. It is recommended that infected (positive) horses shall be permanently identified; and animal once infected is infected for life. Any animal under six months of age which reacts to an official test shall be retested at six months of age and if positive at that time shall be subject to permanent identification and quarantined.
6. It is recommenced that identification of infected animals will be accomplished by freeze branding on hip or neck. It is recommended further that the individual states seek legislative authority to permanently identify without liability, immunodiffusion test reactors as infected

   It is further suggested that freeze branding on the hip or neck with a nationally assigned set of symbols, letters and numerals would be the ideal way of accomplishing this identification. For example, the combination 11A0001 to 11A9999 could be used to provide identification for up to 9999 infected horses in the state of Maine. The A means anemia, the 11 is the identifying number already assigned to Maine for livestock identification purposes. Every state now has such a number. The last four digits will serve as a serial number to identify the particular case in the Maine records.

7. Infected horses should be quarantined. The nature of the disease is such that it is not necessary or desirable to quarantine non-reacting horses that have been in contact with a reactor. It may prove possible to completely eradicate the disease without quarantine of exposed horses.
8. Identification of known carriers would make quarantines self-policing once the significance of the brand became widely known among the horse owning public.
9. It may prove possible to use branded E.I.A. carriers for breeding purposes on isolated farms devoted solely to the purpose of maintaining valuable
bloodlines. Decisions in this area must await research data on the possibility of safely salvaging the foals from such premises.

Additional measures which will prove valuable and which can be recommended to the industry include:

a) Prohibition of untested horses at race tracks, shows, rodeos, trail rides, fairs or similar assembly points.

b) Consideration of the possibility of requiring a negative test for transfer of ownership of registered animals.

APPENDIX 3

RECOMMENDATIONS FOR HANDLING HORSES AT RACE TRACKS AND OTHER LOCATIONS TO AID IN PREVENTION AND CONTROL OF EQUINE INFECTIOUS ANEMIA

1. Maintain at all times systematic and effective insect control. Maintain stables and the immediate surrounding area in good sanitary condition. This includes prompt disposal of manure and other refuse and good drainage to prevent vector multiplication.

2. Restrict the use of hypodermic syringes and other veterinary instruments to authorized veterinarians.

3. Prevent common use of any equipment such as bridles, bits, harness, curry combs, etc. that may produce skin abrasions or absorb body secretions.

4. Clean and sterilize all types of instruments used on horses including surgical, tattooing, dental, and similar items by boiling for 15 minutes before use on each animal.

5. Use separate sterile equipment on each animal when collecting material for the saliva, urine, or other tests.

6. Frequently clean and disinfect paddocks, starting gates, and other equipment subject to contact with different animals. Use 2% trisodium phosphate to clean and disinfect with sodium orthophenylphenate (1 pound to 12 gallons of water at 120 degrees).

7. Require incoming horses to have veterinary health examination and certificates.

8. Stable horses in individual box stalls with separate feeding watering facilities. All horses should be subjected to careful examination by the official track veterinarian.

9. Stable all horses so as to be under the health supervision of the official track veterinarian.

10. Immediately report to the state veterinarian all horses suspected of having an infectious, contagious, or communicable disease.
APPENDIX 4 REVISED

INSTRUCTIONS AND PROTOCOL FOR HORSE INOCULATION TESTS
FOR EQUINE INFECTIOUS ANEMIA

I. Suspect Animal (Donor)
1. Draw 300 ml. whole blood into vacuum bottle containing acid-citrate-dextros or val Alsever’s solution.
2. Submit information on donor animals as follows:
   Name _____________________________________________________________
   Identification No. ________________________________________________
   Breed ___________________________________________________________
   Age __________________________________________________________________
   Location _________________________________________________________
   Owner _____________________________________________________________
   Trainer ___________________________________________________________
   Immunodiffusion Test Results ________________________________________
   Clinical history _________________________________________________

II. Recipient Test Animal Qualifying Steps
Qualify by:
1. Holding in insect free barn six weeks prior to inoculation (30 days absolute minimum), with negative Immunodiffusion tests at beginning and end of observation period.
2. Treating for internal parasites.
3. Establishing normal temperature and hemograms.

   TEMPERATURE GUIDELINES
   A. Obtain a temperature reading two times daily.
   B. Establish base line — consider daily temperature of individual horse, plus the temperature of his stable mates for a minimum of three weeks.
   C. Eliminate variables such as new grooms.
   D. All routines should be the same each day.

   HEMATOLOGY (EDTA LIQUID-PREFERRED PRESERVATIVE)
   A. Determine PCV 5 times a week (to establish a norm), for three weeks minimum prior to inoculation.

III. Animal Inoculation Test
1. Time & Inoculum — sixty days would be standard. One recipient horse should be used. The dose of the inoculum should be 300 ml. whole blood given intravenously immediately following collection.
2. Temperature Readings — same as Temperature Guidelines for recipient test animal qualifying steps, except B which reads —
   B. Consider daily temperature of individual horse, plus the temperature of his stable mates.
3. Hematology (EDTA liquid-preferred preservative)
   A. Perform PCV at least five times weekly following inoculation.
   B. It may be well to perform leptospirosis test and prepare blood smears
in an attempt to identify *Babesia* during febrile periods.

C. Further hematological tests may be used to provide supportive evidence.

4. Immunodiffusion test performed weekly.

5. At end of test period animal should be euthanized and histopathological exam performed.

_Miscellaneous Items_

Post inoculation temperatures should be interpreted relative to the base line for the individual horse. Any elevation in temperature will be considered significant if stablemates remain at their base line on that day. Experience of investigations have shown that a temperature rise of as little as 1° above normal is indicative of infection when accompanied by significant hemograms and clinical signs. Conversion from Immunodiffusion negative to positive is considered infective.
REPORT OF THE 1971 COMMITTEE ON INFECTIOUS DISEASES OF HORSES

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


VENEZUELAN EQUINE ENCEPHALOMYELITIS

The summer of '71 will long be remembered by our nation's equine interests as a period of frustration, indecision, inconvenience, unreadiness, and, in many cases, hysteria. 1971 is the year in which Venezuelan Equine Encephalomyelitis entered the United States from Mexico, occasioning illness and death of hundreds of horses and malaise of dozens of human beings in South Texas.

The first case of VEE was confirmed from specimens taken from a horse near Brownsville, Texas, which initially exhibited symptoms resembling VEE on June 25, 1971, just eight months following the last meeting of this organization. At that time your Committee on Infectious Diseases of Horses stated that VEE constituted a major threat to the United States equine industry at the moment, and that vigorous plans for preventing its introduction into this country should be initiated at once. To this end the Committee recommended and this Association adopted for implementation a series of steps directed toward precluding its entry into the United States and outlined measures which, if followed, should immediately contain its spread.

Among the recommendations made at that time were:

1. A request to begin immediate negotiations to prepare through the cooperation of the Department of Defense ten million doses of VEE vaccine for use in the United States when the disease erupts in the United States.

2. A request that the U.S. Department of Agriculture pursue under a highest priority a research project to obtain data required for the commercial production and use of VEE vaccine in the United States.

3. The immediate establishment of a select advisory committee using all of the expertise on VEE existing in USDA, U.S. Public Health Service, U.S. Army Medical R&D Command, and any other possible sources, including the President of the United States Animal Health Association to represent the several states.

262
4. The proposal of a VEE contingency plan which had been outlined in detail by the United States Animal Health Association for adoption by USDA dealing with the disease when it appeared north of Mexico City, and ultimately for dealing with it when it entered the United States.

Notwithstanding the foregoing recommendations, VEE entered the United States in late June of this year, and we were not prepared for it.

It should be pointed out that from what we have been advised, negotiations with Mexican officials in attempting to establish a buffer zone through the vaccination of horses in North Mexico failed. But the lack of action on the part of the United States Department of Agriculture to have selected an advisory committee to develop emergency operational procedures for immediate implementation when the disease entered South Texas, plus the lack of an adequate backlog of vaccine and indecision by key officials on vaccine usage created a chaotic situation which led to political pressures to correct a situation which could have been avoided had the recommendations of this Association been observed. Hopefully the lessons learned from this near disaster will be of value in future dealings with exotic diseases.

The Committee adopted and referred to the Committee on Resolutions a Resolution relating to VEE control measures for 1972, a copy of which is attached to this report. (See copy attached)

A resolution was received by the Committee which recommended that the USDA extend its VEE immunization program to all states not now receiving the benefit of such service, to the end that all equidae in the United States be vaccinated by May 15, 1972. The Deputy Administrator of the Agricultural Research Service of USDA expressed doubts that sufficient support could be obtained to finance such an extensive program of VEE vaccination. Therefore, no action was taken by the Committee resolution.

**EQUINE PIROPLASMOsis**

In the area of equine piroplasmosis, concern was expressed by members of the Committee that the Resolution concerning the treatment of *Babesia caballi* infected horses disclosed at USDA import stations was not being effected as was recommended in the 1969 report.

The Committee was advised that efforts have been made to get amendments to Title 9 CFR to require the testing of imported horses, but these proposals have not cleared the Office of General Counsel in these intervening two years. However, since January, 1971, horses arriving at import stations have been tested even though the regulations have not yet been approved. The Committee recommends that USDA further pursue this matter so as to obtain legal authority for testing and clearing horses for import into this country.

It was requested that a feasibility study be initiated on eradicating equine piroplasmosis within the United States through the elimination of vector approach, namely, eradication of Dermacentor nitens, the tropical horse tick, which is the only known vector in this country. State and federal officials in Florida where the tick and the disease are most prevalent will initiate this study.
EQUINE INFECTIOUS ANEMIA

The Committee considered the Protocol and Guidelines for conducting the immunodiffusion test for equine infectious anemia as prepared by the American Association of Veterinary Laboratory Diagnosticians in response to the Committee's request of 1970. With minor amendments the Protocol and Guidelines were approved by the Committee and it is herein recommended that the agar gel immunodiffusion test, commonly referred to as the Coggins test, as conducted within the scope of the Protocol and Guidelines, be accepted as an official test for equine infectious anemia by the U.S. Department of Agriculture. Such Protocol and Guidelines are attached to and made a part of this report (Refer to the Proceedings of the AAVLD appearing in this edition.)

The Committee deliberated at length and recommends for adoption the attached Prospectus on Equine Infectious Anemia, which replaces the Prospectus previously approved by this Association.

AFRICAN HORSE SICKNESS

Your Committee is pleased to report that the subcommittee appointed to prepare a Prospectus and Guidelines on African Horse Sickness has completed its charge, which document is herein included and recommended for adoption by the Association.

Respectfully submitted,

C. L. Campbell, Chairman

The EIA Protocol appears in the AAVLD Section.
A PROSPECTUS AND GUIDELINES ON
AFRICAN HORSE SICKNESS — 1971

1. DEFINITIONS FOR THE PURPOSE OF THIS REPORT
   A. **African Horsesickness (AHS):** AHS is a highly fatal, insect borne, febrile, virus disease of equine animals, clinically dominated by an acute pulmonary edema or a hemorrhagic myocarditis associated with localized areas of inflammatory edema and hemorrhage. There are several antigenic strains of the virus which vary in virulence and antigenicity. Recovered animals develop an immunity to the infecting strain but remain variably susceptible to antigenically different strains. Transmitted not by contact but by hematophagus insects, primarily culicoides, it occurs seasonally with its vectors. The incubation period is 3-9 days.

   B. **State Veterinarian:** The chief livestock regulatory official — usually an employee of the State Department of Agriculture or State Livestock Sanitary Board and responsible for the control and eradication of animal disease within his state.

   C. **Advisory Committee:** A group of veterinarians either experienced or trained to work in the control of AHS to serve as consultant to State or Federal regulatory veterinarians.

   D. **Suspect Horse:** A horse with clinical signs suggestive of AHS but yet to be confirmed by a laboratory test.

   E. **Infected Horse:** A horse proven to be infected by virus isolation or a horse having positive clinical signs of AHS in an area where the disease has been confirmed.

   F. **Vector Control:** Daily aerial spraying of the area with insecticides lethal for culicoides and mosquitoes. Application of larvacides to mosquito breeding places.

   - Daily spraying or dressing of horses with insect repellants.
   - Removal of equine animals away from insect breeding areas especially to high altitudes free of insects.
   - Keep horses in mosquito-proof stables during the hours of darkness.
   - Carefully spray the interior of aircraft arriving from enzootic areas.
   - Remove all equine animals from an area with a 10-? miles radius from international airports when feasible.

   G. **Confinement of Suspect AHS Horses:** AHS suspects appearing in a clean area should be quarantined in insect-tight quarters until the diagnosis is confirmed. If there are not such quarantine facilities for all such suspects and positive cases, the positive ones should be promptly destroyed to avoid contact with vectors.

   H. **Positive AHS:** Cases should receive a conspicuous identification which will last until after destroyed or through convalescence if to be held in strict quarantine.

2. BASIC INFORMATION ESSENTIAL TO THE CONTAINMENT OF AHS
   A. **Responsibility for Control:** AHS is exotic to the United States and is
reportable to State and Federal regulatory veterinarians. Emergency animal Disease Eradication Procedures are to be followed in accordance with recommendations of these regulatory agencies.

B. The Virus of AHS: AHS is caused by a small 50 micron pantropic virus of which there are over 40 strains that are grouped into nine immunologically distinct serotypes. It is a hardy virus; retaining virulence for 6 months at 4° C, and with only slight loss of titer after 30 days at 30° C when 2% ether was added. It stores well for months at -70° C. The virus has been cultivated in tissue culture in which strain typing may be done through neutralization tests or fluorescent antibody techniques. Serial passage intracerebrally in mice leads to adaptation and attenuation for the horse. Virus properly attenuated through serial passage in mice or tissue culture is suitable for vaccine production. Vaccines must be prepared from immunologic types appropriate for the region. Tissue culture vaccines are recommended because of possible neurologic damage from the neurotropic, mouse brain vaccine.

C. The Disease: (See definition A. page 1)

(1) Clinical forms for clinical diagnosis-
Clinically, AHS usually takes one of four forms; AHS fever, acute pulmonary, cardiac, or mixed forms.

AHS fever is mild, often inapparent. Animals typically show no more than 1-2 days of mild fever, some anorexia, dyspnea, rapid pulse, conjunctivitis and make a rapid recovery.

The acute pulmonary form typically shows a fever up to 106° for a day or two before the sudden onset of an acute pulmonary edema. The edema is followed by increasingly severe respiratory distress associated with sweating, more labored breathing, nasal dilation, with head extended downward, forelegs apart, and possibly coughing. Within a few hours frothy edematous fluid fills the lungs and often exudes from the nostrils terminally. The mortality rate often exceeds 90%. Surprisingly, animals continue to eat until the respiratory distress becomes acute.

The cardiac form has a longer incubation period and a slower less obviously acute clinical course but the mortality may still reach 80+. It is characterized by areas of inflammatory edema in regions of the supra-orbital fossae, frontal region, eyelids, lips and neck. The ventral surface of the tongue may bear petechiae, often more severely than in equine infectious anemia. There is a fever, and a rapid, pulse, and abnormal heart sounds. In fatal cases, cardiac insufficiency leads to dyspnea, dependent edema and often a terminal pulmonary edema. Animals tend to eat until the last day when abdominal pain and restlessness are associated with hypoxia and cardiac failure.

The mixed form is a frequent clinical combination of the pulmonary and cardiac forms. From the standpoint of pathology, most fatal cases are mixed to some degree.

(2) Differential diagnosis-
Especially when AHS invades a new region, it may be confused clinically with more familiar conditions. In the United States, equine infectious anemia, equine influenza, equine viral arteritis, and babesiosis, should be differentiated. When AHS produces a rapidly fatal pulmonary edema, it
may also be confused with anthrax or some form of poisoning. In Africa, it is also sometimes confused with trypanosomiasis or spirochetosis.

D. Transmission: Natural transmission is accomplished by insect vectors, not by direct contact between animals. Culicoides are considered the principal vectors. They are hardy insects with essentially a world-wide distribution in warm countries. Mechanical transmission by a variety of blood sucking insects and flies including Stomoxys calcitrans has appeared significant in some outbreaks. Although experimental transmissions have been accomplished by the artificial feeding of Anopheles stephens and Culex pipiens mosquitoes on infected blood and later on susceptible horses, there is slight evidence that mosquitoes play a significant role in natural transmission.

There are many species of culicoides whose habits vary, but experience with the African species indicates they are primarily nocturnal feeders. In South Africa, the disease disappears seasonally 9 days after the first insect killing frost, verifying both the incubation period and the dependency of transmission on the insect vectors. An unknown, non-equine reservoir host appears to harbor the virus between outbreaks in equine animals.

As culicoides appear each season, they usually remain free of the virus for a time, then become infected from some unknown source before they infect equine animals.

Since the virus is present in the blood and tissues of infected horses, mechanical transmission via contaminated needles and instruments can readily occur.

E. Spread of the Disease: Although confined essentially to southern and equatorial Africa for over 200 years, since 1959 AHS has spread to North Africa, 11 countries of the Middle East and Spain. In the course of this rapid spread, it has killed roughly 1/2 million equine animals and has infected countless others. Now present in Africa and the Middle East and being readily transmitted by the ubiquitous culicoides, which are being continually redistributed by the wind and some international aircraft, AHS poses a constant threat to equine animals on all continents.

F. Control Measures:

(1) Intercontinental Control-

As long as AHS is not present in the Western Hemisphere, the transportation of infected animals or vectors from Africa or the Middle East poses the greatest hazard.

It is recommended that no equine animals be imported into the United States from countries where there are known to be active cases. All ships or aircraft from such countries must be as carefully disinfected as if infected insects were known present.

African and Middle Eastern countries are considered infected. Conveying ships, or aircraft, and animals must be disinfected upon arrival. All equine animals from these areas for import into the United States are subject to a 60-day quarantine and appropriate serologic testing. When any animal in such quarantine shows any clinical signs suggestive of AHS, its blood should be tested for the virus by animal inoculation or serologic testing or both, depending upon availability, and degree of suspicion.
NOTE:
It is recommended that complement-fixation tests and serum neutralization tests be conducted upon each horse in quarantine during the first and the eighth week in quarantine. Maximum safety from their serologic tests would result by considering any positive tests (indicating past exposure to the virus) to be reason for prohibiting entry to the United States. This would be the safest interpretation at least until more research is conducted to determine the possible presence of the virus in the presence of various complement-fixation and serum-virus neutralization titers. It is possible for a horse to be immune to one antigenic strain, yet infected with another, so a positive serologic test only assures past exposure to the virus or vaccine. (It is recognized that safety from the introduction of AHS is a relative thing, and that it would be still safer to prevent the entry of any equine animal from Africa or the Middle East; i.e., from any country or continental region ever having had AHS. The ultimate would be no imports of equine animals from anywhere. But, obviously, the relative risks must be weighed against the need and pressures for reasonable international trade in equine animals. For this reason, while we as a committee make a recommendation, we also list methods for the various levels of safety so that the USDA regulatory people may decide upon the degree of safety they can enforce without being in undue restraint of trade.)

Once known to be infected with AHS, such countries will remain on the infected list until they have proven to the Director of Animal Health that they are free. However, in no case will they be considered for a free status until they have gone through three annual insect seasons without a case of AHS.

(2) International control should AHS appear in Canada or Mexico—
The following steps would be among the essential:
Closure of the border to the entry of all equine and canine animals.
Disinfect all vehicles and aircraft at the United States' border ports of entry.
Taking advantage of natural barriers, draw a line from 25-50 miles from, but parallel with, the border; require registration and confinement of all canine and equine animals within the zone to prevent their movement; and require daily insect spraying (area fogging) of equines, if cases are known or suspected within 100 miles.
Recommend stabling of equine animals at night, and remove the light bulbs so that lights cannot be turned on.
Cooperate in the elimination and control of insects and insect breeding areas along the border that pose a special hazard.
Send observers into infected areas to keep the United States posted on the situation.

(3) United States control in the presence of AHS—
*Phase I*
Should a confirmed case of AHS appear in the United States, the
following minimal controls appear essential:

a. Immediately quarantine all equine and canine animals on the premises.

b. Initiate insect spray control immediately, and twice daily thereafter.

c. Destroy all equine animals in the infected herd on the premises and, when possible, burn or bury deeply on the premises so as to prevent the exposure of blood from the animal to insects.

d. A zone roughly 15 miles in radius from the infected premises, but taking advantage of natural barriers, should be drawn to indicate the "infected zone." All equine animals in this infected zone should be carefully identified, registered, and immobilized to prevent their movement.

Establish deinsectization stations for all vehicles permitted to enter or leave the quarantine zone.

Spray with residual insecticides all stables, barns, and equine animals starting at the periphery and moving into the center of the zone once every 24 hours if possible. Consider aerial spraying as with helicopters or fog trucks.

Prohibit any movement of dogs within the zone, and destroy all strays.

**Phase II**

Be prepared for Phase II, if warranted by spread of AHS.

1. A source of 1 million doses of multiple strain vaccine should be available on short notice.

2. Private practicing veterinarians should be informed regarding the characteristic of the disease and employed on a fee basis in vaccination brigades.

3. Vaccinated animals must be permanently identified by a lip tattoo (or some other adequate method) applied at the time of vaccination.

A plan should be developed and implemented to provide a stockpile of a minimum of 1 million doses multivalent AHS vaccine. It is proposed that this would be done as follows:

1. The Government of the Union of South Africa be approached to provide and make available to the United States (through the Onderstepoort Laboratory) 1 million doses of polyvalent AHS vaccine. Vaccine stockpile could be handled on a rotation-replacement basis, and the South Africans could use and replace this stockpile each annual vaccination season; provided they maintain a constant stock for us of at least 1 million doses of vaccine of known effective potency, less than 1 year old.

2. If No. 1 is not feasible, AHS vaccine should be stockpiled within the United States.

**NOTE:**

In the decision to follow Phase II, it should be noted that successful killed virus vaccines were being reported in 1968 and should be given consideration for potential use in this hemisphere as soon as their efficacy has been established through field experiences.
Good Morning Ladies and Gentlemen:

I trust you are enjoying yourselves here in Oklahoma City and are benefiting from the many informative sessions the Association has planned for you during your three-day stay.

Let me venture a bit of safe speculation regarding your stay. At dinner last night you probably had beef, pork or chicken. A few of you had fish, perhaps ten percent at most. I'll even go one step further in my safe speculation and say that the meat and poultry portion of your dinner was probably good.

But was it safe? Was it free of animal drug residues that might prove harmful to your health? Of course there is no real way of knowing the answer to these questions the instant you take that first bite of steak. The answer ultimately lies in the chemist's laboratory, in the careful analysis of animal tissue.

I had a steak last night, and I want to assure you that the question of residues didn't even begin to cross my mind. Why? Because after total involvement with the entire question of residues in meat I believe that the producer of my steak was using animal drugs correctly when he raised that steer.

By far the vast majority of today's American farmers are completely responsible individuals. They understand the inherent dangers of misusing animal drugs. They remember thirty years ago when these valuable production tools were only brief paragraphs in the experimental research sections of the agricultural magazines. They've seen agricultural chemistry come a long way, and they respect the fact that in a business where too many farmers have been forced to cross the thin line between profit and loss, an antibiotic growth promotant or a vaccine can often literally make all the difference between "In the Black" and "In the red."

But there is another very good reason why I am confident the meat I eat is safe: Animal Drug Certification. Very briefly, and I'll get to the specifics later, the present voluntary certification program works like this. The livestock and poultry producer certifies in writing when marketing his animals that he has followed manufacturer's directions concerning FDA and USDA required withdrawal periods when administering drugs to food-producing animals. The objective of the program is to assure the consumer that meat, milk and eggs continue to be wholesome and free of unauthorized residues.

This is a guarantee from the producer to the packer, with one exception, which I will cover in a second. The program was launched this last March by a special Committee representing a broad spectrum of the animal protein production industry. This committee, The National Animal Drug Certification Committee, NADCC for short, has designed and distributed almost two million certificates for the producers to use in certifying their food animals.
It is into this introductory context regarding the voluntary certification program that I wish to interject the topic of the recent des residue incident and its impact upon voluntary certification.

On October 8, some two weeks ago, the USDA announced it had found residues in ten samples of cattle and sheep liver tissue. This report appeared to conflict with earlier statements that no des residues had been detected, and at this point various congressional spokesmen for anti-des legislation jumped into the fray, demanding an explanation for the error in reporting procedures and stating that outright banning of the hormonal growth promotant was the next step.

The USDA and FDA lost no time in announcing that additional restrictions and controls would be imposed on des as a result of the residues in the ten liver samples. The FDA changed the 48 hour withdrawal requirement on des to seven days; and the USDA imposed mandatory certification on des, setting it apart from all other animal drugs which utilize the voluntary program.

Needless to say, these two new controls on an animal drug previously under the voluntary certification umbrella, landed with no small impact upon the NADCC. The Animal Health Institute, AHI, which represents the Animal Pharmaceutical and Biologics Manufacturers, is one of the Committee's endorsers and "prime movers" in launching and maintaining the voluntary program. NADCC's administrative responsibilities are handled by AHI. The Institute's reaction to the government action is virtually identical to that of other NADCC members. AHI president, Dr. James G. Affleck, has said that "The Institute will continue to work actively to promote both voluntary animal drug certification and USDA's recently imposed mandatory certification for use of des in cattle feeds and as implants. "I am convinced," says Dr. Affleck, "that the producers will significantly increase their efforts to provide the consumer a safe, residue-free meat supply. This is a goal shared by all of us in government and industry. It is encouraging that the National Animal Drug Certification committee reports significant positive response to the voluntary program in its short six months existence."

The NADCC said in a similar statement, and here I quote the Committee's outstanding chairman, C. W. "Bill" McMillan, of The American National Cattlemen's Association. "We intend to strengthen our certification promotional efforts considerably within the beef cattle industry . . . we will redouble our efforts . . . for swine and broaden our program in poultry production. The voluntary program has served the consumer and cattle industry well. This program is essential to the success of the meat production industry and ultimate safety of the consumer. Be it voluntary or mandatory, we must have a program."

There is a very apparent theme running through these two statements I've just read to you, a theme understood among members of the NADCC: We will cooperate to our fullest with the USDA/FDA, and we are determined to strengthen our efforts to make voluntary certification for all other animal drugs work. But it should be remembered that in the final analysis, the producer holds prime responsibility for proper observance of all withdrawal requirements. They, ultimately, will make . . . or break - voluntary certification.

When will these new governmental measures become official for Des? Our best guess is soon, probably within the next month. "Why, then," you may ask, "does industry and government continue to back voluntary certification, and why are we so convinced that it can work?"
The philosophy behind voluntary animal drug certification is certainly not elaborate, but very, very basic to human nature. The Animal Protein Producers of America asked themselves whether or not they would prefer voluntary or mandatory compliance to animal drug manufacturers’ label directions for proper withdrawal times. Self-determination, self-imposition, self-discipline, call it what you will. The choice was clear: Initiate an industry-based voluntary program, and make it work.

Voluntary certification was destined to enjoy strong endorsement by industry and government from the outset. In addition to endorsements by The American National Cattlemen’s Association and The Animal Health Institute, the committee enlisted and received the backing of the American Feed Manufacturers Association, American Meat Institute, National Broiler Council, National Independent Meat Packers Association, National Livestock Feeders Association, National Pork Producers Council, River Markets Group, and Western States Meat Packers Association.

I’ve saved two important endorsements for the anchor position: USDA and FDA. Both agencies have endorsed the voluntary program as a preferred alternative to more restrictive regulations governing the use of animal drugs. Dr. C. D. Van Houweling, who heads up the Bureau of Veterinary Medicine within the Food and Drug Administration, voiced the agency’s attitude toward the program when he said: “We in FDA look on the certification program as a major step by producers. If properly observed, it will do much to help all of us in our obligation to assure the consumer of safe and wholesome food.” However, he continued, “I urge each of you to support this program, because if a voluntary program such as this is not successful you will in all probability be faced with more restrictive regulations governing the use of animal drugs and the possible loss of valuable meat production tools.”

At this juncture I would point out that Dr. Van Houweling’s words were somewhat prophetic. The Des incident provides a clear enough case in point. I dislike cliches as much as the next man, but the old saying, “One rotten apple can spoil the whole barrel” couldn’t be more true than here. Clearly, whoever is responsible for ten positive residue samples out of 2,500 tested, have through their thoughtlessness only harmed themselves. Mandatory certification was the only realistic alternative, I believe, in view of these violations. Acutally, it is clear that the existence of the voluntary program provided the vehicle for maintaining Des on the market. In other words certification provided an alternative to the banning of an essential product.

Responsibility for monitoring drug residues in food animals as you well know, rests with USDA’s consumer and marketing service. In a letter complimenting the NADCC for its efforts, Assistant Secretary of Agriculture, Richard Lyng endorsed the certificate and said that the USDA and FDA were cooperating in efforts to strengthen their activities in protecting the quality of the nation’s meat and poultry products.

As you can see, there is a considerable amount of “horsepower” behing the program. I hasten to add, however, that signing on the line and genuine participation are at opposite ends of the activity spectrum. The voluntary certification program requires enormous promotional activity if it is to reach America’s animal agriculture community, which last year produced 48 billion
pounds of red meat and poultry.

The National Animal Drug Certification Committee’s prime promotional resource is the public relations committee of the Animal Health Institute. This group was directly responsible to the NADCC for introducing the original program. They determined that the number one audience was the producer, so it was imperative that a thorough education job be effected with the agricultural press. The committee reports receiving excellent cooperation from agricultural editors.

I might add that the public relations committee stresses that FDA and USDA have been extremely helpful in educating producers and such segments of agriculture as County Extension Agents, Radio Farm Broadcasts, and Agricultural College Editors on the certification program. Government agencies have the ability to make the “grassroots” contacts so essential to the success of the program.

This fall the NADCC has requested the PR committee to introduce a special campaign aimed at producer meetings. This promotion in turn has been designed to touch base with the program endorsers and to ask their participation in getting the message of proper drug use to producers. In a way that publicly reaffirms their commitment and will to participate in the program, the endorsers clearly indicate to the consumer, I think, that they remain convinced that voluntary certification is a good thing and that they continue to give it their wholehearted support.

What will certainly prove to be the most important promotional thrust given the certification program since its inception will be provided by the AHI public relations committee after the first of the new year. They plan to approach the agricultural media in a 1972 campaign designed to literally reintroduce the entire concept of certification. The group hopes to draw again from the considerable resources available through the NADCC and the AHI member-companies in aiming its message at, to be more specific, the small farm producers.

These people are often isolated in one way or another from the mainstream of “The New Scientific Agriculture.” They just simply may not have the time to read all the latest requirements concerning drug withdrawal times. The AHI public relations committee feels these producers need to be approached in particular.

In addition, a survey assessment of the certification program is already underway. It should provide valuable information into the areas which need to be re-emphasized for the 1972 campaign. The Animal Health Institute’s Market Research Committee has designed this six-month progress survey in conjunction with Doane’s Agricultural Service, an independent survey group based in St. Louis. Plans presently call for release of the final results after January 1. Incidentally, our plans also call for a second survey later in 1972 to measure the program’s effectiveness over the period covering its first year. This data, of course, will be compared with the six month figures to give us an accurate appraisal of our progress. The goal remains to certify all meat producing animals, using animal drugs requiring withdrawal times, either through NADCC’s voluntary program, or, as with Des, through a government regulated mandatory program.

Now that I’ve talked at some length about the NADCC’s certificate, let’s take a quick look at it. The certificate gives the user the option of certifying either that he has properly withdrawn his animals from animal drugs or that he guarantees that the animals, while in his possession, have not received drugs or feed additives. Carbon copies are to be retained by the person to whom he consigns his animals, a packer for example. In this way both parties have a record that all government
requirements have been met.

Where does this voluntary form stand now that USDA and the FDA have changed the requirements for Des? We hope to have the answer on November 2, when the two government agencies meet with NADCC members to discuss these latest developments. I personally feel that a certificate incorporating both programs would best serve the producers and packers. With two different certificates, one for mandatory certification of Des, the other voluntarily certifying all other animal drugs, the industry would have a veritable paper blizzard on its hands.

What we need to insure the continued success of certification, whatever form it takes, is simplicity. Producers have enough to do within the limited manhours available to them now. Duplicating efforts would, I think, only serve to discourage them.

An encouraging indicator is given by the Government's complete cooperation in discussion and formulating a new program involving both certifications. As I mentioned earlier, NADCC's position is one of complete cooperation regarding the new regulations. I am confident the outcome of our discussions will be beneficial not only for the Animal Protein Industry but for the consumer, whose safety must be the prime consideration.

In the time allotted me I've given you the current information regarding The National Animal Drug Certification Committee's voluntary certification program. In summarizing I again re-emphasize that voluntary compliance can work. It will take considerable promotion by the meat production industry. The final responsibility, however, rests with the cattle producer out in Arizona, and the feedlot operator here in Oklahoma, and the swine farmer in Iowa. They are the people who must understand that animal drugs have given agriculture invaluable production tools. If these tools are abused, as Dr. Van Houweling has implied, they may be gone tomorrow. This would be sad indeed for the cost conscious consumer and for the farmer who treads a fine enough profit line as it is.
THE LIVESTOCK DRUG INDUSTRY
IN THE 1970's

Remarks by
Dr. David A. Phillipson
Vice President - Agricultural Division - The Upjohn Company

I'm pleased to have the opportunity to talk to you today about the future of the livestock drug industry, for I think it's an extremely important and timely topic, especially in this age of constant change and rapid technologic development.

But, before we can discuss what's ahead, I think it would be prudent to take a quick look at the past, to try and discover just where we stand today.

As you know, we're all part of a truly amazing past, for U.S. agriculture during the last 50 years has been full of invention, development, ingenuity, technological advancement, and progress.

At the turn of the century, America's farmers had to feed and clothe 75 million persons. Today they're feeding and clothing nearly 3 times that number, and at a standard undreamed of only a few years ago.

And they're doing it with ease . . . our people are the best clothed in the world. And we even have enough farm products left over to help meet the needs of many other countries.

Yet . . . scarcely 50 years ago, ours was a horse-drawn agriculture, not much different from that which existed even a hundred years before.

What was it that enabled American agriculture to leap ahead at this fantastic pace that has amazed the rest of the world? Why is our nation basking in an abundant food supply, instead of on the verge of starvation?

Much of the credit, of course, must go to our industrious, hard-working farmers who set the pace for this rapid advancement. Their strong desire to possess — and even demand — new and better methods of managing and operating their farming enterprises has literally pulled agriculture up by its own bootstraps to the tremendous level of production we see today.

But, a large measure of credit, I think, must go to our scientists, academicians, and agricultural businesses, who have backed up the farmer all the way, and have provided the technology that has enabled him to make his history-making forward strides.

Their diligent study and far-sighted research and development has made our agricultural enterprise in the U.S. one of the most modern and advanced businesses in the world, and has permitted an output of food and fiber that was thought impossible even 20 years ago.

And, as you gentlemen are fully aware, the animal health and livestock drug production industry has been one of the leaders in this advancing technology.

For without the development and invention of many of our drugs, this great increase in agricultural production would not have been possible. And I think that we too often lose sight of this fact, and tend to take these developments too much for granted.
The list of livestock diseases conquered during the last few years has been almost endless.

Without these disease controls, our modern poultry, cattle and hog production operations would be all but impossible, for who would want to take the great risk that some dread disease would sweep through their operation killing thousands — or in some cases, millions — of animals or poultry.

And our growth promoting products have added literally millions of pounds of extra meat to the grocery shelves of the nation, and have helped keep the cost of these products low enough so that all of us can be well fed.

For example, a recent report issued by the USDA states that these growth promotants save U.S. consumers up to Four Hundred Million Dollars annually, by allowing our farmers to be more efficient in their meat production.

Perhaps even more important, the high standards of modern livestock and poultry production would be literally impossible to maintain without such disease controlling and growth promoting drugs.

So, I think we can say that the animal health industry has certainly contributed its share in the development of our modern-day agriculture.

But, we cannot sit back and smugly remember our successful past, for our battle has only just begun.

Each day, nearly 150,000 new beings are added to this already overburdened planet . . . a staggering increase that will require the additional equivalent of nearly 9 million head of beef cattle, one million dairy cows, 20 million hogs, and 5 billion layers each year in order to keep them well fed.

And even without this additional burden, the world is already in trouble. For, during the 15 to 20 minutes I spend talking to you here today, hundreds of persons are dying of starvation.

So here, ladies and gentlemen, lies our challenge for the 70's . . . to put all the know-how we've got into a giant effort to help feed a starving world.

Meeting the huge demands of our fellow men is not going to be easy . . . but it's a job that must be done. And happily, I think it's a job that we've already set our sites on and the work is underway.

Already, all of us involved in the animal health industry — the manufacturers, the veterinarians, the government agencies and the academicians, are being called upon to contribute all we can during the decade ahead to help solve this world food shortage crisis.

In order to meet this growing challenge, I think that all of us are going to have to be prepared for some big changes in our industry — changes which are going to be necessary for us to help American agriculture attain its giant food production goals.

Probably one of the biggest changes — and the one that I think will have a most profound effect on all of us involved in the drug industry — will be the continually increasing size of our American farming operations.

In order to meet the ever-growing demands being placed on their time and money, farmers will continue to increase the size of their operations during the 70's. This is the only way he'll be able to efficiently employ the resources and new technology that will be required of him.

These larger units are going to place increasing demands on all of us involved in the drug industry and livestock health.

Farm owners will require more technical assistance from all of us as never
before, and I think all of us will experience a re-direction of our activities in order to accommodate the needs of the large, professional farm operator of the very near future.

He will demand from the drug industry — and need — many new, effective and safe drug products to aid him in his constant efforts to produce efficient, profitable disease-free animals that will provide food for our tables.

And, to properly manage this side of his growing business, he’ll need ever-increasing technical assistance and education from us, to enable him to use these new products economically, efficiently and safely.

The academicians will be asked for more basic research and educational assistance; government agencies will be called upon for more management guidance to help him continue delivering an economical, wholesome meat supply to the consumer; and the drug industry will be expected to keep all segments of agriculture informed about its products, and trained in their proper use.

We’re beginning to see a surge of activity in these directions already, as all segments of our industry are rallying to the needs of more and better education for those involved in our nation’s food production.

Witness, for example, the recent joint animal drug industry speci-directed seminars presented to the FDA task force. Or the joint livestock-drug industry’s program to introduce the livestock drug withdrawal certificates to livestock producers everywhere. And industry’s prior efforts to alert producers to follow specified withdrawal times of all drug products.

Livestock feed manufacturers, who will become an ever-more important link between drug manufacturers and the farm operator, are also shoring up their educational program and technical assistance to their customers. They are actively participating in all of the previous programs I’ve mentioned, and are promoting proper use of livestock drugs through their own industry associations, and also through many individual company activities.

The net result of all this activity will be that tomorrow’s farmer will become a highly skilled professional in the proper use of all drug aides that he employs in his livestock operations, and his judgment will command renewed confidence and respect on the part of consumers throughout the world.

I also foresee a change in the operational procedures of tomorrow’s veterinarian... a continuing switch from therapeutic medicine to preventive medicine.

The veterinarian of the 70’s will continue to be the livestock producer’s major defense in keeping his animals healthy and disease free, but he will be just as concerned with preventing the occurrence of disease as he will be with controlling it after an outbreak.

It will be this veterinarian’s job to lend professional livestock management assistance to his clients, so that only more and more rarely will he be called upon to treat a disease outbreak in his client’s herds.

Many of our schools of veterinary medicine are already changing the structure of their educational programs to emphasize techniques of preventive medicine.

And much of the technology to aid the veterinarian in preventive medicine is already with us, so I predict we’ll see this major change of emphasis completed by the end of the 70’s... which will bring us ever-closer to the goal of producing completely disease-free livestock.

Also during the 70’s, I believe we’ll see an ever-increasing cooperation between
all segments of the drug industry — the regulatory agencies, drug manufacturers, the veterinary profession, and livestock feed companies . . . cooperation designed to help assure the safety and wholesomeness of tomorrow’s food supply.

This cooperation will be necessary and vital if we are to attain our goal of feeding a hungry world, and yet retain our country’s solid economic condition. Each of us has a great stake in seeing that these goals are achieved, and only through the pooling of all our resources through solid cooperation can we achieve them.

If we divide and go our separate ways, to only work on our own special interests, we’ll dilute ourselves to such an extent that we may seriously hinder the continued progress of American agriculture.

We, therefore, must be careful to guard against letting emotionalism affect our actions, and must never lose sight of our scientific goals as we face these new challenges in the years ahead.

I’m particularly concerned that we do not get too deeply caught up in the wave of emotional, crusader consumerism that now poses a threat to the entire drug and livestock industry.

Consumer protection is, of course, a useful function in our society. But it can do great harm when it cases aside all scientific reasoning and falls largely in the hands of the uninformed publicity seekers . . . who choose to ignore scientific facts completely.

I think that many of our most important drugs and pesticides are in jeopardy right now, and with very little, if any, scientific basis for concern.

Antibiotics in livestock feeds are under criticism for fear they’ll cause people to become immune to them, should they ever need the same drug for treatment of diseases in their own bodies. Yet, the facts show that more than 100 billion meat animals, fed on antibiotics, have been consumed without documented cases of antibiotic resistance being recorded as a result.

Diethylstilbestrol is currently under fire because the crusaders say that it is a cancer-causing compound, and is showing up in the meat we eat. Yet, they fail to tell us that a person would have to eat up 2,200 pounds of cattle livers, containing DES residues, at one sitting, just to get a therapeutic dose of this drug that many physicians actually prescribe in the treatment of certain human disorders. And the crusaders fail to balance their criticism by informing the public that we all have many of these compounds circulating naturally through our boides in far greater concentrations than we’d ever get from eating meat products.

As another example, the usage of 2, 4-D and 2, 4, 5-T were recently severely limited by the Environmental Protection Agency — and this agency must have reacted largely to the emotional demands on the part of these crusaders, as its own scientific advisory committee voted 9-1 in suggesting continued use of these products.

This type of reasoning just doesn’t make much sense, when it completely ignores advice from the scientific community. This disbelief in our scientists is frighteningly similar to that which was found in Europe during the Dark Ages, when that continent was set back decades through emotionalism and fear.

What would have happened to world progress if we would have had this same type of unscientific reasoning in years past?

Would one death in a million due to allergic reaction to penicillin have stopped
development of that drug . . . and have condemned millions of others to death who would have been denied its life-saving use?

Or would stringent residue requirements have stopped the development of DDT, which has since saved the lives of millions who would otherwise died of malaria?

Many of us in the drug industry are beginning to fear that this kind of situation may well be with us during the 70's, if, indeed, it isn't already.

It now takes up to seven years and may cost several million dollars to bring a new major drug product to the market. And this cost goes up every day as the result of new restrictions placed on us, too often as the result of some who cry "wolf."

What this means is that only drug products that will have widespread usage . . . enough to return that several million dollar investment . . . will be developed. And many very important, and much needed compounds, will not be brought to the market because they may only see uses in rare, or isolated disease cases, and forecasted usage will not warrant their production.

For example, the Delaney Amendment to the Food and Drug Administration Act allows only a zero residue of any substance known to cause cancer. On the surface, and to the general public, this sounds like a reasonable restriction.

But, when our scientists are coming increasingly to believe that almost anything - if given in high enough dosage - can cause cancer, one wonders just what drugs will be permitted in the future.

And when our scientists tell us that it takes astoundingly high dosages of even the most potent carcinogens to cause cancer in a human being, we wonder about the rationality of allowing new and beneficial products to be shelved when sophisticated measuring can find only one minute part per billion - or maybe only a few molecules - of the substance in residue studies.

Our scientists tell us these amounts are so insignificant as to be ridiculous. But our crusaders and publicity-seeking politicians tell us to cease their development.

Requirements and restrictions on drugs and pesticides have gotten so out of hand in this country that - if these same restrictions were applied to the foods we eat, almost nothing would be left on the table.

Regulations require up to a 100-fold safety factor in non-cancer-causing substances, and a sero safety factor in known carcinogens. Applying these factors to naturally occurring chemicals that are found in common foods, we'd find the following items removed from our tables: Vitamins A, D, and K; bananas; strawberries; pineapple; nutmeg; eggs; peaches; pears; spinach . . . just to name a few.

The safety record of the drug and pesticide companies has been remarkable, even during the time when we were largely self-policied. This is obvious in the fact that the crusaders usually fail to document their charges with facts. That's because there are none that will constructively back their cause.

You really have to dig - and I mean dig - to find documented cases of human death or injury that has resulted from faulty drugs. And the few cases of pesticide poisoning we've seen during the last few years have almost all stemmed from accidents due to the improper usage of compounds.

I don't mean to imply that there are absolutely no risks involved in introducing these new compounds to the public. There are risks in everything we do. Indeed, I have heard that hundreds of people have died from allergic reactions to many of
our most common drugs, like penicillin, tetanus vaccine, and the like. But, we've
wisely decided that the benefits of these drugs far outweigh their risks, and so,
while a very few suffer from them, millions owe their lives to them.

Yet, let one farm worker contract pesticide poisoning and you'll see an uproar
across the nation that is truly amazing. And, immediately, the millions of pounds of
extra food this product may have provided to the world's hungry is forgotten.

I sometimes think that, if these crusaders hadn't been raised in a country that
has always kept them fat and healthy, and more than provided for their needs, they
wouldn't be quite so quick to criticize our attempts to provide food and health for
the world.

If they were the head of a slowly starving family in India, I'm sure they would
think twice before making the front page with many of their unfounded
accusations.

So I think the challenge is there for all of us involved in the livestock drug
industry — the manufacturers, the regulatory agencies, the veterinarians and the
feed companies — to form a cooperative, united front that will help return scientific
reasoning to its rightful place in deciding these matters.

I don't personally feel emotionalism has any place in decisions of these kind,
especially when there's so much at stake throughout the world.

And I also think there's a great challenge here for our country's press, to try even
harder to give fairer, more balanced coverage to these issues . . . which will provide
a more realistic picture to the public even though it might not sell as many
newspapers as some of the more screaming headlines.

Too often, these emotional individuals are able to grab front-page headlines with
their charges. And then it isn't too long before these allegations become fact in the
minds of the public and some politicians.

But, let one of our country's scientists try to refute these charges, and his
statements are buried somewhere in the back of the paper, if printed at all.

I think we all have to work hard to overcome this unfortunate trend toward
emotionalism and I submit that if it continues, not only will the world's tables
become increasingly short of food, but our own country's development will also
suffer.

We now only spend 16 per cent of our earned dollars for food in this country,
compared to up to 55 percent in other nations of the world. This means we don't
have to spend as much time working to keep bread on our tables, and we have time
and money left over to tackle many other problems our nation is facing.

So, I think it's time we all stop and take a long look at where we're heading.

Are the 70's going to rival the 60's in agricultural development and progress? Or,
are we finally going to begin to revert and leave a great portion of the world
without adequate food and fiber.

We certainly have the technology to get the job done, and our brilliant record of
the past 50 years has proven that we have the ingenuity and ability as well.

So, let's all accept the challenge of this progressive, rapidly changing agriculture
that we're all a part of, and lead it through to a dramatic new high in the 70's.

We in agriculture have no choice but to accept this challenge — and see it
through to fruition. Because, for millions of hungry people throughout the world —
we are their only hope for survival.
SAFETY EVALUATION OF AN AMICARBALIDE ISETHIONATE FORMULATION IN PONIES

Weidle,* Vance K., Jr., and Edds,** George T.

Equine Piroplasmosis (EP) is an infectious hemoprotozoan disease of solipeds characterized by fever, anemia, icterus, and other clinical signs arising from hemolytic anemia caused by Babesia caballi and Babesia equi. Developments in the knowledge about the activity of antibabesial drugs have coincided with the recent need for therapy of EP in the United States. Among these antibabesial drugs are the aromatic diamidines, which include an amicarbalide isethionate formulation (AIF). The developments involved safety and efficacy studies comparing AIF to other aromatic diamidines in babesia-infected cattle, mice, horses, and dogs. Injection of 8.8 mg/kg amicarbalide as AIF intramuscularly on each of 2 successive days was shown to remove the carrier state of Babesia caballi infection, but insufficient information had been obtained concerning the margin of safety and pharmacological mechanisms by which side reactions are produced.

It was the purpose of this investigation to gain information about the organ and tissue response of ponies to dosages of AIF at and greater than the above therapeutic level.

Specifically, studies conducted included monitoring the changes in packed blood cell volume, kidney function, serum enzymes liberated by liver and muscle, plasma glucose, and total and differential white blood cell counts. Following these in vivo studies, post-mortem gross and histologic tissue examinations were made in recognition of the need for relating quantitatively the influence of dose level on the amount of tissue response and the values obtained in the ante-mortem tests.

LITERATURE REVIEW

Among the babesicidal agents studied recently, aromatic diamidines have shown the most promise of being efficacious, but establishment of any one drug as a treatment of choice for babesiosis was hampered by the frequent side reactions occurring with their usage. Prior to the use of aromatic diamidines,

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a Diampron, May & Baker, Limited, Dagenham, England. Aqueous solution containing 3,3'-diamidinocarbanilide 50 per cent w/v, N-monomethylacetamide 27 per cent w/v, and chlorocresol 0.1 per cent v/v.
6,6'-di(-N-methylquinolyl) urea dimethosulfate (quinuronium sulfate) was the treatment most commonly used for bovine babesiosis. During the search for a new drug which would be at least as effective as quinuronium sulfate, but without its toxic effects, a large number of compounds had been screened for activity against *Babesia rhodaini* in mice. The most promising of these were then tested against *Babesia divergens* in splenectomized calves.1,2

In 1960, the initial field trials of AIF in cattle were performed, but the data pertaining to tolerance and safety were gathered only by gross observations and incidental to the evaluation of the curative properties of the drug. It is a white, crystalline, anhydrous solid, molecular weight 548.6, melting point 209°C (decomposing at 256°C), and is approximately 100 per cent w/v soluble in water.

Compared to quinuronium sulfate, AIF did not cause release of histamine in significant amounts in mice or in sheep diaphragms, but did in rats, as evidenced by slight excitement and some face washing. In rats, where quinuronium and AIF released similar amounts of histamine, quinuronium was distinctly more toxic.9 The acute toxicity (LD₅₀) of AIF in mice was found to be 120 mg/kg subcutaneously, compared with 8 mg/kg of quinuronium sulfate.1 However, when the 2 compounds were injected subcutaneously in non-infected, splenectomized calves at a site behind the shoulder, the maximum tolerated dose of AIF was greater than 40 mg/kg, while that of quinuronium sulfate was less than 4 mg/kg. There were no obvious signs of toxicity in the calves that received this dose of AIF.

Shone et al.17 described experiments demonstrating certain tissue reactions at site of injection of AIF. On this basis, these workers advised against use of the subcutaneous route of administration. Local reaction to intramuscular injection of 10 mg/kg of amicarbalide as AIF was limited to swelling observed at the site of injection on the day following administration.

In studies comparing AIF and phenamidine isethionate by Shone et al.17 against *Babesia bigemina* in calves, AIF showed a much earlier effect on the parasites as judged by the appearance of degenerate forms. Animals in the AIF group were less seriously ill and never stopped feeding entirely, whereas phenamidine-treated animals stopped eating for 2 days.

An extensive investigation of the pharmacological action of 4 aromatic diamidines, 4,4'-diamidinostilbene (stilbamidine), 4,4'-diamidino-1,3-diphenoxypropane (propamidine), phenamidine, and pentamidine has been reported by Wein.24 The diamidines reduced the pressor and other effects of adrenalin. The fall in blood pressure was reduced but not abolished by full doses of atropine.

Beveridge et al.4 reported that local tolerance of adult dairy cattle to subcutaneous and intramuscular injections of 5 to 20 mg/kg amicarbalide was good, particularly when 20 per cent rather than 40 per cent w/v solution was used.

It was the aim, in establishing a dosage for treating animals for babesiosis in enzootic areas outside the United States, to reduce the parasitemia only to a point at which the defense mechanisms of the patient are able to set up a balance between parasite and host. This so-called state of premunition, wherein the host enjoys a valuable measure of resistance to further infection, is dependent upon the continuing presence of the infecting organism in the tissues of the animal throughout its life. Complete sterilization of the blood is therefore inadvisable in an enzootic area, since the patient emerges from a successfully treated attack of
babesiosis as susceptible to reinfection as it was before.16

The history of EP and its appearance in the United States has been reviewed.20,21 To date, the disease has been localized and essentially confined to south Florida due to the presence of the horse tick, *Dermacentor nitens*, as principal vector.21

Evaluation of chemotherapeutic agents for safety and effectiveness against EP in Florida has been most important in order that an agent be discovered that could be used to completely eliminate the carrier state of the disease. Finding such an effective agent has permitted inclusion of a therapeutic phase in the current EP control program in the United States.6,13 This also involved quarantine of animals identified as infected and/or carriers by complement-fixation and the control of vector ticks.6 It has been somewhat difficult and time consuming to determine a minimum dosage schedule that would eliminate the carrier state of the *Babesia caballi* infection, produce minimum side effects, and still be practical for use.14 Clearing the animal from circulating organisms was dependent upon the dosage and frequency of drug administration. It was possible to eliminate the infection with a single large dose, but this resulted in increased signs of intoxication. By decreasing the daily dose, but administering the drug for several successive days, reduced toxicity to the host animal resulted.14

On the basis of extensive trials involving natural and experimental EP infection on both intact and splenectomized horses, with diagnosis by complement-fixation and blood smear, and subsequent negative subinoculations into susceptible horses, it has been concluded that 3 drugs are capable of removing the carrier state of *Babesia caballi*.8,14 The drugs are Berenil, 5 mg/kg of body weight intramuscularly on each of 2 successive days, phenamidine isethionate, 8.8 mg/kg of body weight intramuscularly on each of 2 successive days, AIF, 8.8 mg/kg of amicarbalide isethionate of body weight intramuscularly on each of 2 successive days.8,14

A successful treatment for clearing *Babesia equi* infection has not been developed,14 although reevaluation of dosages of the above drugs, as well as investigation of new drug combinations, is continuing.21,22 Workers in Florida have compared physiological and tissue response to Berenil, phenamidine, and AIF.8,14 Berenil produced swelling and necrosis at intramuscular injection sites, respiratory embarrassment, and intoxication.14 Undesirable side effects also occurred with phenamidine, for when administered subcutaneously, as recommended by the manufacturer, it produced extensive swelling, which often progressed to dry necrosis with subsequent sloughing of the skin. In an attempt to avoid tissue destruction, phenamidine was administered intramuscularly to several horses. However, the toxicity of the drug was apparently increased. This toxicity, more damaging to the liver than other organs, was considered a serious and highly undesirable side effect, and caution was urged when the drug was to be used in horses known to have impaired liver function.16

At therapeutic dosage, AIF produced fewer side effects than either of the 2 previously described drugs.

Respiratory or digestive disturbances were noticed in some animals. In the early trials, 2 horses exhibited swellings in the area of the eyes and muzzles, in addition to areas over the back and sides.14 The horses responded to symptomatic treatment.

May and Baker, Limited, has indicated that N-monomethyl-acetamide (NMNAA)
in incorporated into AIF as a solubilizing agent\textsuperscript{5} and the chlorocresol for its antibacterial action.

**MATERIALS AND METHODS**

Ponies of primarily Shetland breeding were purchased from private sources through auction markets. The ages ranged from 2 1/2 to 10 years. Six weeks prior to the initiation of the projects, they were dewormed with a commercial preparation of 1,1-dimethyl, 2,2,2-trichloro-1-hydroxyethyl phosphonate, micronized phenothiazine, and piperazine dihydrochloride\textsuperscript{b} administered through stomach tube at the manufacturer's recommended dosage. The ponies were kept separated from other animals, fed approximately 2 pounds of a ground grain mixture once daily, with good quality grass hay and fresh water \textit{ad libitum}. No signs of systemic infectious diseases were observed at any time during the experiments. Immediately preceding each phase of the project, the ponies to be used were weighed and placed in groups of predetermined size. The hair was clipped from the ventro-lateral aspect of the neck of each animal to facilitate location of the jugular vein.

Sampling of blood was by jugular venipuncture using 1 1/2 inch 20 guage Vacutainer needles.\textsuperscript{c} Blood for serum biochemical analyses was collected in 10 ml Vacutainer tubes with silicone-coated interiors and no additives.\textsuperscript{d} For tests involving whole uncotted blood, 10 ml Vacutainer tubes containing 143 U.S.P. units of sodium heparin were used,\textsuperscript{d} and for plasma glucose determinations, blood samples were collected in 5 ml Vacutainer tubes containing 10 mg sodium fluoride and 10 mg potassium oxalate,\textsuperscript{d} which supplied the glycolytic inhibition required for this test.

Packed Blood Cell Volumes (PCV) were obtained by the microhematocrit method\textsuperscript{3} with the use of plain capillary tubes and a Model MD International Micro-Capilalry centrifuge.\textsuperscript{e}

Blood Urea Nitrogen (BUN) values were obtained by the use of the Urograph paper chromatography method,\textsuperscript{3} and reported in mg of urea nitrogen per 100 ml of blood.

Sorbitol dehydrogenase (SDH)\textsuperscript{11,19} and creatine phosphokinase (CPK)\textsuperscript{18} determinations were made by measuring optical density changes with an Eskalab Alpha\textsuperscript{g} at ultraviolet wavelength 340 mu. Values are reported in Sigma units, 1 Sigma unit of SDH being that amount of the enzyme representing 1 millimicromole of DPNH oxidized per hour at 25° C under the assay conditions of Sigma Chemical Company procedure number 50-UV.\textsuperscript{19} One Sigma unit of CPK will phosphorylate 1 millimicromole of creatine per minute at 25° C under the conditions of Sigma Chemical Company procedure number 40-UV.\textsuperscript{18}

\textsuperscript{b} Dyrex, T. F., Fort Dodge Laboratories, Fort Dodge, Iowa.
\textsuperscript{c} Becton, Dickinson and Company, Rutherford, New Jersey
\textsuperscript{d} Becton, Dickinson and Company, Rutherford, New Jersey.
\textsuperscript{e} International Equipment Company, Needham Heights, Mass.
\textsuperscript{f} Warner Chilcott Laboratories, Morris Plains, New Jersey.
\textsuperscript{g} Smith Kline Instrument Company, Philadelphia, Pa.
White cell counts were performed using standard application of the Coulter Electronic Cell Counter."\(^h\)

Plasma glucose was determined by the o-toluidine method of Dubowski, and reported in mg of glucose per 100 ml of blood.

Euthanasia was accomplished by intravenous administration of a saturated solution of sodium pentobarbital in 50 per cent dimethylsulfoxide. Samples of tissue taken at necropsy were fixed in 10 per cent buffered formol-saline, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Group 1 consisted of 2 mares and 2 stallions weighing from 152 to 207 kg each. Values for SDH, CPK, PCV, and BUN were determined 3 times during the week preceding the injection of the therapeutic dose, 8.8 mg/kg amicarbalide as AIF intramuscularly with a 1-1/2 inch 18 guage needle in the gluteal region on each of 2 successive days. The tests which had been performed prior to the administration of the drug were made again on the first, second, third, and fifth day following the second injection.

Group 2 consisted of 5 geldings weighing from 168 to 218 kg each. Values of SDH, CPK, PCV, BUN, plasma glucose, and total white blood cell counts were obtained 3 times during the week preceding drug administration. Two ponies were given 26.4 mg/kg amicarbalide as AIF, or 3 times the therapeutic dose, intramuscularly on each of 2 successive days. A solution was made of 500 mg crystalline anhydrous amicarbalide isethionate per ml of sterile water (SIW). Individual doses of AIW were prepared immediately prior to use. Two ponies were given 26.4 mg/kg amicarbalide as AIW intramuscularly on each 2 successive days. Finally, one pony was infected intramuscularly on each of 2 successive days with a volume of sterile water corresponding on a body weight basis to the volume of AIF and AIW given to the other ponies in this group. The tests for possible disturbed functions which were performed prior to drug administration were repeated on the first, second, third, fourth and eighth day following the second injection. Post-mortem examinations of animals that succumbed were made and tissues were collected for histological examination.

Group 3, consisting of 1 gelding, 2 mares, and 1 stallion, weighed from 132 to 204 kg each. Values of SDH, CPK, PCV, BUN, total and differential white blood cell counts were obtained 3 times during the week preceding drug administration. The mares and stallion were given 17.6 mg/kg amicarbalide as AIF, or twice the therapeutic dose, intramuscularly on each of 2 successive days. The gelding was injected intramuscularly on each of 2 successive days with an amount of sterile water corresponding on a body weight basis to the volume of AIF given the other ponies in this group. The same tests which were performed prior to drug administration were measured on the first, second, third, fourth, and ninth day following the second injection. One mare was euthanized 120 hours after the second injection. Post-mortem examination was performed and tissues were collected for histologic examination.

Group 4 consisted of 2 stallions weighing 187 and 200 kg. Intramuscular injections in separate sites were made in both ponies simultaneously as follows: 8.8 mg/kg amicarbalide as AIF, 8.8 mg/kg amicarbalide as AIW, and 27 per cent

\(^h\) Coulter Electronics, Hialeah, Florida
solution NMMAA in sterile water equal in volume to the other 2 injections. One pony was euthanized 48 hours and the other 24 days post-injection. Post-mortem examinations were performed, and tissues were collected for histologic examination.

RESULTS

**Group One.** Ponies in group 1, receiving 2 injections of 8.8 mg/kg amicarbalide as AIF, the dosage recommended for clearing the carrier state of *Babesia caballi* infection, showed minimal changes in the parameters measured following administration of the drug. As shown in Figure 1, serum levels of SDH increased from 342 Sigma units pre-injection to 796 Sigma units as a peak on the second day post-injection, and by the fifth day post-injection, the value had declined to 628 Sigma units. Figure 2 shows a second day post-injection value of serum CPK of 4.7 times the pre-injection mean, and decline to 1.6-fold value on post-injection day 5. The PCV pre-injection mean was 33.9 per cent and post-injection values ranged from 32 per cent to 34.5 per cent. The amount of blood urea nitrogen ranged from 16.6 mg/100 ml to 19.1 mg/100 ml following injection, while the mean pre-injection value was 18.7 mg/100 ml.

**Group Two.** All ponies in group 2 that received injections of 26.4 mg/kg amicarbalide were anorectic for 2 days post-injection and exhibited signs of abdominal pain starting 1 hour after each injection and continuing for approximately 8 hours. These signs consisted of kicking at their abdomens, mild sweating, looking around at their sides, and repeatedly lying down for short periods of time. One pony in group 2 was found dead on the morning of the second day after the second injection of 26.4 mg/kg amicarbalide as AIW. Time of death was estimated at 36 hours post-injection. Another gelding in group 2 died 72 hours following the second injection of 26.4 mg/kg amicarbalide as AIF. The pony was found recumbent, and during the 30 minutes preceding death exhibited dyspnea, sweating, opisthotonus, cyanotic mucous membranes, and semi-rigid extension of all legs. Thus, the data on group 2 on post-injection days 2 and 3 were from tests on 3 ponies and from 2 ponies on days 4 and 8.

**Blood Biochemistry and Hematology.** Figure 1 shows a 915-fold mean increase in SDH in group 2, from 273 Sigma units pre-injection to a 250,00 Sigma unit peak on day 2 post-injection. The increase in CPK on ponies in group 2 is shown in Figure 2, the peak being 7.7 times the pre-injection value. Figure 3 and Table 1 show the transient leucopenia that occurred at this dosage level. Table 1 also shows that only minor changes occurred in blood urea nitrogen, PCV, and plasma glucose values. The samples which had 29 mg per 100 ml blood urea nitrogen, 58 per cent PCV, and 26.3 mg plasma glucose per 100 ml were all drawn from the same pony within 20 minutes of death 72 hours post-injection.

**Pathologic Findings.** On post-mortem examination, the ponies were found to be in excellent nutritional condition. The gross pathology was essentially the same and lesions were widespread in both ponies that died following administration of amicarbalide at the 2 injections of 26.4 mg/kg level. Large and small intestine mucosal surfaces showed hemorrhagic enteritis. Extensive ecchymoses were visible on urinary bladder mucosa, endocardium of both ventricles, and parietal pleura in dorsal mediastinal area. Both lungs were dark and congested, but not atelectatic. All lobes of the liver exhibited multiple areas of severe fatty degeneration. These areas
were 5 to 80 square centimeters in size, and tan to yellow in color. Injection sites in gluteal muscles were a light gray color, well delineated, and involved approximately 30 cubic centimeters of tissue. Serosal surfaces of spleen showed extensive petechial hemorrhage.

The histologic picture of the liver was primarily one of necrosis at the peripheral part of the lobules. The hepatic cells in these areas either had disappeared or exhibited varying degrees of degeneration, consisting of swelling of their cytoplasm with vacuolation and karyorrhexis. Lobular pattern was preserved and hepatic cells in the central part were less severely affected, although several lobules showed extreme dilatation of sinusoids with red blood cells and hemorrhagic extravasation.

In the renal cortex, tubule epithelium showed severe degeneration and necrosis, and in several tubules it had even disappeared and had been replaced with granular eosinophilic material. Many tubules contained hyaline casts and microcalculi were seen in occasional tubules. Intertubular and blomerular capillaries were highly congested.

Microscopic lesions were evident in skeletal muscle only at the injection sites, where acute myositis was manifested by hyalinization and disappearance of sarcoplasm. Muscle fibers were fragmented and an inflammatory exudate consisting of fibrin and polymorphs was present between fibers.

In the small intestine, surface epithelium of the mucosa was necrotic and desquamated in several places. The lamina propria was edematous and infiltrated with inflammatory cells, mostly of the mononuclear and polymorphonuclear variety.

Acute enteritis was the overall picture in the large intestine, slightly less severe than in the small intestine, though at certain areas of the submucosa, the infiltration of inflammatory cells was more severe.

The urinary bladder exhibited lakes of extravasated blood under the epithelium and in the submucosa. At places, the epithelium appeared desquamated, but no inflammatory reaction was apparent in any part of the wall.

Subendocardial hemorrhage occurred at several places in the heart, and extensive extravasation into the myocardial was seen between muscle bundles and around occasional muscle fibers. Myocardial fibers exhibited normal striations.

Splenic pulp was severely congested; sinusoids were dilated, and some hemorrhage had occurred into the subcapsular area. Lymphoid elements appeared somewhat atrophic and stray areas of focal necrosis were evidenced by smudged cells in the pulp.

Group Three. Ponies receiving injections of 17.6 mg/kg amicarbalide as AIF on each of 2 successive days showed anorexia of 1 day’s duration and signs of mild abdominal discomfort lasting approximately 3 hours.

On the second day post-injection, the ponies' serum showed a mean peak SDH value of 27 times the pre-injection value. (See Figure 1) Serum CPK increased to an 8.2-fold peak on the third day after administration of the drug. (See Figure 2) Both enzymes were at normal levels on the eighth day post-injection. The leucopenia manifested at this dosage level did not reach the minimal point reached by the ponies in group 2, but the means approximated those of the higher dose group. (See Figure 3) BUN, PCV, total and differential white blood cell counts are shown in Table 2.

Pathologic Findings. The mare euthanized 120 hours after the second injection
was in excellent nutritional condition and was an estimated 120 days pregnant with a normal fetus.

The kidneys exhibited some hyperemia on cut surface of sagittal section, but other grossly visible pathology was limited to skeletal muscle at injection sites. In these sharply delineated areas, approximately 20 cubic centimeters of tissue had a pale gray cooked appearance.

Histologically, the periphery of liver lobules was composed of hepatic cells exhibiting cytoplasmic vacuolation and eosinophilic degeneration. Some degree of proliferation of biliary epithelium was seen in the portal areas.

Examination of renal tissue revealed increased cellularity in occasional glomeruli, hyaline droplets in the cytoplasm of stray tubular epithelial cells, and focal mononuclear infiltration around tubules.

As suggested by gross appearance, the condition at the intramuscular injection sites was one of acute myositis, showing inflammatory edema, and inflammatory cells between muscle fibers.

Diffuse leucocytic (eosinophilic) infiltration was seen in the lamina propria and submucosa of the large intestine.

**Group Four.** During the period of observation between administration of the drugs and euthanasia, the 2 ponies in this group receiving 8.8 mg/kg of amicarbalide isethionate showed no signs of adverse reaction to the drugs. At necropsy, both ponies were in excellent nutritional condition. No pathologic lesions were visible grossly, except at flutal intramuscular injection sites.

A 10 cubic centimeter portion of skeletal muscle from injection sites in the gluteal region was again a pale gray color, well demarcated from surrounding normal colored muscle. The only difference seen grossly in samples from the 2 ponies was a slightly more condensed and organized appearance of the injection sites in the pony examined 24 days post-injection.

Kidney tissue of both ponies exhibited some granular material and microcalculi in occasional tubules in the medullary area.

The histopathologic changes seen in the tissue from the amicarbalide injection sites 48 hours post-injection were those of inflammation, specifically an exudate consisting of fibrin, macrophages, polymorphs, and red blood cells between fibers and muscle bundles. Striations of several fibers were indistinct or lost, and many fibers were fragmented and infiltrated with mononuclear cells. In skeletal muscle at the amicarbalide injection sites in the pony euthanized 24 days post-injection, areas of necrotic muscle tissue by a zone consisting of degenerated muscle fibers, mononuclear cells, and irregular clumps of proliferated sarcolemmel nuclei.

In both ponies, tissue from the neck site where NMMA had been injected showed only focal infiltration of mononuclear cells between fibers.

**DISCUSSION**

The therapeutic dose recommendation of 8.8 mg/kg amicarbalide as AIF was established for *Babesia caballi* infection in previous work at this institution. However, adequate studies of margin of safety and tolerance of AIF in uninfected horses were not performed at the same time. Uninfected ponies were used for the present study and deaths occurred at levels similar to the amounts producing deaths in *Babesia caballi*-infected horses, i.e., 26.4 mg/kg amicarbalide as AIF injected intramuscularly on each of 2 successive days in the present study, as compared to
33 mg/kg in infected horses, which was the next higher dose after 22 mg/kg administered in the above-mentioned work.

Following injection of 8.8 mg/kg amicarbalide as AIF on each of 2 successive days, ponies in this group 1, continued to eat normally and exhibited no signs of abdominal discomfort, depression, lameness, or swelling at injection sites, such as had been described in other species\(^4\) and at higher doses\(^8,14\).

The signs of abdominal pain exhibited by ponies following intramuscular injection of 26.4 mg/kg amicarbalide on each of 2 successive days could possibly be attributed to parasympathomimetic activity produced by the known anticholinesterase property of AIF\(^10\). At least partial inhibition of this activity and relief of the signs could probably be expected from atropine administered in appropriate doses, since it was shown\(^24\) to reduce a diamidine-induced fall in blood pressure.

Division of group 2 into subgroups receiving amicarbalide isethionate in 2 forms allows evaluation of the properties and effects of NNMAA in AIF. Analysis by Student's test of serum SDH and CPK levels on day 1 post-injection revealed no significant difference (\(p > 0.2\)) between ponies that did and did not receive NMMAA with the amicarbalide. This would agree with Guidoux' work in rats\(^12\) in that the dosage of NMMAA represented by the amount administered in AIF at the group 2 level did not produce hyperglycemia in that study. On the basis of the above CPK comparison and comparison of injection site lesions, the irritant property of AIF cannot be attributed to NMMAA, as amounts of affected muscle tissue did not vary appreciably between subgroups.

Changes in PCV throughout all phases of the present study appeared to be directly related to the amount of excitement of the individual pony immediately preceding and during collection of the blood samples.

The large elevations in SDH from pre-injection established values indicates adequate sensitivity of this as a test of liver damage quantitatively correlated with liver histopathology. Earlier post-injection peaks were reached and more rapid return to normal was seen in this study than in similar investigations\(^22\). The perilobular nature of the fatty degeneration observed in the liver at 3 times the therapeutic level of amicarbalide is typical of changes seen in acute toxic hepatitis.

Serum CPK elevations were considered to have been due to the release of the enzyme by skeletal muscle fibers damaged at injection sites, based on the fact that elevation of this enzyme is known to be a highly specific indicator of myocardial and skeletal muscle damage\(^18\). No gross or histopathologic changes were seen in myocardium.

The transient leucopenia observed in groups 2 and 3 is similar to that seen following therapy with drugs that act by interfering with cell protein synthesis. This leucopenia was unexpected, since it had not been previously described in studies of this or other diamidines. In the present study, the leucopenia was confirmed by collecting additional blood samples from the same affected ponies and performing manual leucocyte counts using standard hemacytometer techniques\(^3\).

Plasma glucose deviations from pre-injection values were not appreciable, and on an overall basis appeared to correlate only with the degree and duration of anorexia.

Blood urea nitrogen, as an indicator of kidney damage, did not appear to have the sensitivity required of parameters used in a study of this type, since only minor changes were seen in serum from animals with severe renal histopathology.
It should be considered noteworthy that twice the therapeutic dose of AIF had no deleterious effect on the fetus developing in the group 3 mare that was euthanized 120 hours post-injection. Sections of uterine wall revealed no abnormalities histologically. The other mare in this group was bred repeatedly during normal cycles of estrus before and after receiving AIF, but did not conceive.

Ponies in group 4, receiving a total of 17.6 mg of amicarbalide per kg, were considered as having received the therapeutic dose for purposes of evaluating tissue changes, even though it was administered at one time. The histopathologic features of amicarbalide injection sites in the skeletal muscle from the pony euthanized 24 days post-injection suggest some muscle regeneration. The very minor changes seen at NMMAA injection sites in both group 4 ponies do not reflect drug reaction and could easily be attributed to needle trauma.

**SUMMARY AND CONCLUSIONS**

*In vitro* investigations of the response of ponies to a range of dosages of AIF included measurements of changes in PCV, BUN, SDH, CPK, plasma glucose, total and differential white blood cell counts. Post-mortem examinations of ponies that died or were euthanized during the course of the study were conducted.

Ponies which received the recommended therapeutic dose of amicarbalide, consisting of intramuscular injection of 8.8 mg/kg on each of 2 successive days, showed very minor serum enzyme changes, no changes in PCV, or BUN, and pathologic tissue changes consisting of necrosis of approximately 10 cubic centimeters of skeletal muscle at injection sites.

The groups receiving 2 injections of 17.6 and 26.4 mg/kg amicarbalide demonstrated the capability of the drug to elicit quantitative responses from the ponies with regard to the levels of the 2 serum enzymes. The PCV showed transient leucopenia following injection. Two ponies succumbed to 2 injections of 26.4 mg of amicarbalide and showed liver damage reflected by the 915-fold increase in SDH. Twenty-seven-fold increases in this enzyme were seen following 2 injections of 17.6 mg/kg. This 35.2 mg/kg total dose did not alter the normal pregnancy of approximately 120 days duration in one of the mares. Quantitative response was also demonstrated by the 20 and 30 cubic centimeter volumes of muscle necrosis at injection sites of the double and triple therapeutic doses respectively. Marked organ pathology was observed only at the total 52.8 mg/kg level and consisted of perilobular hepatic cell degeneration, necrosis of renal tubular epithelium, and ecchymotic hemorrhages on serosal and mucosal surfaces of structures in thoracic and abdominal cavities.

No significant differences were noted between the ponies receiving injections of amicarbalide with and without NMMAA. Thus, one may conclude that NMMAA does not delay or enhance absorption of amicarbalide from the injection site or have additive or inhibitory effect on the properties of amicarbalide as presented in AIF.

The other principal conclusions that can be made from the results of this study are that under these experimental conditions AIF appears to be safe at the dosage of 2 intramuscular injections of 8.8 mg/kg amicarbalide and that the maximum safe dose is between 2 and 3 times that amount.
Figure 1. Pre- and post-injection mean serum SDH levels in ponies receiving 8.8, 17.6, or 26.4 mg./kg. amicarbalide on each of 2 successive days.
Figure 2. Pre- and post-injection mean serum CPK levels in ponies receiving 8.8, 17.6, or 26.4 mg./kg. amicarbalide on each of 2 successive days.
Figure 3. Pre- and post-injection means and ranges of total leucocyte counts in ponies receiving 17.6 or 26.4 mg./kg. amicarbalide on each of 2 successive days.
### TABLE 1
HEMOGRAMS OF 4 PONIES BEFORE AND AFTER 2 INJECTIONS OF 26.4 MG/KG AMICARBALIDE (Group 1)

<table>
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<tr>
<th>Day (Preceding) or Following Drug Injection</th>
<th>Total WBC/μm</th>
<th>BUN (mg/100 ml)</th>
<th>PCV (%)</th>
<th>Plasma Glucose (mg/100 ml)</th>
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### TABLE 2
HEMOGRAMS OF 4 PONIES BEFORE AND AFTER 2 INJECTIONS OF 17.6 MG AMICARBALIDE PER KG (Group 3)

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<th>Day (Preceding) or Following Drug Injection</th>
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<td>91-95</td>
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BIBLIOGRAPHY

REPORT OF THE PHARMACEUTICALS COMMITTEE

Chairman, S. F. Scheidy, Bryn Mawr, Pa.
Dan J. Anderson, Fort Worth, Texas; C. C. Beck, Ann Arbor, Michigan; G. T.
Edds, Gainesville, Florida; Arthur Freeman, Chicago, Illinois; Roland Gessert,
New York, N.Y.; H. E. Gouge, St. Joseph, Missouri; James Hanson, St. Paul,
Minnesota; Hilmer L. Jones, Rohway, New Jersey; Fred Kingma, Fairfax,
Virginia; Karl Mayer, Naperville, Illinois; George Mitchell, St. Louis, Missouri

Committee Members Present for Meeting October 25, 1971
Drs. Anderson, Edds, Freeman, Gessert, Hanson, Mayer, Mitchell and
Scheidy.

Guests

During the past year there has been continued and increased surveillance by
governmental agencies in the use of chemical and biological agenty by the livestock
industry. This has insured the safety and wholesomeness of animal products as food
produced in this country.

For many years the federal control agency and especially the section of the
Food and Drug Administration, now the Bureau of Veterinary Medicine, operated
under the authority and regulations of the Food-Drug and Cosmetic Act and
amendments adopted over a period of many years.

The 90th Congress passed the Animal Drug Amendments of 1968 and enacted
Public Law 90-399.

The regulations to administer these amendments became effective October 14,
1971. These have simplified somewhat the filing of information regarding chemicals
for use in animals. However, there now is a greatly increased amount of work that
must be done and reported to the Food and Drug Administration prior to the
approval of new drugs useful in the production of animal products (meat and milk
and eggs). The Committee is hopeful that the additional effort and expense
incurred will not adversely affect the continued development of new drugs.

The Pharmaceuticals Committee of the USAHA urges the cooperation of the
livestock industry, stock yards, slaughter houses, U.S.D.A. Poultry and Meat
Inspection, and other regulatory agencies, in the use, recognition, and promotion of
a voluntary medication withdrawal certification program.

In recognition of the many years successful treatment of disease conditions by
various veterinary pharmaceuticals, and in recognition of the high cost of veterinary
drug research in relation to the limited dollar market for many of these
pharmaceuticals, the Pharmaceuticals Committee recommends that, in considering
the veterinary drug efficacy reviews by the NAS/NRC, regulatory agencies consider
also the successful use experience of veterinary practitioners in addition to carefully
controlled experiments by veterinary drug manufacturers and research workers.

Further, it is recommended that the “Grandfather Clause” pertaining to new
drugs in the law be retained in both letter and spirit, so that unnecessary research
efforts will be avoided.

296
The Committee endorses the continued use of fixed combination drug products for use in animals when: The safety and efficacy of the combination have been determined; the components are effective in the presence of each other; and all components are used at proper therapeutic dosages.

The Committee commends the Bureau of Veterinary Medicine for their consideration of the value and practicality of combination drugs for use in control of animal disease.

The Committee commends the view of Dr. Charles C. Edwards, Commissioner of Food and Drugs, with respect to the Ritts Committee Report of 1971, wherein he states, “I think FDA clearly must oppose the recommendation to abolish the present Bureau of Veterinary Medicine. This Bureau is performing a good and useful service that is perhaps not as visible as some of our other programs, but vital in its special areas of performance.”

Concern is expressed for the dairy industry in controlling mastitis if the RDA Regulations for approval of new antibiotics, antibiotic combinations, and other combinations of therapeutic agents for the treatment of mastitis are made significantly more stringent.

In the matter of feed additives, and particularly growth promotants, FDA now are asking for additional efficacy data. Possibly such a request may be in order in some instances in which a drug may have been approved on the basis of minimal efficacy data. But to require complete detailed efficacy studies repeated for each combination of growth promoter and prophylactic or therapeutic drug is unnecessary.

Also, FDA want to require more definitive dose titration of feed medications with the view of possibly limiting dosage recommendations to only the (one) most effective dose. While a definitive dosage titration provides excellent guidance to the veterinary practitioner, poultry pathologist, and livestock man, elimination of a practical dosage range fails to take into account variations which may occur in severity of infection, environmental conditions, and geographical location.

The Committee re-endorses the report of the Pharmaceutical Committee of 1969, and again recommends that those responsible for promulgating rules and regulations with respect to use of drugs in animals not de-emphasize or overlook the benefits derived from the use of these drugs because of overemphasis of the potential hazards and risks; and, further, that those responsible interpret the provisions of the law and apply them on the basis of concrete evidence of harm rather than on the basis of theoretical or speculative hazards.

We recommend this report to the Executive Committee for their consideration.
THE NEWCASTLE SITUATION IN THE UNITED STATES

Earl E. Grass, D.V.M., M.P.H.

Newcastle Disease was first recorded on the island of Java in 1926 by Kraneveld. A disease of similar characteristics was described in that same year by Doyle in England. Subsequently, Newcastle Disease has been diagnosed worldwide. The disease may have been present in the United States as early as 1935 but it was not positively identified as Newcastle Disease until 1944. Brandley and Hanson in Poultry Diseases, states that few, if any of the avian species, are resistant to the disease.

It should be pointed out that while there are several strains of the virus, the strains are usually grouped for convenience into one of three classifications, depending on the virulence of the virus. The three general classifications used are lentogenic, mesogenic and velogenic. Lentogenic strains produce a mild or inapparent disease and require 100 hours to kill inoculated embryos. Mesogenic strains produce a mild disease which rarely result in death but will kill embryos in about 50 hours, while velogenic strains develop severe disease, often resulting in death, and inoculated embryos are killed in 24-48 hours. All three types will cause egg production losses in laying birds but the velogenic strains are particularly characterized by central nervous system symptoms, sometimes viscerotropic lesions, and high mortality in unvaccinated birds.

Outbreaks of Newcastle Disease in the United States have usually been attributed to the lentogenic strains and an occasional mesogenic strain. Velogenic strains of the virus have occurred from time to time in this country but are generally considered to be exotic to the United States and when encountered, are considered to be of foreign origin. Knowledgeable persons at the Veterinary College, Texas A & M, are of the opinion that there may have been five introductions of velogenic Newcastle Disease into that state since the late 1940's. This is a report of the latest of these outbreaks.

Ranch A filled a laying house with 12,000 started pullets on May 5, 1970. The birds came into production normally, but five weeks after coming to the ranch, they experienced a laying slump from 59% down to 11% with approximately a 1,500 bird mortality. The adjoining house was emptied in June, cleaned and refilled on July 4 and 5 with 12,500 started pullets. On August 9th, this second group of pullets began showing signs of respiratory disease by gasping, respiratory distress and bloody mucous. Egg shells became rough and egg whites were watery. In one week’s time there were 1,650 birds left alive. Birds were submitted to two private laboratories as well as Texas A & M University where Dr. C. F. Hall isolated a Newcastle virus. The disease ultimately spread to seven other houses on this ranch, all of which had been vaccinated for Newcastle Disease. Estimated death losses on this ranch were 21,698 birds or 11% mortality.

It is disturbing to me that rather than taking precautions in such a situation, sales representative of primary chicken breeders sent live birds via commercial airlines back to their home laboratories. So in addition to potential exposure of poultry being contacted in airports and freight terminals, the possibility of
infecting genetic stock existed.

Extra pullets, not needed to fill the house on July 5, were sold to a small egg producer in El Paso, who did not experience any disease problems at that time. Labor for the operation is recruited from both sides of the Rio Grande River. Feed is ground and mixed at the ranch. Eggs are sold to any and all buyers, who generally return used egg cases and flats to the ranch. Dead birds were disposed of by placing them in barrels for the local garbage feeder to pick up. This man also collected dead birds from Ranches B, C, D, and E.

Ranch B, located one-fourth of a mile from A, suffered a production drop from 70% to 38% in June but production recovered and the birds were kept until January when one house was restocked. In February, a heavy death loss in the new ones was attributed to Marek's Disease and no further diagnoses was made. Ranch C experienced problems in early November as will be shown later. Ranch D suffered a production loss beginning November 21, and Ranch E’s problem began about February 3, 1917. These four ranches received birds and feed from the same source, sold eggs to one company and had a common serviceman. Here again, diseased birds were shipped to a commercial laboratory for diagnosis but virus isolation was not attempted. When a survey was made in June, all of the birds samples on these ranches revealed HI titers of at least 1:320 with one exception. The exception was a house of birds on Ranch E which had been placed in May 25th, three weeks prior to the survey. The highest titer in this house was 1:80. This indicates that the older birds had a higher HI titer from field exposure than one would expect from vaccination titers.

In late November, the disease spread into small farm flocks and game birds (fighting roosters) in El Paso, Texas area. In some cases a direct connection could be found between the commercial flocks and the small flocks. One small group of birds was transported to Dallas, Texas and was followed by mortality of 63%. In other cases a neighborhood would become infected and the disease appeared to spread by visits. One feed mill, using burlap sacks for feed sales, appeared to be implicated in the spread of the disease to the small flocks. The majority of these small flocks were unvaccinated for Newcastle Disease. Symptoms reported by the owners were greenish diarrhea, gasping and sudden death. Some central nervous system disturbances were observed. Mortality in these flocks ranged upwards up 100% loss.

The disease persisted in small flocks in the El Paso area until about the middle of February. No flocks have been discovered in El Paso which broke after the first week of February. The survey in June showed HI titers of 1:320 or greater in these flocks also.

In early March 1971, Newcastle Disease was diagnosed in a commercial laying operation in New Mexico, Ranch F. The signs seen here were gasping, rales, mucous from the nostrils, tracheitis, hemorrhages on the submucosa of the proventriculus and inflammation and necrosis of the cecal tonsils. Mortality ranged up to 25% in some houses and egg production dropped to near zero. One group of growing pullets, suffered 90% mortality. It was suspected that these pullets had not received any of its Newcastle vaccine. The severity of the disease in any particular house appeared to be directly related as more severe as the time elapsed since the last vaccination increased. All of these birds had been vaccinated with a B1 type, water administered vaccine. Older birds did not recover completely as far as egg quality is
concerned. Egg production in one group of birds dropped from 59% to 14% and came back to about 51% after a month’s time. Another group of birds dropped from 80% to 24%. Tissues were submitted to Dr. R. P. Hanson at the University of Wisconsin for virus isolation attempts, but there were unsuccessful.

Personnel control was attempted to keep the break isolated but eventually more than 500,000 birds were affected.

All of the houses on this ranch are environmentally controlled houses. A large die off of sparrows and starlings occurred in the surrounding pecan orchards but in retrospect, no one could say if chickens or wild birds began dying first.

Two weeks later, the disease broke about twenty miles away in another large commercial operation, Ranch G. Once again, this was an environmentally controlled housing. In emptying and filling houses, it was possible to have had a common labor source with Ranch F as well as the El Paso area. The disease spread throughout the entire operation in less than two weeks. Estimated mortality was about 23%. Egg production in individual buildings dropped from 69% to 11%, with many of the eggs being unfit to use. This operation was somewhat different, in that additional replacements were added at six week intervals. Subsequently, a replacement flock which became infected in May, five weeks after being placed, yielded a Newcastle virus which is being characterized.

Many small flocks became infected in the New Mexico area at the same time. Some of these were owned by workers on the commercial farms, some were adjacent to the farms. At least one owner from El Paso bought feed in Las Cruces which may be another link in the chain. In addition, cock fighting is legal in New Mexico, and at least three pits are located in Las Cruces which might have been an important factor in the spread of the disease. Egg wholesalers also bought eggs where they could and returned cases.

In April, Ranch H became infected with Newcastle Disease. Death losses ranged about 21% in this operation. This flock was located in a small village which also had numerous small flocks. Death losses occurred in several small flocks as well as the commercial flock in such close proximity that an orderly sequence could not be determined. In addition, feed for the commercial flock was delivered to the premise in burlap sacks from the mill which supplied the infected flocks in Texas.

It is of interest to note that velogenic Newcastle Disease appeared in Canada also.

Presently, an outbreak of Newcastle Disease involving an interstate shipment to California from Florida is under investigation. The Newcastle Disease virus has been isolated and is being characterized by Dr. R. A. Bankowski and Dr. R. P. Hanson with the possibility that it may be a velogenic strain. Death losses have occurred in California and Florida but appear to be confined to a few flocks. This outbreak involved game birds and there is a suspicion of imported game birds as a source of exposure.

SUMMARY

In retrospect, the following observations can be made concerning this Newcastle Disease outbreak:

1. Based on documented cases, the disease appeared in Texas in August (and possibly as early as June) and persisted until late February. It then appeared in New Mexico the first week of March and persisted until the end of May. Because Newcastle Disease is not a reportable disease in most states, the
presence of the velogenic virus was not made known to responsible authorities until the outbreak was almost over. In my opinion, this severely hampered the investigation of the disease.

2. The disease affected commercial egg laying flocks, small farm flocks and game bird breeders. It would appear that there was adequate opportunities through ranch personnel, feed sacks, egg cases and fighting cocks to spread the disease in the affected areas and aerosols played little, if any role, in the spread of the disease.

3. Of the eight commercial ranches affected by the outbreak, one raised his own replacements while the others brought in pullet replacements from Nebraska and East Texas. No evidence of the severe form of the disease was found in these other areas. One secondary outbreak was located east of Dallas, Texas when a flock of 50 pullets was transported to that area.

4. Losses in commercial egg laying flocks were mainly economic, due to the loss of egg production and egg quality. Death losses did, however, reach to 20%. The current practice of having several different age groups of birds on one premise served to perpetuate the disease. Interestingly, although an outbreak in one house would fall in the accepted incubation range for velogenic Newcastle Disease, the new additions would be on the premises for four to five weeks before the new group would show signs of the disease.

5. Work conducted at Plum Island by Veterinary Science Research Division, indicates that the B1 type Newcastle Disease vaccines now in use are adequate to protect birds against this disease. However, experience in the field indicates that in the face of the challenge described here, the route of administration of the vaccine may be all important to produce adequate immunity. The losses of 90% of pullet growing flock and 90% of a 27 week old laying flock suggests that not all birds are being properly vaccinated. The isolation of the velogenic virus from primary and secondary cases shows that the vaccinated birds, while protected to varying degrees, still became infected.

6. Death losses in unvaccinated small flocks ranged up to 100%. A NcD virus was isolated from one of these flocks and in other flocks, hemagglutination titers of 1:320 or greater, along with clinical signs were used to make a diagnosis of Newcastle disease.

Four and possibly five outbreaks of Newcastle Disease in the past year caused by a viscerotropic velogenic virus should give us cause to review our vaccination recommendations, at least in some areas of the country. In fact, it may be time to review our whole approach to the Newcastle problem in the United States. Practices currently in vogue in the poultry industry are conducive to a wide spread of virulent strains of virus. While the reported outbreak of Newcastle Disease remained fairly well contained, this does not mean the next outbreak may not become widespread.
NEWCASTLE DISEASE REPORTED IN THE UNITED STATES 1968-1970

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</table>

* all ages combined
EGG PRODUCTION LOSSES TO ND
TEXAS - NEW MEXICO 1971

RANCH A

JUNE 6, 1970

RANCH C

NOV. 1, 1970
EGG PRODUCTION LOSSES TO NCD
TEXAS - NEW MEXICO 1971

RANCH F
MARCH 8, 1971

RANCH G
MARCH 22, 1971

WEEKS OF LAY
ESTIMATED DEATH AND ECONOMIC LOSSES IN COMMERCIAL FLOCKS ATTRIBUTED TO A NEWCASTLE DISEASE OUTBREAK IN TEXAS AND NEW MEXICO.

<table>
<thead>
<tr>
<th>RANCH</th>
<th>DISEASE ONSET</th>
<th>MORTALITY</th>
<th>%MORT.</th>
<th>EST. LOSSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Aug. 10, 1970 (June 6?)</td>
<td>21,700</td>
<td>11%</td>
<td>$220,000</td>
</tr>
<tr>
<td>B</td>
<td>Jun. 14, 1970 Feb. 6, 1971</td>
<td>3,100</td>
<td>19%</td>
<td>$28,900</td>
</tr>
<tr>
<td>C</td>
<td>Nov. 1, 1970</td>
<td>3,000</td>
<td>20%</td>
<td>$27,200</td>
</tr>
<tr>
<td>D</td>
<td>Nov. 21, 1970</td>
<td>3,300</td>
<td>19%</td>
<td>$30,600</td>
</tr>
<tr>
<td>E</td>
<td>Feb. 3, 1971</td>
<td>9,000</td>
<td>17%</td>
<td>$83,300</td>
</tr>
<tr>
<td>F</td>
<td>Mar. 4, 1971</td>
<td>110,000</td>
<td>22%</td>
<td>$700,000</td>
</tr>
<tr>
<td>G</td>
<td>Mar. 17, 1971</td>
<td>80,000</td>
<td>23%</td>
<td>$600,000</td>
</tr>
<tr>
<td>H</td>
<td>Apr. 10, 1971</td>
<td>1,500</td>
<td>21%</td>
<td>$12,500</td>
</tr>
</tbody>
</table>

ESTIMATED TOTAL LOSS 233,400 $1,702,500
COMPARISON OF PROTECTION PROVIDED BY TWO COMMONLY USED VACCINE STRAINS OF NEWCASTLE DISEASE VIRUS.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>BL TYPE</th>
<th>LA SOTA TYPE</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEXAS ISOLATE</td>
<td>1/21</td>
<td>1/22</td>
<td>23/23</td>
</tr>
<tr>
<td>FLORIDA ISOLATE</td>
<td>0/20</td>
<td>1/22</td>
<td>23/23</td>
</tr>
<tr>
<td>TEXAS GB-NCD VIRUS</td>
<td>2/21</td>
<td>0/22</td>
<td>23/23</td>
</tr>
</tbody>
</table>

VACCINE CHALLENGE TRAILS CONDUCTED AT PLUM ISLAND

REFERENCES
TRANSMISSIBLE DISEASES OF POULTRY COMMITTEE

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

The Transmissible Diseases of Poultry Committee met on Monday, October 25, 1971 at 1:30 P.M.

The Chairman afforded the Committee the following report involving a meeting held August 31, 1971 concerning a highly virulent Newcastle Disease outbreak:

PART A INTRODUCTION

Newcastle Disease is a highly contagious and destructive vital disease, primarily of the avian species. It attacks chiefly chickens and turkeys but various other birds as well as certain mammals, including man, may also contract the disease. In poultry, the infection can vary from subclinical to fatal with different forms of the disease causing systemic or localized nervous, respiratory, or gastrointestinal involvement. This report is concerned with the highly pathogenic or viscerotropic, velogenic form of Newcastle Disease recently identified in the United States.

The presence of viscerotropic, velogenic (highly pathogenic) form of Newcastle Disease virus in the United States constitutes a problem that has the potential of causing devastation in domestic flocks.

The present situation with viscerotropic, velogenic Newcastle Disease (as differentiated from the milder forms normally seen in the United States) is at the stage of an emerging disease similar to Venezuelan Equine Encephalomyelitis which is not usually found in the United States.

This form of Newcastle Disease has appeared in epidemic proportion and became established in Europe, Asia, Africa, and South and Central America; however, only recently a few outbreaks, limited in scope, have been identified in the United States. Canada reported and instituted a slaughter stampout program of its first outbreak of the highly pathogenic form of Newcastle Disease in August 1971.

PART B GENERAL RECOMMENDATIONS

The recommendations of this Committee should be applied to this exotic disease expeditiously.

*These Recommendations are developed by the following poultry research scientists: Dr. R. A. Bankowski, Dr. Harry E. Goldstein, Dr. L. C. Grumbles, Dr. R. P. Hanson, Dr. S. B. Hitchner, Dr. B. S. Pomeroy.
The scientific committee recommends the eradication of viscerotropic, velogenic Newcastle Disease present in the United States and any further outbreak that may occur. We further recommend that steps be undertaken to minimize the risks of reintroduction of this highly virulent form of Newcastle Disease into the United States from outside sources.

PART C SPECIFIED RECOMMENDED VISCEROTROPIC, VELOGENIC NEWCASTLE DISEASE PROGRAM

1) Quarantine infected areas.
2) Apply vaccination program as specified under Part D.
3) Attritional slaughter of birds from infected areas under supervision.
4) No introduction of replacement birds into infected areas until 30 days after last infected or exposed birds removed.
5) Negotiable indemnity.
6) We recommend that procedures be implemented for stopping reintroduction of viscerotropic, velogenic Newcastle Disease into the United States from outside sources. The U.S. Department of Agriculture, U.S. Public Health Service, and U.S. Department of the Interior recognize an imperative need for quarantine stations, or importers need to provide adequate isolation facilities in the United States to house imported birds.
7) We recommend that importers of exotic avian species establish quarantine facilities approved and supervised by USDA at peripheral ports of entry for the purpose of preventing entry of viscerotropic, velogenic Newcastle Disease and other diseases transmissible to domestic poultry.

PART D
RECOMMENDATION FOR USE OF CURRENT USDA LICENSED VACCINES

High Risk Areas
- Increase frequency of vaccine application.
- Spray immunization on revaccination.
- Individual application of vaccine is a superior method and should be utilized as birds are handled.
- Vaccination program should be worked out for each individual flock based on professional consultation.

PART E RECOMMENDED VACCINE RESEARCH

1) Currently licensed Newcastle Disease vaccines need to be re-evaluated in depth in light of these new developments and the appearance of the highly virulent form of Newcastle Disease in the United States. For example, the re-evaluation should include research on dosage, method of vaccination, timing, duration of immunity, and vaccine seed virus specificity in chickens, turkeys, geese, ducks, and upland game birds such as pheasants, quail, and partridges.
2) Investigate present Newcastle Disease vaccines for application to psittacine and ornamental birds.
PART F

IT IS RECOMMENDED THAT ALL FORMS OF NEWCASTLE DISEASE BE A REPORTABLE DISEASE IN ALL STATES

The Committee endorsed the preceding recommendations and further recommended that all Newcastle Disease outbreaks or any unusual poultry disease problems be investigated by appropriate state officials. The Committee further directs attention to the fact that since the August 31st recommendations were made, the disease has been diagnosed in two additional states.

This Committee recommends that the USDA initiate immediate quarantine measures where Asiatic Newcastle Disease has been diagnosed to provide adequate protection for the poultry industry of the nation.

The Committee reviewed the previously recommended Uniform Rules and Methods for the Eradication of Pullorum Disease and Fowl Typhoid. The Committee endorsed the recommended revision of Memorandum 565.1 to be compatible with the National Plans Program, to allow free interstate movement of products.

Your Committee commends the State of Minnesota for advancing to the point of requesting Phase II Status in their Turkey Program.

SITUATION REPORT – DUCK VIRUS ENTERITIS

There have been six confirmed outbreaks of Duck Virus Enteritis (DVE) in 1971. All were in captive waterfowl flocks. (See figures 1 & 2)

Maryland

At Patuxent Wildlife Research Center, Laurel, a single black duck was found dead on May 14 and 18 respectively. In both instances DVE was confirmed by virus isolation and neutralization. There were no additional death losses.

On July 6, mallards that tested negative to DVE by the serum neutralization test were placed as sentinals in contact with the flock.

The sentinals were again tested negative to the SN test on August 30, 1971.

There was an agreement that no birds would be moved off the Center until the sentinals were tested the second time. No quarantine was placed.

Pennsylvania

No. 1 April 12, 1971, three Canada geese were found dead in a flock of approximately 350 waterfowl on a premises in Chester County. DVE was diagnosed on the basis of gross lesions, liver intranuclear inclusion bodies, and three of five serum pools positive to the serum neutralization test in contact birds.

June 8, pre-bled muscovy sentinal ducks were placed in contact with remaining waterfowl. All sentinals were negative for DVE by the serum neutralization test. The sentinals were negative for DVE by the SN test on the second bleeding July 26, 1971. The state quarantine was then lifted.

No. 2 Approximately 300 muscovy ducks died early in June on a premises in Berks County. DVE was diagnosed by gross and microscopic lesions and duck embryo inoculation. The few remaining waterfowl were destroyed and after cleaning and disinfecting the premises the quarantine was released August 25, 1971.

Long Island, New York

In late April and early May an occasional bird of various species of waterfowl was found dead on the premises in Suffolk County, New York.

Suspect material from specimens from each premises was inoculated into duck
embryos and seven-day-old ducks at the Plum Island Animal Disease Laboratory. Mortality occurred with lesions of DVE. The causative agent was isolated and inhibited DVE hyperimmune serum.

On June 22, 1971, waterfowl on these premises were vaccinated with modified live virus DVE vaccine, furnished by the Long Island Duck Research Laboratory, under the supervision of a New York State Veterinarian. There are plans to revaccinate these flocks in October 1971. Provided no further death losses occur, consideration will be given to releasing the quarantines at that time.

For Transmissible Diseases of Poultry Committee, USAHA, 1971
Submitted by Claude J. Pflow, ANH, ARS, USDA

The Committee recommended that the poultry industry be urged to use only vaccines that have been approved by State or Federal Agencies. The Committee discourages the use of turkey blood and other unapproved products because of possible transmission of highly infectious diseases such as Asiatic Newcastle Disease, and the inconsistency of the resulting immunity.

The Committee was made aware of a projected program that would enhance the marketing of poultry products to England and other countries. Your Committee commends the USDA for exploring this potential and recommends that the USDA take steps to implement this program.
FIGURE 1

DUCK VIRUS ENTERITIS
Order of Counties Affected

TRANSMISSIBLE DISEASES OF POULTRY
FIGURE 2

DVE CONFIRMED OUTBREAKS

- Migratory
- Captive
- Commercial

Outbreaks

CLINICAL AND PATHOLOGICAL SIGNS, OR VIRUS ISOLATIONS
DVE ALSO KNOWN AS DUCK PLAGUE

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
ENVIRONMENTAL HEALTH ASPECTS AND ANIMAL DISEASES ASSOCIATED WITH ANIMAL WASTES

J. H. Steele

Health problems associated with animal wastes have been of concern to public health agencies for generations. As medical science has developed, this concern has evolved from a vague regard for eliminating or minimizing a public nuisance to a growing awareness of an important factor in the complicated cycle of diseases common to human and lower animal life. A large number of diseases of microbiological etiology are transmitted through animal wastes. Animal waste material also may contribute to man's body burden of potentially toxic chemicals. These may be introduced into the animal as therapeutic agents or growth regulators; these may be passed through the animal, as in the case of the pesticides.

Animal wastes are considered to include all by-products of animal life, including excreta and inedible by-products of animal processing for food and fiber production.

Over 100 diseases of animals may be transmitted to man. Not all of these zoonoses are transmitted through animal wastes, but many can be. Some of the most significant bacterial zoonoses are salmonellosis, staphlococcal and streptococcal infections, tetanus, tuberculosis, brucellosis, leptospirosis, and colibacillosis.

Animal wastes also play a significant role in the distribution of fungal diseases by providing nutrients for the survival and growth of fungi in man's environment.

Q fever, a rickettsial disease, is transmitted to man primarily through air laden with dust containing animal wastes. It is largely an occupational disease of cattlemen, slaughterhouse workers, and woolsorters, but may also attack those residing adjacent to feedlots and stockyards. A troublesome parasitic disease transmitted through animal wastes is trichinosis which persists even though the practice of feeding swine raw garbage has been greatly reduced in the past 15 years.

Less is known regarding the role of animal wastes in the direct transmission of viral diseases than in bacterial diseases. However, the importance of animal wastes in the reproduction of insect vectors of many viral diseases is well documented.

While the transmission of disease agents from animal to man is uppermost in our concern, animal wastes also contribute to man's problems by increasing the prevalence of disease in animal hosts and thus increasing the potential for human contact with and infection by pathogens.

Anthrax is one of the oldest diseases identified with animals that is transmissible to man. Greek physicians wrote about the danger of contaminating the environment and how the shepherds could become diseased from the fleece of dead animals. Virgil, a Roman author of the first century B.C., described anthrax in animals and how it was transmitted to man through hides, fleece, and meat. As late as the 18th century people frequently died when they were fed meat of animals that had died of anthrax. The first law prohibiting such practice was enacted as recently as 1810.

Anthrax has been present in the United States for the last 100 years, if not longer. Frequently is has been transmitted by effluents from hide and hair
processing plants. These effluents contaminate pastures downstream as well as cause disease in cattle that drink the water. There are many water courses in the United States that are still contaminated with anthrax spores and probably will remain so for decades and centuries.2 We are fortunate that there are good vaccines available today; otherwise, this would be a serious problem.

While it is difficult to determine an order of importance for the zoonozes transmitted by animal wastes, one would certainly place salmonellosis high on the list. There are more than 1100 serotypes of the Salmonella which have been isolated from animals, from man, and from their environments. Many of them are known to produce disease in man. Salmonellosis is a disease ranging in severity from mild gastroenteritis to septicemia, enteric fever, and meningitis leading to death. Severity of disease depends on the infecting serotype, the number of organisms in the infecting dose, and, of course, the general physical condition of the individual. It is primarily a food-borne disease in that exposure is by the oral route.

Salmonellosis is a major public health problem. In 1969, 21,113 isolations from man were reported and 17,645 cases3. It is authoritatively estimated that a million or more cases occur each year in the United States. Animal excreta and inedible by-products of food processing, such as viscera, bones, and feathers, are vehicles that carry salmonella organisms from their animal hosts to man4. Direct contact with such wastes constitutes an occupational hazard for livestock producers and slaughterhouse and rendering plant workers; contamination of edible food products and feces provides a menas of carrying the organism to the consumer, and to the home and institutional environment.

Animal wastes are also a vital factor in perpetuating and extending the prevalence of animal hosts of the Salmonellae5. Feeding of animal feces to poultry, swine, beef, and dairy cattle is one means of increasing the incidence of animal salmonella hosts, as is the use of contaminated animal protein supplements in animals feeds.

Salmonella has been found in many water supplies in the United States. The big waterborne outbreak of southern California a few years ago affected some 16,000 people. How the water supply of the city of Riverside became contaminated is unknown, but Salmonella typhimurium (Phage II), the cause of the outbreak is widely disseminated in animals not only in California, but throughout the world. There is speculation that the water table may have become contaminated by seepage hundreds of miles away. Geologists have pointed out that in western states there are extensive earthquake faults along which water could meander for many miles. Contamination could originate in feedlots where cattle may be passing Salmonella typhimurium.

A recent study in southern Arizona indicated that salmonella was an important disease in feedlot cattle. The only reason that the disease was not transmitted further in Arizona was the high temperature, evaporating the water very rapidly so that it had little opportunity to contaminate subsoil water.

Other examples of gross pollution of water courses with salmonella have been reported by investigators studying the Chicago drainage canal, New Jersey offshore waters, and rivers in southern United States.

Efforts are now under way to combat salmonellosis. Health and agricultural agencies are beginning to develop and apply standards of food processing to minimize or prevent salmonella contamination of foods, and food processors are
looking such product ingredients as salmonella-free egg powder. A national effort to reduce the incidence of salmonellosis and ultimately bring it under control will require extensive changes in methods of operation in the rendering industry to prevent contamination of products which have been heat treated with raw materials containing viable pathogens. It will also require extensive evaluation of feeding feces as feed ingredients to provide assurance that such practices do not add to the animal reservoir of the Salmonellae.

Tuberculosis must still be considered as an important disease related to animal wastes. While bovine tuberculosis caused by Mycobacterium bovis has been effectively controlled in this country, it is occasionally found in some wild animals as well as in food animals and in pets. Its ultimate eradication will depend on eliminating the disease in these species as well as in cattle. Mycobacterium tuberculosis, the human type of tubercle bacillus is capable of infecting cattle, swine and household pets. Mycobacterium avium, the etiologic agent of tuberculosis in gallinaceous birds, is capable of producing tuberculosis in swine and of infecting cattle to such an extent that reactions are produced in routine tuberculin testing of cattle.

The bovine tubercle bacillus is transmitted to man through respiratory secretions, feces, and milk. The reduction of the disease in cattle and the pasteurization of milk have resulted in human infections from the bovine tubercle bacillus rarely occurring today. In those few cases where infection of man with the bovine tubercle bacillus is known, there usually is an occupational contact with cattle.

Debate continues over the ability of the avian tubercle bacillus to produce disease in man. The occurrence of such disease is rare. The avian tubercle bacillus is important economically, however, due to the food loss associated with disease in egg-producing flocks and the condemnation of diseased swine at slaughter.

The exposure and/or feeding of poultry feces to swine and cattle thus presents significant hazards in reducing our food supply and in further complicating the case-finding procedures in cattle.

Brucellosis in man has dropped dramatically in the past 15 years, due mostly to reduction of the disease in the cattle population and to pasteurization of milk. It is now mainly an occupational disease of those with close contact with cattle and swine and their viscera and excreta.

[Brucellosis in man and animals is caused by any one of three species of Brucella. Brucella abortus is predominantly of bovine origin, Brucella suis of swine origin, and Brucella melitensis primarily infects goats. Cows may become infected with Brucella suis or Brucella melitensis as well as Brucella abortus. Swine may become infected with all three species; however, they are most susceptible to Brucella suis.]

Many outbreaks of brucellosis have been traced to contaminated water courses from meat-processing plants, rendering plants, and contaminated farms. Our rural water supplies must not be vehicles that could disseminate this disease in the future.

Control of brucellosis in man depends on breaking the cycle of infection between man and the animal hosts of the disease. Ultimate elimination of the disease in the animal population. Since man is susceptible to infection by the three major species of Brucella, and since the causative organisms are transmissible between cattle, swine, and goats through excreta, it is important that animal wastes
be handled to eliminate possibilities of distributing this human and animal pathogen.

Leptospirosis is a disease of large proportions and is world-wide in distribution. The disease in man shows a wide range of symptoms and severity, depending on the species of leptospira involved, exposure, and the health of the individual. A number of animal species host the leptospira, including the domestic food-producing species. Cattle and swine are the principal domestic animals involved — leptospirosis occurs in epizootic form in stabled and feedlot herds. Dogs and rodents are frequently infected.

The true incidence of leptospirosis in man is unknown. It presents symptoms similar to influenza, enteric viral infections, infectious gastroentiritis, and a number of other diseases. Leptospiroae are transmitted from the animal host to man through a number of routes — nasal, oral, through abraded or lacerated skin. Leptospiroae are frequently found in water. The source of water contamination is animal excretions. A few years ago an Iowa outbreak affected several hundred people. A study revealed that cattle grazing in pastures along a river were carriers of L. pomona. These organisms were discharged by the cattle and eventually found their way to the river. In late summer a number of people bathed in this river — many of them became infected.

More recently, an outbreak was reported in the state of Washington when teen-agers swam in polluted irrigation water. The source of water pollution was found to be infected cattle which had access to the irrigation canal.

One of the most unusual causes of stream pollution is tularemia bacillus. This organism normally is found in rabbits, small rodents, and occasionally sheep. All of these animals can discharge the tularemia organism in their excretions. That, in turn, pollutes streams. The unusual thing about this organism is that it can be left in the sediment of the stream; hence, the organism multiplies and the stream becomes a source of infection of animals and man.

E. coli has a broad spectrum of pathogenicity and virulence. Some of the serotypes, such as 108 and 111, are dangerous for both man and animals and can be the cause of death of infants and of newborn animals.

There is considerable evidence that many animal viruses — such as foot-and-mouth disease, rinderpest, hog cholera, distemper and canine infectious hepatitis — can be transmitted by water. Fortunately none of these diseases affect man except foot-and-mouth disease on rare occasions.

In addition to providing a means for pathogen spread in the environment, animal wastes may contribute to man's environmental exposure to a number of potentially hazardous chemicals. Pesticides and their toxic metabolic products are both excreted in the urine and feces and stored in body tissue. Veterinary therapeutic agents are similarly excreted. While the direct exposure of man is probably of no concern the feeding of excreta containing such chemicals provides a means of added exposure of the animal population to such agents. The resulting tissue deposits of these materials in food-producing animals may provide a significant added source for man of chemicals which may have long-term subtle or dramatic health effects. The long-term toxic effect of such materials warrants extensive research and suggests a cautious approach to further utilization of animal excreta for animal feed.

The rapid population growth creates a continually changing pattern or urbanized
living. Cattle feedlots and broiler-raising operations which were, only a few years ago, well isolated from community populations may now be surrounded by housing developments. A poultry-processing plant which currently gets by with disposing of its wastes in a series of lagoons or by spreading them on open land may, by next year, be providing a source of bacterial pollution to a community water supply. The recharging of subsoil water, as in California is creating concern.

Municipal regulation of location of feedlots, broiler raising plants, and animal slaughtering operations will continually affect more animal-producing and processing operations. The continuing spread of urban populations, with the reduced availability of open areas for animal waste disposal, will require new disposal methods. New methods should be carefully evaluated to determine that they do not present an opportunity for multiplication of insect and rodent vectors of disease; for increasing the animal reservoir of zoonotic diseases; for providing direct means of transmitting diseases from animals to man; for draining or leaching materials containing pathogens to a groundwater source; for building up, in an animal population, the levels of potentially toxic chemicals; or for supporting added sources of fungal contamination of the environment. The evaluation of health aspects should become a significant part of the development of waste-disposal or waste-utilization methods.

Pollution results when large herds of livestock are concentrated on small land areas. There has been a trend toward food animal production throughout the U.S. in the past decade to grow more animals on less land. Today there are more than 80,000 beef cattle feedlots which will probably double in the next decade. In addition to the cattle feedlots there are 250,000 broiler farms and 3,000 swine lots. These animals produce almost 2 billion tons of waste each year. Recycling conversion of fuel oil is the only hope for solution of this problem. One dry ton of waste or eight wet tons can be converted to one barrel of fuel oil under a high pressure carbon monoxide atmosphere based on research of the U.S. Bureau of Mines. This method of utilization holds much promise for all types of agricultural wastes.

A 10,000 head feedlot will produce waste equivalent of 200,000 people. A 250,000 broiler farm is equivalent to 25,000 people. Swine are about one to one ratio with man.

These farms will continue to grow as our society grows and becomes more affluent. Without exaggerating one can predict that animal waste will increase 2 or 3 fold in the next decade. The solution of the problem will call for new methods of recycling that insure healthy animals and chemical methods of conversion to fuel oil and byproducts. The challenge will effect all society especially the public health.
REFERENCES

The Committee met and heard four presentations. Dr. Stanley L. Diesch read a brief paper entitled “Microbiologic Contaminants of Livestock Wastes as a Public Health Problem.”

Today, quality and ecology of environment is of concern to every segment of society. Environmental pollution has increased the responsibility and inquiry for all persons associated with animal and human health. On August 10, 1970, President Nixon, in presenting the Report of the White House Council on Environmental Quality, stated, “in dealing with the environment, we must learn not to master nature but how to master ourselves, our institutions, and our technology.”

In the United States, increasing centralization of livestock, milk, poultry and egg production has increased disposal and recycling problems associated with more than 1.7 billion tons of animal wastes produced annually. Approximately half of this amount is produced by concentrated systems.

Despite the fact that the total volume of animal wastes produced in the U.S. is about 10 times the human population wastes, little concern has been given to agricultural wastes during the last decade. In the past, animals were largely produced in unconfined areas where wastes were assimilated by the environment. The livestock industry is rapidly growing from a small farm enterprise toward an agricultural industry and wastes are becoming concentrated in an unprecedented amount — for example, the number of beef cattle fattened in feed lots have doubled since 1950 — to more than 16,000,000.

The biochemical oxygen demand of wastes of a large midwestern feedlot may be equated to one million people living on 320 acres of land. If these animals are infected an infinite number of pathogenic agents may be shed into the environment.

In the past, economics of agricultural operation has largely determined the management of livestock wastes. In past unrestricted development and construction of feedlots, minimal consideration was given to public health or to the resultant interaction of man and lower animals in their common microbe-laden environment.

Livestock wastes, which include dead animals, meat industry wastes and animal manures, constitute a massive volume of organic and inorganic materials that must be disposed of or recycled.

New systematic approaches of waste disposal are being developed in part as a result of society’s demand for environmental quality. Although human disease problems associated with agricultural occupations have been documented, there is
growing recognition and concern of man's contact with livestock wastes through recreational and outdoor activities.

More than 150 zoonotic diseases are transmitted between animals and man. The causative organisms of many of these are shed in animal wastes, or contaminate animal wastes where adequate nutrients for survival and growth may be found. A literature survey concerned with recovery of specific microorganisms from urine and feces of inoculated, infected cattle indicated that 14 microorganisms of specific disease entities were recovered from the feces of infected cattle (adenovirus, anthrax, brucellosis, Coxsackie A., Coxsackie B, enterovirus, foot and mouth, leptospirosis, psittacosis, ornithosis, Q-fever, reovirus, rinderpest, tuberculosis and tularemia); and 7 microorganisms from urine of inoculated infected cattle, (brucellosis, foot and mouth, leptospirosis, Q-fever, rinderpest, tuberculosis and tularemia). This report excluded most intestinal diseases.1

Epidemiologic investigations of human cases have associated pathogenic microorganisms of animal wastes with outbreaks of disease. In a report by Decker and Steele2, there is documentation of human health problems created by bacterial zoonoses, such as leptospirosis, salmonellosis, staphylococcal and streptococcal infections, tetanus, brucellosis, tuberculosis and colibacillosis and other classes of pathogenic agents which occurred following contact with wastes. Animal wastes also serve as breeding grounds for many vectors essential for viral transmission.

In an extensive literature survey titled Solid Waste/Disease Relationships, Hanks3 states that the literature fails to supply data which would permit a quantitative estimate of relationship between solid waste and disease. He further states that circumstantial and epidemiological information presented in reports, does support the definite relationship of diseases and solid wastes — including animal waste. He further states that in developed countries incidence, prevalence and severity of human infection due to animal fecal wastes is low — if one looks at reported outbreaks — but suspicion is that the amount of disease is actually much higher.

In implicating livestock wastes as vehicles of disease, many variables affecting the host-agent-environment realtime relationship are found under field conditions.

In recent years, Local, State and Federal Governmental regulations have developed and guidelines or are in the process of developing laws to minimize the public health hazards of livestock wastes and its subsequent environmental effect. In 1971, the State of Minnesota developed and implemented regulations for control of wastes from livestock feedlots, poultry lots, and other animal lots. In these regulations, standards govern storage, transportation, and disposal of animal wastes, and the registration and issuing of permits for construction and operation of animal waste disposal systems for the protection of the environment.

Transportation and rendering of carcasses of animals, poultry, fish and other renderable products are usually controlled by rules and regulations of state agencies such as the Minnesota Livestock Sanitary Board.

In order to comply with regulations, and because of economics, new systematic approaches to livestock waste management are being developed and utilized. As modern technology for treating concentrated livestock waste is more commonly utilized, the accompanying health hazards to man and lower animals must be evaluated.

In an effort to determine the public health effect of a new method of animal
manure disposal, research to measure the survival of pathogens in manure disposal of the oxidation ditch (extended aeration method) is being conducted at the Department of Veterinary Microbiology and Public Health, University of Minnesota. *Salmonella* was selected as a model of pathogens shed in the feces and *Leptospira* as a model of pathogens shed in the urine. In acute cases calves have shed 10,000,000 organisms per gram of feces.\(^4\) As many as 100,000,000 leptospires have been reported shed per ml. of cattle urine.\(^5\)

The following research was conducted in liquid manure in a 10:1 scale laboratory model located in our laboratories. This is a model of an operational field oxidation ditch located at Rosemount Agricultural Experiment station, where beef cattle were fattened over the ditch on slatted floors. Salmonellae and leptospires were seeded into manure of this model and in a model settling chamber. Summer and winter environmental conditions were simulated.

Survival times measured are summarized below.\(^6,7,8\)

Salmonellae were measured by cultural techniques to have survived:

*Summer temperatures (20°C)*

- Model oxidation ditch: 17 days
- Settling chamber: 14 days

*Winter temperatures (2°C)*

- Model oxidation ditch: 47 days
- Settling chamber-sludge: 87 days
- Settling chamber-effluent: 66 days

Leptospires were measured by cultural techniques to have survived:

*Summer temperatures (20°C)*

- Model oxidation ditch: 126 days (preliminary)
- Settling chamber-effluent: 5 days
- Settling chamber-sludge: 4 days

*Winter temperatures (2°C)*

- Model oxidation ditch: 18 days
- Settling chamber-effluent: 9 days
- Settling chamber-sludge: 11 days

These findings indicate the capability of salmonellae and leptospires to survive manure of a model oxidation ditch, and in the sludge and effluent of the settling chamber. Since infected livestock are capable of shedding salmonellae of leptospires for weeks to months, their wastes must be considered long term sources of pathogens. We are presently engaged in quantitative research to determine if these pathogens found surviving in animal manures are capable of infecting animals and potentially man.

The purist could state that all animal manures and wastes must be disinfected or rendered pathogen-free before allowing contact with air, soil or water environment. It would be possible to chlorinate the effluents of the oxidation ditch prior to discharge. However, the sludge, due to the high BOD, would be extremely difficult to render pathogen free. Difficult problems are found in chlorination of liquid manure because of interference of pH, organic materials and other substances. Today, a large part of animal wastes are recycled as fertilizers, by pastured animals depositing manure on the ground. Other common methods are composting, direct spreading on land, lagooning, or gun-spraying. Major changes in waste management methods would be costly for livestock producers. Restrictions are being placed on
agriculture Guidelines, applicable to geographic conditions, which protect the livestock producer, the environment, and the health and welfare of the public are essential. To protect public health and reduce disease transmission, animal wastes are being restricted from selected natural waters, and land with run off potential, especially in areas of human population density. Zoning should be considered.

New methods of concentrated and high density livestock production and waste management present a unique opportunity to the agricultural industry and those professions concerned with improving the health of man and lower animals. The fact that feces and urine, major vehicles of disease transmission, are being increasingly confined to specific holding areas and separated directly from animals and man is significant in disease control and prevention. Qualitative and quantitative research findings and field application together with proper waste management will solve present day problems. The concentrations of domestic animals are man-created factors and must be controlled by man.

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*This investigation was supported by PHS Research Grant No. EP-00302 from the Division of Research and Development, Bureau of Solid Waste Management, Consumer Protection and Environmental Health Service.

**Department of Veterinary Microbiology and Public Health, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota 55101.
Dr. J. F. Stara presented a paper entitled “Effects of Environmental Pollution on Domestic Animals.”

The biosphere, which provides man with most of his material needs, is being degraded due to two major factors:

1. The rapidly growing number of people which requires the consumption of constantly larger shares of earth’s resources and which competes more and more with other forms of life;

2. The steadily increasing demand for a rising standard of living with disregard to consequences of increased pollution and general degradation of our natural environment. In order to preserve the ability of the biosphere to continue to sustain life, a shift of emphasis from materialistic to aesthetic and spiritual values seems to be the only avenue with some assurance of success to preserve the necessary ecological and environmental balance.

Allow me to quote from a presentation by Wm. Ruckelshaus, the Administrator of the Environmental Protection Agency before the National Press Club in Washington, January, 1971:

“We must all understand that man has reached this critical point in human development not by deliberate intent to pollute and desecrate, but rather by his single-minded pursuit of isolated, short-range objectives which seemed desirably and beneficial at the time. The road to our environmental crisis, like the road to hell, was paved with good intentions.

“We must all learn that we are the victims of a point-of-view, in relation to our environment, which has long been obsolete. This viewpoint holds that man must conquer nature. We have clung to this vision with such tenacity that we now inherit the spoils of a three-hundred-year war against nature. A war marked by many victories for our side and many tragic defeats for our apparent enemy. Certainly when our ancestors conceived the notion that the land and its resources were inexhaustible, their direct experience seemed to confirm their view. They could exploit this place and that resource and, move on westward to the next place and next resource. Indeed, this frontier attitude may have served us well as the Nation expanded westward, and it probably accelerated and encouraged the economic and technologic advances which followed. Today, however, there is no excuse for such an attitude. We can no longer blind ourselves to the fact that thoughtless aggression against nature and the earth are self-destructive.

“We now understand that there is no place left for the American frontiersman to move on to, and that we can no longer stand back and gaze with awe and wonder at where the forces of technology are taking us. We must act now and place these forces under our conscious control. We are making some beginnings.

“By establishing the Council on Environmental Quality and EPA, President Nixon has placed our Nation in a much better position to meet the dual environmental challenge — to attend to what is urgent and, at the same time, to foresee and respond to what is ahead. Indeed, this dual challenge is not only national, but also global. I believe that our country has a unique opportunity to play a part in leading international efforts to save the planet.”

An important segment of the complex and intricate organization of all living things on this planet are domestic animals, such as cattle, sheep, goats, swine,
horses, dogs, cats, rabbits, chickens, turkeys, ducks, geese, pigeons and honey bees. All of these are important to man; their relationship with him is interdependent; they do affect his health and welfare.

Food producing animals — the domestic livestock — are "the great converters", just like the plants are called "the great anabolizers". This is due to the fact that they provide energy and other basic needs to mankind. Most importantly, they provide "renewable" sources of energy in contrast to "fixed" sources such as fossil fuels. All efforts must be made to prevent any possible alteration of this most important segment of the biosphere.

Many diseases and intoxications affecting domestic animals are due to ingestion of the harmful agent by water or feed; or by inhalation of polluted air. Recently a blue ribbon committee listed the "key environmental pollutants", i.e., those that have the most significant global effects on aquatic and terrestrial ecology; they have world-wide distribution pattern and a residual effect. They include: carbon dioxide, carbon monoxide, particulate matter, sulphur dioxide, oxides of nitrogen, toxic heavy metals, e.g., mercury, arsenic, cadmium, lead, nickel, chromium, manganese, copper, zinc, oil, chlorinated hydrocarbons (esp. DDT and polychlorinated biphenyls), other hydrocarbons, radionuclides, nutrients, food additives, heat and noise. In an attempt to control pollution one must assess the method as well as cost of control in the different media. For the terrestrial ecosystem the air is the intimate medium; for the aquatic, it is the water.

Air pollutants that have caused the most significant effects on livestock in the United States are fluorine (fluoride) and arsenic. Major sources of airborne fluoride is the heavy chemical industry, e.g., phosphate fertilizers, aluminum production, fluorinated plastics, etc. Fluoride settles on forage and is ingested by animals. Sources of airborne arsenic are industrial smelters. The route of exposure in animals is similar to that of fluoride.

Other air pollutants of interest are: Smoke, auto exhaust, beryllium, carbon monoxide, dust, hydrocarbons (formaldehyde methane), lead, manganese, mercury, molydenum, nitrogen dioxide, ozone, sulphur oxide, vanadium, zinc, pesticides, pollens, poultry fecal matter, aircraft emissions, etc.

Pollutants most commonly carried in water are: pesticides, such as aldrin, endrin, chlordane, DDT; mercury, arsenic, silver, cadmium, chromium, copper, lead, nickel, ammonia, cyanides, sulfides, fluorides, detergents, pathogenic organisms, tar, petroleum wastes, paper manufacturing wastes, etc.

Solid wastes, the nuclear power production and the liberal usage of pesticides represent the major potential vehicles for additional environmental pollution.

When the toxic effects are considered combined effects, in addition to the action of single agents in atmosphere or water, and in other environmental vehicles, should be considered. There are always interacting systems that co-exist in the environment, e.g., air pollutants and infectious agents. Multiple casualty as a factor of a disease is well established but frequently overlooked.

Various forms of poisoning resulting from low and high levels of nitrates in food and water was presented to the Committee by Dr. R. H. Singer. Constant ingestion of nitrates can result in poisoning due to the effect of the nitrate ion, its reduction products — nitrates, nitrogen oxide gases, hydroxylamine, or ammonia, or by the formation of toxic reaction products of nitrates with amines in certain animal feeds — all of which cause conditions other than methemoglobin formation in man and
animals. These conditions included hypochloremia, interference with thyroid function, sudden death due to cardiovascular collapse, abortion, pulmonary adenomatosis, and severe liver damage. This report pointed out the many areas of the nitrate problem that will require further investigation in order to cope with the excesses of nitrates in feed and water now experienced through many parts of the country.

Dr. Fred Clayton explained to the Committee the National Library of Medicine Toxicology Information Program, Special Informational Services.

In 1966, the President, following recommendations of his Science Advisory Committee, directed that the Department of Health, Education, and Welfare establish a computer-based Toxicology Information Program.

A Departmental Toxicological Information Coordinating Committee was established and, in 1967, the National Library of Medicine was directed to develop a Toxicology Information Program (TIP). The general objectives of the Toxicology Information Program are: (1) to create computer-based toxicology data banks from the literature of files of collaborating Government agencies; and (2) to establish toxicology information and data services for the scientific community.

Extensive collaboration with the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) has resulted in the development and implementation of several automated data banks and systems, including: (1) a central data management system for the Division of Pesticide Community Studies, (EPA), containing epidemiological studies of individuals occupationally exposed to pesticides; (2) an on-line, whole-text search system containing 6,000 abstracts from Health Aspects of Pesticides Abstract Bulletin (1966-1971), which will be made available to multiple users via terminals; (3) use of the same system for query and preparation of indexes for the Report of the Secretary’s (DHEW) Commission on Pesticides and Their Relationship to Environmental Health (Mrak Report); and (4) initiation of a data bank of toxicological data extracted from 400 pesticide petitions from the files of FDA/EPA. Files of articles concerning biological effects of an FDA-selected list of food additives are also being prepared through conventional literature searches.

A new information analysis center, the Toxicology Information Query Response Center (TIQRC), has been established at the Oak Ridge National Laboratory (ORNL) to provide specialized reference services for TIP.

TIP/NLM and FDA have continued with Chemical Abstracts Service to maintain the Common Data Base (CDB) of 35,000 biomedically important chemical substances.

A project to identify and process articles for a Drug Interaction Bibliography with Selected Excerpts (1967-1970) has been initiated; it will cover human and animal studies. The bibliography will be ready for publication at the end of 1971.

A new computerized quarterly information service called “Toxitapes” for industrial and pharmaceutical toxicology, based on specially processed articles selected from the Biological Abstracts data base, has been initiated in collaboration with BIOSIS. Tapes from this service will be available gratis for evaluation by interested participants.

The development of an on-line, interactive, computer-based roster of authorities in toxicology and related fields has been completed and is operational at TIP/NLM. NLM’s on-line Abridged Index Medicus retrieval system (AIM-TWX) has been
enlarged through the addition of some 15 journals from the area of clinical toxicology.

Other activities include: (a) the development of a new abstract journal on the *Health Effects of Environmental Pollutants*, and a companion tape service, as a collaborative undertaking by NLM and BIOSIS; and (b) continuing work on the development of a Toxicology Vocabulary as a thesaurus to assist in indexing the published scientific literature and specialized data files.

These activities, and others in progress, will help meet the recommendations of the President's Science Advisory Committee report on "Handling of Toxicological Information".

Dr. R. L. Parker briefly reviewed the Venezuelan Equine Encephalitis outbreak in Texas during 1971.

Discussions followed each presentation. The Committee noted that at the present most state veterinarians lack the power of quarantine of animals for strictly public health causes, such as known ingestion of toxic materials, and that there is not mandatory reporting to public health agencies of diagnostic laboratory findings of public health importance.

The Committee adopted two resolutions which will be submitted for approval.
CHARACTERIZATION OF PROTECTIVE ANTIBODIES TO TRANSMISSIBLE GASTROENTERITIS VIRUS IN SERUM AND MILK OF SOWS EXPOSED TO THE VIRUS

by
Abou-Youssef, M. H., D.V.M., Ph.D. and Miodrag Ristic, D.V.M., Ph.D.

Theoretical and applied immunologic approaches which have been successfully used for preventing and controlling many viral and bacterial diseases have been of little value with respect to TGE.\(^1\),\(^2\),\(^3\),\(^4\) Although systemic active immunity can be induced in baby pigs,\(^5\),\(^6\) little is known about activation of local intestinal immune responses in such young animals. Moreover, rapid replication of TGE virus in the intestines of baby pigs offers little time for activation and expression of immune responses. Safe immunogenic agents suitable for stimulation of intestinal immunity against TGE in young pigs are not available. Major research efforts have been focused on inducing anti-TGE immunity in pregnant swine which in turn would transfer it passively to their newborn litters.

Sows which recovered from natural exposure to TGE virus at least 3 weeks prior to farrowing conferred protection against the disease to their baby pigs.\(^3\),\(^7\),\(^8\) Also, experimental studies have shown that pregnant sows exposed orally to gut-derived virus develop adequate immunity to the virus and on challenge protect their baby pigs.\(^9\) Attempts to use the high cell culture passage of TGE virus in the same manner resulted in development of \textit{in vitro} neutralizing antibodies in exposed sows, however, on challenge these animals failed to protect their baby pigs.\(^10\) Recognizing this difference between gut and cell culture propagated viruses, we decided that our future immunologic studies should be focused on gut-origin virus. We felt that baseline data obtained from such studies would correlate closely with those occurring in the field.

It was known to us that the milk taken from sows exposed orally to the gut-origin virus contained antibody that neutralized the virus and protected baby pigs against the disease. This was done by taking colostrum and milk from immune sows and mixing each with a fixed quantity of TGE virus (100 baby pig ID-50). The mixture was incubated at \(37^\circ\) C for 1 hour and then given orally to baby pigs. However, sera from these sows, used in a similar manner, failed to protect baby pigs against TGE. In spite of a striking difference of pig protective properties between antibodies occurring in the milk and serum of sows exposed to gut virus, both antibodies reacted by \textit{in vitro} cell culture neutralization test to a similar titer. This

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This study was a part of the North Central Regional Project NC-62. The study was jointly supported by funds from the Illinois Agricultural Experiment Station, the Department of Agriculture, State of Illinois, and the National Pork Producers Council.

329
finding lead us to speculate that there might be a difference between anti-TGE antibodies in the milk and that of the serum, and that the protective antibody produced by TGE virus of gut origin might be of the secretory type and thus occurring in the milk rather than in the serum. Therefore, the objective of this research was to detect, isolate, and characterize antibodies in the serum and milk of sows exposed to gut-origin TGE virus and to determine the class of immunoglobulin that is largely responsible for protection of baby pigs against the disease.

I. Immunoglobulins to gut-origin TGE virus

A. Detection of anti-TGE antibody: Susceptible pregnant sows were exposed to the gut-origin TGE virus by oral, intramammary, and intramuscular routes, at 5 and 3 weeks before farrowing. After farrowing, colostrum, serum, and milk samples were collected and the anti-TGE antibody titer was measured in each fluid by the in vitro neutralization test using the cell culture TGE virus. Results of these tests demonstrated that each route of inoculation resulted in production of in vitro neutralizing antibodies in the milk and serum of exposed sows.

B. Isolation and identification of immunoglobulin classes: A known quantity of protein contained in the serum and in the whey portion of the colostrum and milk was fractionated by Sephadex G-200 column chromatography and 5 ml fractions were collected. Phosphate buffer saline (PBS) at pH 7.5 and at a flow rate of 15 ml/hr was used. Selected protein peaks were collected and concentrated by Diaflo ultrafiltration. Concentrated fractions were then processed through a diethylaminoethyl cellulose (DEAE) column. Elution was accomplished by following stepwise changes of phosphate molarity at pH 7.5: 0.01, 0.02, 0.05, 0.075, 0.125 and 0.2 M.

Immunoglobulin classes IgM, IgA, and IgG contained in eluates obtained by Sephadex G-200 and by DEAE column chromatography were identified by precipitation in gel using specific antisera kindly provided by Dr. Philip Porter* (Figs. 1 and 2). These purified immunoglobulins were also used to produce monospecific antiserum to swine immunoglobulin classes in rabbits. The purity and quantity of each of the immunoglobulins were determined by immunoelectrophoresis and radial-immunodiffusion, respectively. The patterns observed in these tests are shown in figures 3 and 4.

C. In vitro neutralization of TGE virus with immunoglobulins of different classes: In the colostrum of a sow orally exposed to TGE virus, the antibody detected by in vitro neutralization test was found distributed in the IgA and the IgG fraction (Fig. 5). These results were further verified by absorption of each fraction with monospecific anti-IgA and anti-IgG antiserum. Analysis of the serum profile of this sow showed that preponderance of in vitro neutralizing antibody was in the IgG (Fig. 6).

Analysis of immunoglobulin profile of colostrum from a sow injected intramammarily at 5 and 3 weeks prior to farrowing showed that most of in vitro neutralizing antibody resided in the IgA fraction (Fig. 7). This was also verified by the absorption test. The antiviral activity in the serum of this sow was found in the IgG fraction. Finally, all in vitro neutralizing antibody in

* Unilever Research Laboratory, Shainbrook, England.
colostrum and serum of a sow injected intramuscularly with the virus was confined in the IgG class (Fig. 8).

D. Protection of baby pigs by immunoglobulins: On challenge, baby pigs nursing sows exposed orally and intramammarily to the gut-origin virus were protected. On the other hand, sows which were exposed to the virus by intramuscular injection did not protect their pigs.

Protective properties of the colostrum, milk, and serum of these sows were ascertained by allowing these fluids to react in vitro with the virus and then assaying infectivity of the reacted virus for baby pigs. IgA and IgG were selectively removed from these fluids by precipitation with monospecific antisera. After removal of a specific immunoglobulin from these fluids, protective effect for baby pigs of the remaining immunoglobulin was ascertained. Results of these experiments are shown in tables 1 and 2. It is evident that most of the pig protective effects resided in the IgA fraction of colostrum and milk of these sows.

Conclusion

This study shows that exposure of pregnant swine by various routes to TGE virus of gut and/or cell culture origin stimulated production of milk and serum antibodies which reacted in vitro with the cell culture propagated virus. This in vitro neutralizing effect resided in the IgA and IgG immunoglobulin classes. On the other hand, protection of baby pigs resided in the IgA immunoglobulin class found in the colostrum and milk of sows exposed orally and/or intramammarily to the gut-origin TGE virus only.

These findings indicate that the development of safe prophylactic measures for TGE will require additional studies designed to isolate the prototype TGE virus from guts of infected baby pigs and to propagate it in a medium which will assure maintenance of its immunogenic stability.
Figure 1. Immuno-diffusion of IgG, IgA, and IgM from swine serum and whey against specific antisera.

a. 1, 3, and 5 contain IgG of swine serum; 2, 4, and 6 contain IgG of swine whey. The central well contains rabbit anti-swine IgG.

b. 6 contains IgA of swine whey and 12 contains IgA of swine serum. The central well contains rabbit anti-swine whey IgA.

c. 13, 14, 16, 17 contain IgM of swine serum; 15 and 18 contain IgM of swine whey. The central well contains rabbit anti-swine serum IgM.

Figure 2. Immunodiffusion of swine whey IgG, IgA and IgM against rabbit anti-whole swine serum. 1 and 4 contain whey IgG; 2 and 5 contain whey IgA and 3 and 6 contain whey IgM.
Figure 3. Identification of swine IgM, IgA and IgG immunoglobulin classes by monospecific rabbit antisera by means of immunoelectrophoresis.
Figure 4. Radial Immunodiffusion Test. Appearance of precipitin ring in anti-IgA antibody-agar plate after 24 hours. Four dilutions of a standard whey fraction of known IgA concentration were placed in wells of row “e” of the plate. Dilution of the test samples were placed in the remainder of antigen wells. Rows a and b contained colostral dilutions of sows exposed to TGE by intramammary injection, rows c and d contain colostral dilutions of sows exposed orally to TGE virus. Row f contained serum dilutions of a sow exposed orally to TGE virus.
Figure 5. Results of *in vitro* neutralization of Purdue-108 TGE isolant by colostral whey eluates of sow exposed orally to TGE virus of gut origin. IgM and IgA were found in FI, and IgG was found in FII.
Figure 6. Results of *in vitro* neutralization of Purdue-108 TGE isolant by serum eluates of sow exposed orally to TGE virus of gut origin. IgM was found in FI, IgA was found in the valley between FI and FII, and IgG was found in FII.
Figure 7. Results of in vitro neutralization of Purdue-108 TGE isolant by colostral whey eluates of sow exposed to TGE virus of gut origin by intramammary injection. IgM and IgA were found in FI and IgG was found in FII.
Figure 8. Results in vitro neutralization of Purdue-108 TGE isolant by colostral whey eluates of sow exposed to TGE virus of gut origin by intramuscular injection. IgM was found in FI, IgA was found in the valley between FI and FII, and IgG was found in FII.
Table 1. *In vivo* neutralization of TGE virus (assay in baby pigs) by serum and colostrum absorbed with anti-lgA and anti-lgG antisera. The samples were from a sow exposed orally to TGE virus of gut origin.

<table>
<thead>
<tr>
<th>Sample Used for Virus Neutralization</th>
<th>No. of pigs with TGE</th>
<th>No. of pigs Exposed</th>
<th>Time Signs of TGE Developed after Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum* Absorbed with Anti-lgA</td>
<td>2/2</td>
<td></td>
<td>72 hours</td>
</tr>
<tr>
<td>Serum* Absorbed with Anti-lgG</td>
<td>2/2</td>
<td></td>
<td>24 hours</td>
</tr>
<tr>
<td>Colostrum** Absorbed with Anti-lgA</td>
<td>2/2</td>
<td></td>
<td>72 hours</td>
</tr>
<tr>
<td>Colostrum** Absorbed with Anti-lgG</td>
<td>0/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control***</td>
<td>2/2</td>
<td></td>
<td>18-24 hours</td>
</tr>
</tbody>
</table>

*Serum had anti-TGE titer of 1/160 against 10^2 CPE ID-50 of Purdue-108.

**Colostrum had anti-TGE titer of 1/160 against 10^2 CPE ID-50 of Purdue-108

***Control pigs were exposed to 500 ID-50 of Z-1 TGE virus.
Table 2. *In vivo* neutralization of TGE virus (assay in baby pigs) by serum and colostrum absorbed with anti-IgA and anti-IgG antiserum. Samples were from a sow exposed to TGE virus of gut origin by intramammary injection.

<table>
<thead>
<tr>
<th>Sample Used for Virus Neutralization</th>
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<th>No. of Pigs for Virus Neutralization</th>
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<td>72 hours</td>
</tr>
<tr>
<td>Serum* Absorbed with Anti-IgG</td>
<td>2/2</td>
<td>2/2</td>
<td>24 hours</td>
</tr>
<tr>
<td>Colostrum** Absorbed with Anti-IgA</td>
<td>2/2</td>
<td>2/2</td>
<td>48 hours</td>
</tr>
<tr>
<td>Colostrum** Absorbed with anti-IgG</td>
<td>0/2</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>Control***</td>
<td>2/2</td>
<td>2/2</td>
<td>18-24 hours</td>
</tr>
</tbody>
</table>

*Serum had anti-TGE titer of 1/40 against $10^2$ CPE ID-50 of Purdue-108.

**Colostrum had anti-TGE titer of 1/160 against $10^2$ ID-50 of Purdue-108.

***Control pigs were exposed to 500 ID-50 of Z-1 TGE virus.
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COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE

Chairman: D. P. Gustafson, Lafayette, Indiana
Co-Chairman: L. R. Barnes, Indianapolis, Indiana


The Committee addressed itself to those transmissible diseases of swine of current importance and which were not assigned to other committees. A sub-committee on Trichinosis was appointed in 1970 at Philadelphia. The first meeting of the sub-committee was held in the Federal Center Building, Hyattsville, Maryland, on September 28, 1971. Members present were: L. F. Van Gorder, ANH, Chairman; J. S. Andrews, VSR, (Beltsville, Md.); L. R. Barnes, ANH (Indianapolis, Indiana); G. P. Combs, ANH (Hyattsville, Md.); D. D. Juranek, CDC, (Atlanta, Georgia); T. A. Ladson, State Veterinarian, Board of Agriculture, (College Park, Md.); P. L. Zillman, President, Livestock Conservation, Incorporated, (Hinsdale, Illinois); and D. E. Zinter, Pathology Group C&MS, (Beltsville, Md.).

The purpose of the September meeting was to bring the sub-committee up-to-date on happenings in the area of trichinosis eradication since the last meeting of the full committee at the Annual Meeting of the USAHA in Philadelphia, Pennsylvania, last October. It was envisioned that the duties of the subcommittee were to be, to keep up-to-date on development of the proposed trichinosis eradication program, to transmit pertinent information to the full committee, and to reflect to other organizations what the USAHA wants done concerning the program. The immediate task of the meeting was to bring the sub-committee up-to-date on all facts concerning the program, and with information on progress in regard to its implementation.

Since the conclusion of the Fort Dodge Project, little has been done to continue the use of the pooled sample technique in commercial packing-houses. A Program Planning Budget (PPB) Model for a proposed Trichinosis Eradication Program had been developed, approved by the Food Safety Committee, and interdepartmental group, and submitted to the Secretary of Agriculture. No authority has been given yet for implementation of the program. In November 1970, there was formed an ARS/C&MS Interagency Work Group and enabling legislation for the proposed Trichinosis Eradication Program was drafted. This bill defined terms, provided authority for establishing and carrying out the test in the plant, controlling the shipment of hogs, garbage feeding, experimental testing, compulsory individual hog identification and quarantine of infected premises.

The problems involved in implementation of the proposed program were rather fully discussed. The essence of their deliberations is to be found in the report of the Trichinosis Eradication Subcommittee which is appended to this report.
SWINE DYSENTERY

In the report for 1970, it was presented by resolution that a nationwide, economically important problem in swine production is resulting from an inability to cope with swine dysentery. Furthermore, it was recommended that the Agricultural Research Service set aside funds in support of intramural and extramural research programs to identify the agent or agents causing the disease, to develop better methods of diagnosis, and to evaluate biological and/or pharmaceuticals for its prevention and treatment. Support for this resolution's position has been obtained from the National Pork Producers Council, the American Veterinary Medical Association, the American Association of Swine Practitioners, the National Hog Farmer publication, and Livestock Conservation Incorporated (LCI). The latter group has established a Swine Dysentery Advisory Committee to coordinate moves to inform and assure the officials of the U.S.D.A. and appropriate members of the Congress of the United States of the seriousness of the problem and the necessity for research support and programming. Information will be developed from producers with regard to dollar value losses to emphasize the National concern.


The LCI Advisory Committee met during the meeting of the USAHA at Oklahoma City, Oklahoma, and developed its program to attain its goals. The essence of the meeting was presented to the Committee. In a spontaneous expression of concern the Committee and assembled interested audience, invited and received a report of progress among vendors of pharmaceuticals, on drugs to be used to control and treat swine dysentery.

At the present, the industry, through the National Pork Producers Council, has provided initial support for research at two universities. In addition, for the next 5 years, Swine Dysentery is to be included as a section of the Regional NC-62 project entitled, “Enteric Diseases of Swine”; thus further emphasizing industry-wide recognition of the importance of the disease.

TRANSMISSIBLE GASTROENTERITIS

An evaluation of the performance of Diamond Laboratories' TGE-Vac was presented with refreshing candor by Drs. R. F. Marshall and D. A. Fuller of Diamond Laboratories, Inc.

One condition of the TGE-Vac special license from the Veterinary Biologics Division of the ARS requires that Diamond Laboratories conduct investigations of all reported cases of TGE-like disease in TGE-Vac Vaccinated sows and/or their pigs. Such investigations were conducted with care and in apparent good faith. The company was receptive to reports of favorable results from the field also. A total of 260,130 doses were sold between August 13, 1970 and June 1, 1971. Since 2 doses are required this represents vaccine for 130,065 sows. It is believed that a high percentage of vaccine sold was applied because of pressures generated by the national economic climate.

Forty-seven favorable results were obtained from herds in which it was
reasonably clear that challenge of resistance by TGE virus had occurred. Morbidity occurred in 21 of the 47 herds and ranged between 5 and 95% of the sows in them. Some degree of morbidity occurred in the pigs in 16 of the 47 herds with a survival rate of 94%. On-the-farm evaluations were not made by representatives of Diamond Laboratories. The reports of practicing Veterinarians form the basis of this phase of the report. It is recognized that such a report is incomplete and may or may not be representative.

There were a total of 54 cases of suspected TGE involving 1708 vaccinated sows reported. This is 1.3% of the sow doses sold. Twelve cases or 341 sows were determined to have non-related illness. Four cases (142 sows) were improperly vaccinated. There were 38 cases (1225 sows) in which TGE occurred and the vaccine was properly administered.

The Diamond Laboratories' scientists find that there continues to be a need for uniform, more accurate diagnostic procedures. In addition, their efforts revealed that sow morbidity tends to increase baby pig morbidity and mortality and that baby pig survival is often adversely affected by premature weaning (less than 3 weeks of age) when sows are lactating satisfactorily. The Diamond Laboratories scientists stated that, when properly administered, TGE-Vac meets the standards established for evaluation. These standards state that the vaccine is "an aid in the prevention of losses (both morbidity and mortality) in baby pigs due to Transmissible Gastroenteritis."

The report of the Sub-Committee on Transmissible Gastroenteritis is appended to this report.

The Committee's attention was invited to a program for eradication of leptospirosis in swine. The Committee respectfully declined to include the proposal on its program and referred it to the Committee on leptospirosis.

A brief discussion of African Swine Fever was conducted. The Veterinary Sciences Research Division of the ARS was commended for its contributions to the store of scientific knowledge of the disease relating to diagnostic methodology, immunology and epidemiology specifically. Concern was expressed for the availability of adequate funds to continue and expand such efforts in view of the continuing threat of the disease to the swine industry of the United States. Interest was expressed in the activities of the Animal Health Division's ability to monitor at ports of entry by air, land, and sea materials which might initiate an outbreak of ASF in the United States. The Committee wishes to refer possible resolutions and recommendations of the Association to the Committee on Foreign Animal Diseases.
REPORT OF THE SUB-COMMITTEE* ON TRANSMISSIBLE GASTROENTERITIS OF SWINE

Chairman, E. H. Bohl, Wooster, Ohio  
B. C. Easterday, Madison, Wisconsin; E. O. Haelterman, Lafayette, Indiana; George Lambert, Ames Iowa; M. Ristic, Urbana, Illinois; T. W. Tamoglia, Ames, Iowa.

A brief resume is given of the present status of TGE in respect to the following topics.

Etiology

Only one serologic type of virus has been described as causing TGE. This virus has characteristics similar to those of the coronavirus group.

Diagnosis

Clinical signs and history are often sufficient for the presumptive diagnosis of TGE. Examination of the small intestines for evidence of villous atrophy has proven very helpful, but should not be considered specific. For example, pigs with the "three-week-old scour" syndrome (of unknown etiology) may have villous atrophy. Confirmatory laboratory methods include: fluorescent antibody examination on intestinal epithelial cells, serologic test (viral neutralization), and viral isolation either by the use of cell cultures or piglets. A diagnostic problem may be posed when the disease occurs in piglets which are nursing immune or partially immune sows since the epidemiological features of the disease may not be typical. This situation is especially apt to occur in herds which are on a continuous or frequent farrowing program, where susceptible piglets (especially in the 2- to 5-week-old group) are continuously present to perpetuate the infection.

Immunity

The mechanism of passive immunity in TGE is receiving considerable attention. Recent findings indicate that there are different types or classes of antibodies, and that some classes of antibodies are more effective than others in providing immunity against certain infectious diseases. Present evidence suggests, that TGE antibodies in milk are more effective if they are of the immunoglobulin (Ig)A class rather than of the IgG class. Thus, methods are being sought for immunizing pregnant swine so as to provide adequate titers of TGE antibodies of the IgA class in colostrum and milk.

* This is a sub-committee of the Committee on Transmissible Diseases of Swine of the United States Animal Health Association.
Vaccination

Only one licensed product is commercially available (TGE Vac, Diamond Laboratories, Inc.). This is a modified live virus vaccine of porcine tissue culture origin, and is to be administered intramuscularly at approximately six and two weeks before farrowing, for the purpose of providing passive immunity to suckling pigs. Available evidence indicates that this type of vaccine used in the manner described is of limited value in preventing infection and clinical signs of TGE in suckling pigs, but tends to reduce the high mortality associated with the occurrence of the disease in neonatal pigs.

TRICHINOSIS ERADICATION SUBCOMMITTEE REPORT

The newly appointed Trichinosis Eradication Subcommittee reports that the proposed Swine Trichinosis Eradication Program, strongly supported by USAHA RESOLUTION at the 74th Annual Meeting in Philadelphia, is currently being studied in the Department of Agriculture with a view to implementation. The subcommittee further reports that:

1. **Swine Identification** The compulsory identification of swine moving in intrastate or interstate commerce, serves many industry needs, and is also an essential to any effect Trichinosis Eradication Program. Such identification should be made a program requirement to facilitate traceback activities. It is recognized that many disease control and eradication programs require that the individual identification of swine be maintained from farm of origin to point of disease detection.

2. **Field Trials** In-depth field trials or studies should be initiated in known trichina infected herds to aid in the further development and improvement of appropriate epidemiological techniques; herd management procedures; and in the application and evaluation of trichinosis tests in the live animal.

3. **Research**
   a. Current trichinosis research is directed toward reducing the cost of the Pooled Sample Technique by shortening the digestion time, and by recovering and recycling the pepsin remaining after digestion is complete.
   b. A fluorescent antibody test for use in detecting trichinae in live hogs is being readied for field testing.
   c. Future research will include studies on the biochemical differences between trichinous and non-trichinous pork using the techniques of spectrophotometry and pyrolysis gas chromatography.
   d. Efforts will also be made to perfect a skin test using cyst contents as the antigen.

4. **Liaison with Industry** Interested producer groups should be encouraged to organize the stimulate intra-industry meetings where possible to further develop understanding and support for the proposed trichinosis eradication program.

5. **State Committees** Committee activities at the State level are essential to the implementation of the proposed trichinosis eradication program; therefore it is recommended that presently existing swine disease committees within the various states broaden their functions to specifi-
cally include trichinosis eradication.

6. **Public Health** The continued danger to public health from trichinosis is illustrated by recent epidemics, including those in Arizona, California and Ohio. In recent years no reduction has been effected in the 100+ cases diagnosed annually. These reports of diagnosed cases fail to reflect in any satisfactory measure the impact of trichinosis on public health. Most trichina infections go unreported and undiagnosed. By conservative estimate, approximately 120,000 persons are infected with trichinosis each year. Sampling techniques indicate that at least 12,000 of these persons develop symptoms which require medical attention and result in lost time, and other expenses, with hospitalization in some cases. Ninety-five percent of diagnosed cases are attributed to the ingestion of commercial pork products. Prevention of human trichinosis cannot be insured under existing control and a "cook pork well" philosophy. These procedures are inadequate because (1) certain low-volume processing establishments are exempt from the inspection requirements of the Wholesome Meat Act; (2) apparent failures are reported in the application of the heat or cold treatment required to kill trichinae in "ready to eat" pork products; and (3) many consumers fail to cook pork adequately prior to consumption.

7. **Case Reporting for Traceback Purposes** Each case of human trichinosis should be reported promptly to CDC; and a prompt, efficient method of cross-reporting between CDC, State Health Officials, C&MS, and ANH is required at all Staff and Field levels. This is particularly important for ANH traceback purposes; and the attached diagram illustrates the various reporting channels involved in the full-coverage reporting of each case.

8. **Visual Aids** Visual aids should be developed for training and public information purposes. The existing trichinosis pilot project film should be completed; however, the film should be expanded to illustrate producer aspects of the program including the traceback and herd management activities involved.

9. **Wildlife** Conservation, and other wildlife officials should be invited and encouraged to cooperate with the appropriate National and State Swine Health Committees to discuss and develop procedures for the disposal of garbage and wildlife carcasses in both State and National Parks and by hunters and fur dealers.

The Committee commends the initiative of the States of Wisconsin, Illinois, and Kansas in conducting pooled sample examinations of swine in their States including the follow-up traceback of infected swine. The Committee recommends the appropriate steps be taken by State and Federal officials to implement, or otherwise effect, the points raised and proposals made in the subcommittee report.
PROCESSING FOOD WASTE INTO A FEED INGREDIENT

Thomas W. Powell, D.V.M.
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Garbage or food waste has traditionally been disposed of by feeding it to swine. Traditional ways of doing things may no longer be acceptable. What has been acceptable for many years may be in direct conflict with human and animal health today.

Let's ask ourselves a question. Why do we have waste materials? The answer is that a satisfactory method has not been developed for its use.

My colleague: Dr. Charles N. Dobbins, Jr. and I have been working several months on a process by which food waste can economically be used to produce an attractive, desirable, safe feed ingredient. We became interested in this possibility when it was determined that garbage or food waste feeding was implicated as the cause of over 50 percent of the hog cholera cases in 1969 in Georgia.

It has been demonstrated by traditional garbage feeding that food waste does have nutritional value and should not be discarded since waste is merely a resource out of place. Food waste feeding has served a purpose, but many feeders do not meet the heat treatment standards, and constant supervision is not possible. As a result, according to a recent USDA release, in 1970, garbage related outbreaks of hog cholera amounted to 11 percent of the total in the United States.

We have seen a transition period in which control and eradication of hog cholera has taken many steps. Some of these have been the elimination of live virus vaccines, the use of modified live virus vaccines, killed vaccines, serum and finally the elimination of vaccination. It would seem to me that a giant step forward in the total eradication of hog cholera would be the discontinuance of garbage feeding, especially if an alternate use of this material could be developed.

In addition to being quite costly in regulation and control, garbage feeding in its present form serves as an excellent vehicle to spread other diseases of swine such as Salmonellosis, Trichinosis, as well as other bacterial, viral, and parasitic diseases. Garbage feeding also contributes to pollution of ground and surface water. It enhances conditions favorable to the breeding of insects and rodents, it creates a nuisance with offensive odors, unsightliness and is quite difficult to maintain in a sanitary manner. Incineration and the use of disposals are definitely not the answer — in that they are expensive and contribute to other pollution problems. Sanitary landfills offer a temporary answer, but it too is expensive and may lead to subsurface water pollution.

For these and other reasons, many states have, or are in the process of prohibiting the feeding of food waste to swine in any form. The main difficulty with the legislation is that it leaves no alternative other than sanitary landfill, incineration or disposing of food waste through our already over taxed sewage disposal system.

The world population is increasing daily and the demands for protein is increasing proportionately. It is imperative that we find ways and means to utilize
PROCESSING FOOD WASTE INTO A FEED INGREDIENT

waste products by recycling them through various food producing animals. In the future, cereal grains for livestock feed will be competing with the human population for their use. A process by which food waste can be economically changed into an attractive, desirable and safe feed ingredient that can be recycled through livestock and poultry will relieve some of this pressure.

The process we are developing, overcomes all of the disadvantages of present garbage feeding. In addition, the end-product use is not limited just to swine, but may be expanded to cattle, poultry, pet foods and other animal feeds.

The process removes a portion of the fats and oils, reduces the moisture content from 75 percent to around 10 percent as well as eliminating pathogenic organisms which may be present in the raw material. Particle size is reduced, making it a consistent product, which has the capability of blending with other feed ingredients to make complete feeds. The end-product analyzes approximately 23 percent protein, 10 percent fat, with a low fiber content. Previous work with food waste indicates that the digestibility is good and can be utilized by various classes of livestock and poultry. Current testing indicates that it is quite palatable as well as nutritious. The intended use is that of a feed ingredient rather than a complete ration. Due to our commitments to the University of Georgia, and their involvement in patent applications, details of the process are not available for publication at this time.

I would like to acknowledge the cooperation of the Animal Health Division of Agricultural Research Service, especially Dr. Milt Tillery and Dr. C. J. Mikel. We have also had the support of Dr. Jim Andrews, State Veterinarian, Georgia Department of Health, University of Georgia as well as The Department of Army.

The development of the process has been approached with the objective of having a practical, safe, economical solution to the food waste problem. We feel that it will help solve a serious waste problem and help eliminate sources of animal diseases, thereby aiding the termination of a successful disease eradication story.
THE STATUS OF THE STATE-FEDERAL HOG CHOLERA ERADICATION PROGRAM

D. R. Stauffer*

INTRODUCTION
Completion of 9 years of activity has disclosed that FY 1971 was perhaps one of the most significant periods in the State-Federal hog cholera eradication program. Additionally, six States became Hog Cholera Free. With the continued progress made by most of the States, several, however, experienced an increase in hog cholera incidence. For the first time in the history of the hog cholera eradication program, a concerted effort known as the task force approach was used to stamp out the disease in areas where it had apparently become endemic. This successful effort had the desired effect of reducing hog cholera incidence perceptibly during FY 1971.

PROGRAM STATUS (Slide 1)
By July 1, 1971, 50 States had moved from hog cholera control, represented by Phase II, to hog cholera eradication represented by Phase III and Phase IV. No States remain in Phases I or II.

Hog Cholera Free States increased from 15 a year ago to 21 by July 1971. Including the Free States, the number of Phase IV States increased from 25 to 44 by July 1971.

Significant advances have occurred since July 1970, such as Illinois and Nebraska, advancing to Phase IV, Missouri becoming the 44th Phase IV State, and Georgia and Iowa qualifying as Hog Cholera Free.

It is interesting to note that the large western area which is hog cholera free has been able to maintain that status during the fiscal year. Hog cholera incidence in the North Central States and elsewhere has dropped perceptibly. Populous swine States; such as, Wisconsin, Minnesota, Missouri, Nebraska, South Dakota, Illinois, and Iowa are included in Phase IV or Hog Cholera Free. The advancement of Alabama, Arkansas, Georgia, Louisiana, Mississippi, Kentucky, South Carolina, Tennessee, and Virginia to Phase IV or Hog Cholera Free maintained the same level of progress attained by the hog belt. With 44 of the States in Phase IV, about 75 percent of the swine population is located in States where hog cholera is not known to exist.

Although advancement has proceeded as anticipated, some States have been unable to move ahead in the program as scheduled. Presence of the disease has not contributed to these delays, but funding and other program inadequacies have proven to be stumbling blocks along the way. Since salvage was stopped March 1, this problem should no longer prove a deterrent to advancement. All States should

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* Acting Senior Staff Veterinarian, Animal Health Division, Animal and Plant Health Service, U.S. Department of Agriculture.

350
be encouraged to move ahead as rapidly as they are able to comply with program standards. This is necessary to maintain the national interest and effect a successful eradication effort.

Changes in indemnity ratios in Phase IV and Free States have been proposed whereby the Federal Government will pay the greater share of indemnity in these States. This amendment to Federal regulations has subsequently passed and should prove most helpful to those States that have had to spend large amounts of money the past several years to control the spread of the disease. It will also benefit those States where only limited funds have been available for hog cholera eradication.

INCIDENCE (Slide 2)

Hog cholera incidence decreased dramatically in FY 1971 from 1970. Reports of suspicious cases have increased from the outset of the program. With an increase in suspicious cases, we have experienced a 67 percent reduction in positive cases. A dramatic change has also been observed in the ratio of positive cases to suspicious cases. Earlier in the program, the number of positive cases reported exceeded the number of suspicious cases. The past several years, the number of positive cases has approached the ratio of one positive case for each four suspicious cases reported. A great change has occurred in FY 1971 in that only one positive case of hog cholera is reported for every 15 suspicious cases. It is obvious that there is less disease present and that our reporting system has improved also.

(Slide 3)

The number of counties in which hog cholera occurred in FY 1971 decreased by 319. The number of States experiencing the disease decreased by nine.

(Slide 4)

Statistics reveal that 81 percent of the infected herds were confined to six States. During the last half of FY 1971, 50 percent of the positive cases were found in just two States.

The disease incidence has been almost completely reduced in these six States. The application and placement of quarantines on larger areas, better control of movements within and from quarantine areas, more comprehensive surveys, plus locating and depopulating more exposed herds, have all been invaluable in these efforts. The task force approach to disease eradication, the progressive thinking and action of the involved States, contributed greatly to their successes. It is quite evident that a more concerted thrust using all program procedures will be required to effect total eradication in any State where infection remains a threat.

EPIDEMIOLOGY (Slide 5)

Vaccine-associated cases continued downward during FY 1971. Vaccines were incriminated in 0.2 percent of the total cases for the year. Another milestone along the way in hog cholera eradication was the termination of hog cholera product licenses by the biological companies.

For the first time in 70 years, anti-hog cholera serum is no longer being produced in this country. Without the dedication and courage of industry and other allied groups, these significant steps could not have been taken. Additionally,
another first in the program was the termination of the last Class “A” feedlot for serum production operating in the United States.

Serum production was the foundation upon which a prosperous veterinary biologics industry was built. Even in the early years of hog cholera eradication, hog cholera biologics were a significant source of revenue for this industry. Knowing well that a successful program would eliminate the need for such biologics, this industry has supported hog cholera eradication from the outset. The industry, as a whole and individually, should be recognized for its efforts toward hog cholera eradication.

The spread of hog cholera through swine movement, primarily marketing, continues. This movement, with the closely related area or neighborhood spread, continues to be the primary source of positive cases in the hog cholera eradication program. Closer adherence to market standards would prove most helpful in this regard.

Another continuing epidemiological problem is hog cholera associated with garbage feeding. Strong circumstantial evidence continues to point to this as a method of perpetuating the disease. It would appear that the feeding of garbage will remain a potential hazard to the hog cholera eradication program until the virus of hog cholera is eradicated, or until such time that a more effective means of handling the problem is found.

Development of a source for about 20 percent of the positive cases continues to elude our technically competent people. The chronic nature of these cases, plus the masking of hog cholera by other disease conditions for long periods of time, makes the establishment of a source doubly difficult. More and more it will test the ingenuity and patience of our trained diagnosticians and epidemiologists to establish a source of many of our remaining cases.

Consideration should be given to insect vectors as a means of hog cholera spread. Extensive epidemiological investigations have produced substantial evidence to support this theory, especially in those areas of heavy swine concentrations.

OUTLOOK

With the favorable pattern of decline the past 12 months, we are rapidly approaching the desired goal of Hog Cholera Free. It appears only a matter of time until the last case of hog cholera is eliminated from this country. Sporadic cases are expected to occur from time to time. However, most States, calling on past experiences with the disease should be able to confine and stamp it out promptly. Any indication that the disease is regaining a foothold, will result in a concerted effort as demonstrated in the Dismal Swamp area of North Carolina and Virginia.

With the goal of hog cholera eradication appearing on the horizon, our thoughts should be directed toward preventing infection gaining entrance to this country from those areas where hog cholera is known to exist. Consideration should be given now to restricting imports from these countries. Further consideration should be given to those facilities involved in the disposition of waste materials from foreign ships and aircraft. These facilities should be regulated so that the introduction of hog cholera or any other exotic disease does not occur. Finally, we should develop a timetable for the cessation of research activities with hog cholera virus. Plans should be formulated to prevent these strains from ever developing into a threat to the nation’s swine industry. A continuing effort to
uncover unused supplies of vaccines throughout the country should remain a priority consideration.

Perhaps one of the greatest obstacles to eradication that remains is the threat of complacency. As positive cases become fewer in number, the concern for the disease disappears. Maintaining this interest will be a challenge to industry and agriculture until eradication is complete.

In summary, the hog cholera eradication program has progressed more rapidly than many people had anticipated. After nine years of program activity, hog cholera eradication has definitely become a reality.
Cooperative State-Federal

Hog Cholera Eradication Program

July 1, 1971

PHASES I & II (O)

ERADICATION:
(Indemnity can be paid)

III ☑ Elimination of outbreaks (5)

IV ☑ Protection against reinfection
(45+P.R.—21 FREE)
HOG CHOLERA REPORTED, FISCAL YEAR 1971

27 STATES AND PUERTO RICO

138 COUNTIES IN WHICH ONE OR MORE CASES WERE REPORTED

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
81% of positive hog cholera cases found in six states

FISCAL YEAR 1971
SOURCES OF HOG CHOLERA CASES

% of Positive Cases

50

40

30

20

10

0

Same State

Other State

HERD ADDITION

AREA SPREAD

VACCINATION

RAW GARBAGE

NO SOURCE ESTABLISHED

FY 1969

FY 1970

FY 1971

1/ COMPLETED INVESTIGATIONS

U.S. DEPARTMENT OF AGRICULTURE

ANIMAL HEALTH DIVISION

AGRICULTURAL RESEARCH SERVICE
REPORT OF THE COMMITTEE ON
NATIONWIDE ERADICATION OF HOG CHOLERA

Chairman, David L. Smith Indianapolis, Ind.
Co-Chairman, J. B. Taylor, Montgomery, Ala.


Progress in the cooperative State-Federal hog cholera eradication program has been extremely good during the past year.

From January 1 through October 20, there have been only 108 cases of hog cholera - versus 614 for the same period during 1970. Even more striking, since July 1 there have been only eight infected herds: one in Mississippi on July 1; one in Rhode Island on July 22; one in Texas on July 29; three in Puerto Rico on August 2, 26, and 30; one in Texas on September 7; and one in Pennsylvania on October 8.

It is possible that the Pennsylvania case will be the last case of hog cholera located in the United States. If this is so, a year from now this Nation will achieve its goal of being hog cholera free - 3 months ahead of schedule.

This progress in reducing the incidence of the disease is reflected in the status of the various states in the four-phase program. Twenty-eight states are now hog cholera free; all but one state - Texas - is in Phase IV. If no further outbreaks occur, these 22 states and Puerto Rico will be eligible for "free" status as follows:

New York - anytime; Connecticut in November 1971; Alabama and South Carolina in December 1971; New Hampshire and Tennessee in January 1971; New Mexico and Louisiana in February 1972; Minnesota, Virginia, Arkansas and Missouri in March 1972; Ohio, Massachusetts, and New Jersey in April 1972; Oklahoma and Indiana in May 1972; Illinois and North Carolina in June 1972; Mississippi and Rhode Island in July 1972; Puerto Rico in August 1972; and Texas in September 1972.

This achievement is not an accident. It is due, rather, to 9 years of hard work and concerted effort. It is the result of sacrifice and dedication on the part of many.

But we must not let the current rosy outlook lull us into a sense of complacency. Your committee strongly urges that all involved with this program - State, Federal, and industry - maintain the sense of urgency and alertness in hog cholera eradication activities that has brought us to our present position. We must remain vigilant. We must continue to report all sickness in swine promptly. We must continue to "Suspect Hog Cholera First."

Your committee recommends that efforts to locate the eliminate sources of virus - whether vaccine or other - be continued and intensified.

359
To protect against the dangers of reinfection, it is recommended that regulations be instituted to restrict the importation of live hogs and pork products from countries where hog cholera exists.

Your committee recommends that the preliminary report of the Ad Hoc Committee on Hog Cholera Surveillance, a copy of which is attached, be adopted as a part of the program and that it be implemented at the earliest opportunity. In this respect, it is the feeling of this committee that the United States should be recognized as being officially "hog cholera free" when a period of one year has passed since hog cholera has been diagnosed in the United States of America. This reflects the same standard for the Nation as a whole as has been applied to each individual state during the course of this eradication program. It is also consistent with the standards the British used to declare their nation "hog cholera free".

Your committee also wishes to recognize action taken in the past year to implement two recommendations made a year ago. In March of 1971, salvage was discontinued as a part of this eradication program. In August 1971, Federal regulations were amended to increase the Federal share of indemnity payments in states in Phase IV and in "hog cholera free" states.

Federal regulations are now being prepared which would bring Part 76 of the Code of Federal Regulations into line with program standards established by this organization and the U.S. Department of Agriculture. Specifically, these regulations would allow a "hog cholera free" state which has primary, unrelated outbreaks which are promptly stamped out to retain its "free" status. Further, if the state did lose its "free" status, it could regain that status after a period of 6 months without hog cholera had elapsed. Your committee recommends that this regulation be carried forward as rapidly as possible and recommends its adoption following publication in the Federal Register.

PRELIMINARY REPORT OF THE AD HOC COMMITTEE ON HOG CHOLERA SURVEILLANCE
(Held in Washington, D.C., September 8 & 9, 1971)

General:

In order to determine that hog cholera has been eradicated from the United States, and in order to detect it in the event of reintroduction at the earliest possible moment, the following surveillance activities are suggested:

I. Disease Reporting

The reporting of all swine disease conditions that may possibly resemble chronic or acute hog cholera is urged. Owners, caretakers, and veterinarians must be constantly reminded, stimulated, and encouraged to report to regulatory officials in order that a complete investigation with lab diagnosis can be made. To insure that this activity continues at an acceptable level, it is recommended that a veterinarian with advanced training in pathology be assigned in each State (with a sizeable swine population) and given the full-time responsibility to promote swine disease reporting. His activities should include, but not be limited to:

1. communicate with swine owners through information officer and other agencies
2. keep members of the State Veterinary Medical Association informed
NATIONWIDE ERADICATION OF HOG CHOLERA

and stimulate reporting by personal contact
3. assist practitioners and diagnostic labs in obtaining differential diagnosis in problem cases
4. encourage continued cooperation in reporting by extension service and other agencies that communicate with swine owners.

II. Monitoring
A. Laboratory Testing
1. Screening of swine specimens at diagnostic labs
   a. It is recommended that a lab test for hog cholera be run on all cases presented for diagnosis for 1 year after the last positive case. After that period, the test should be run on non-suppurative cases cases selected by the pathologist or epidemiologist.
   b. Because of funding and staffing difficulties, it is recommended that Animal Health (ANH) subsidize State Diagnostic Labs for this service where and when needed.
2. Specimens from swine retained for septicemic conditions that resemble hog cholera by meat inspectors should be obtained and lab tested for hog cholera. (The extent of this activity will depend on geographic areas, volume, cost and epidemiological need).
3. It is suggested that epidemiological and serological surveys be conducted in selected high-risk populations, e.g., cull and junk pigs at slaughter to determine the presence of SN antibodies to hog cholera. This type of activity would be limited in time and place as special investigations.
B. Clinical examinations that constitute monitoring activity. These activities need not be charged to hog cholera as they are ongoing programs to monitor for all diseases.
   1. It is recommended that inspection of garbage feedlots be continued and that well trained and competent inspectors, able to detect possible hog cholera, be used.
   2. It is recommended that veterinary inspection at livestock markets be continued to monitor for all swine diseases.
   3. It is recommended that on-the-farm inspection, prior to release from quarantine of interstate shipments, be phased out at the discretion of the State veterinarian, as far as hog cholera monitoring is concerned.
   4. It is recommended that on-the-farm inspection, prior to sale of feeder pigs, be maintained by industry groups to insure that quality pig production is continued.

III. Regulations
A. To prevent entry from outside the United States it is recommended that regulations be passed, as soon as possible, to prohibit and/or restrict the importation of swine and pork products from countries where hog cholera exists, and that special attention be given to increased surveillance along the Mexican border.
B. To improve marketing regulations in the United States
   We are cognizant of promises made to the swine industry to relax
restriction on marketing of swine as eradication was obtained. Therefore, it is recommended that a review of marketing standards be made by swine industry and regulatory officials, that new regulations be formulated that will be acceptable to the industry and also conducive to the control of all swine diseases, and that such items, as swine identification, market, and transportation vehicle sanitation, restrictions on “pig dealer” etc., be considered.

In regards to the question — “When can we safely declare the United States free of hog cholera?” — The three expressed are listed; please check the one you favor.

☐ The United States will be declared free when the last State reaches free status.
☐ The United States will be declared free 1 year after the last positive case.
☐ The United States will be declared free 2 or more years after the last positive case.

Several of the committee members felt that the Herd Health Program or the VIP Program should be included as a part of the surveillance program. Will you please indicate if you approve or disapprove this point.

The following guidelines are suggested by the Ad Hoc Subcommittee on the disposal of hog cholera virus:

1. Locate and determine the amount of hog cholera virus, in whatever form, in the United States.
2. Request the voluntary disposal of all virus found. Destruction should be by autoclaving or incineration.
3. Some laboratories and biological companies may be reluctant to destroy certain strains of hog cholera virus.
4. It was suggested that some virus would be needed for certain tests; positive controls for the Fluorescent Antibody Tissue Section Test and the Fluorescent Antibody Tissue Culture Test; for use in the Serum Neutralization Test and other research and surveillance needs. The virus will be needed for these purposes until routine surveillance for the hog cholera virus is stopped.
5. Should diagnostic laboratories, university research laboratories and biological companies keep hog cholera virus or continue to use the virus in their work, certain safety criteria must be developed and applied.
6. It is recommended that no more interstate permits for movement of hog cholera virus be issued, except for official program needs.
7. Following the hog cholera surveillance period, a repository or repositories for all remaining hog cholera virus must be established. Four suggestions advanced:
   a. Plum Island, New York
   b. Other countries
   c. American virus typing center
   d. Located under USDA seal with inspection at those laboratories maintaining such virus and which meet the criteria on safety set forth above.
These recommendations should be implemented in two stages:

**Stage I** Destruction of all hog cholera virus as recommended. Establish controls in those laboratories where hog cholera virus is retained during the hog cholera surveillance period.

**Stage II** Destruction of all hog cholera virus, or its relocation in approved repositories within the United States or outside the country. This stage should be implemented at the end of the surveillance period and before hog cholera can be declared exotic to this country.

Dr. R. S. Shaman
Dr. R. S. Shaman, Chairman
Mr. Neil Black
Mr. Keith Myers
Mr. Norval Duorak
Mr. Donald Kruger
Mr. Larry Mark
Mr. Sid Moore
Dr. V. C. Beal, Jr.
Dr. T. L. Beals
Dr. W. O. Coward
Dr. H. W. Dunne
Dr. D. P. Gustafson
Dr. G. H. Wise

Comments by committee members on preliminary report of Ad Hoc Committee:

### SURVEILLANCE

**I. Disease Reporting**

A question raised on advance training and whether it has to be pathology! Trained veterinarian should also be responsible for developing and maintaining reporting and surveillance program. How many “pathologists available”? — short-term nature — individuals must not be left in a dead-in situation. Laboratory should be spelled out — instead of pathologist training — should be animal disease surveillance. Must keep veterinary practitioner informed. Need to maintain close liaison with State and Federal meat inspection officials. Each and every positive case should be thoroughly investigated by regional epidemiologist.

**II. Monitoring**

Projections need to be made on how many laboratories and probable cost and might provide a basis for regional groupings and a basis for a coordinated laboratory effort as standards adopted and enforced — may be relatively short termed. After 1 year from last positive case, selection should be by field diagnostician, State, Line, Staff. A system of documentation and reporting needed. Cases checked should be identified as non-suppurative. Add — “Because of funding and staffing difficulties it is recommended that — B.2 — add but not under the guise of hog cholera alone — first sentence on pg. 3 — lab should be spelled out — add B-1 — This is conjunction with other or an overall inspection — B-1 — We should work toward discontinuing this operation in present form. B-3 — ambiguous — As long as we have surveillance, inspection...
should continue. B-4 — on-farm inspection should be required during surveillance.

III. Regulations

IIIA — Must move as rapidly as the cooperative aspects of the surveillance effort. IIIB — important to swine industry, ANH and States in terms of an orderly transition from the hog cholera program to general swine health — We must either designate hog cholera infected countries or designate free countries. Individual identification on all feeder pigs moved through markets is most important. The report does not stress the importance of an identification program.

When to declare country FREE

II vote for when the last State reaches Free status
7 vote for 1 year after last positive case
1 vote for 2 or more years

DISPOSAL OF HOG CHOLERA VIRUS

1. Statement — No comments
2. Some certification or recommendation of disposition should be made even during the surveillance period, for all locations with virus that dispose of their stocks. Insert virulent before virus in statement. (Dr. Gustafson) Police powers should be used and confiscate virus (Mr. Dvorak)
3. If maintained virus should come under surveillance program.
4. Clear differentiation between needs during surveillance and one eradication is declared. This will be emphasized if the recommendation in No. 6 is adopted.
5. A “reasonable” time for research to be completed (12-31-72) (Dr. Brinkmeyer sees little need for this section.)
7. Need to develop as restrictive regulations as we can, American Type Culture Center — Other countries and Plum Island not too desirable.

Stage I & II — Such relocation should be under supervision — insert virulent before hog cholera virus.
THE RESULTS OF FEEDING SALMONELLA-CONTAMINATED MEAT MEAL TO GROWING-FINISHING SWINE

William F. Nape, D.V.M., and Chasteen Murphy, D.V.M., M.S.

Studies have shown that salmonella organisms are frequent contaminants of animal protein used for livestock feeds. High contamination rates (40% and 52% for fiscal years 1969 and 1970, respectively) have occurred in the finished-product samples collected in blender plants. For this report a blender is defined as an animal protein processor that does not cook the product.

Salmonellae in feeds may establish inapparent animal infection with subsequent contamination of animal products for human food. Groves et al. showed that swine play an important role in the salmonella cycle by the selection of serotypes from contaminated feed, cross infection in swine pens, and finally in edible pork products. In one instance, S. heidelberg was isolated from feed samples and from swine on the farm as well as from animals slaughtered in an abattoir from the same farm. S. heidelberg was also isolated from the abattoir environment, carcasses, and washings of edible pork products in the same abattoir.

In the midwestern part of the United States, blender plants provide most of the animal proteins for livestock and poultry feeds. To kill salmonellae a few blenders have installed terminal heaters to heat treat the animal protein prior to shipment. Feed mills in the area of such blenders usually have the option of purchasing either a nonheat-treated or heat-treated animal protein.

This study was designed to determine the differences in infection rates, pen contamination, and performances between two groups of swine. Salmonella-contaminated meat meal from a blender was fed as a feed ingredient to the test group of swine. Meat meal from the same contaminated lot was heat treated and fed the control group.

MATERIALS & METHODS

A well-managed research farm in Illinois was selected for this study. Over 2000 pigs are born on the farm and marketed annually. One group of 50 swine was fed a ration containing salmonella-positive meat meal. The control group of 50 swine was fed the same ration, except the meat meal was salmonella negative.

A. Swine

The 100 test pigs selected for uniformity from approximately 300 farrowed October 1970, were allotted to 10 pens with 10 animals each. This allotment was made on the basis of weight, breed, and sex. The 10 pens of pigs

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The authors thank Dr. Gerald G. Jelly, Regional Diagnostic Laboratory, Peoria, Illinois, for isolation and identification of the organisms, and Dr. Billie O. Blackburn, Leader, Enteric Bacteriology, Diagnostic Services Laboratory, Ames, Iowa, for serotyping and MPN.
occupied the 10 northernmost pens in the barn. The pigs in pens 1 through 5, on the east side of the alley, were fed a ration with salmonella-contaminated meat meal. Pigs in pens 6 through 10, on the west side of the alley, were fed the same ration with salmonella-negative meat meal. A buffer pen was kept empty between the test pigs and other pigs being fed for market. Prior to this study, the test pigs had been raised on swine concentrates in which animal protein (meat meal) had been heat treated. All pigs were earnotched and tattooed. Both groups of swine were fed the test rations for 99 days (December 23, 1970, through April 1, 1971) and were slaughtered April 2, 1971. Each pig was weighed every 2 weeks.

B. Feed

The test rations were fed in meal form and compounded at the research farm. When the pigs averaged under 100 pounds, the rations contained 4.2% meat meal and when over 100 pounds, 2.5%. The rations contained no antibiotics. Feed consumption was recorded by individual pen.

C. Sampling For Salmonella

1. Meat meal for the test rations: One thousand pounds each of nonheat-treated and heat-treated meat meal were purchased from a local blender in 100-pound paper bags. Four samples were collected during bagging from each of 20 sacks. To eliminate variation in protein quality, the nonheated and heated meat meal were from the same lot.

2. Swine: Prior to animals being fed the test rations, they were sampled on 3 occasions to determine their salmonella status. On December 11, 1970, 110 pigs were sampled by rectal swabs. This sampling was representative of a group of approximately 300 pigs. On December 16, 1970, pigs from this group of 300 were selected for the feed study. Rectal swabs were obtained from each of the 100 test pigs on December 17, 1970, and December 23, 1970.

Each of the pigs on test rations was sampled by rectal swabs at 2-week intervals. Rectal swabs were taken on the farm prior to loading and at the packing plant before unloading. The mesenteric lymph nodes (MLN), bile ducts, and caecal contents were collected from each marketed test pig on the kill line. An attempt was made to secure suitable specimens free of exogenous contamination. Each MLN and each bile duct were placed in individual containers. Each tissue specimen was dipped in alcohol and flamed at the laboratory. The centrally located tissue was minced and cultured. Three to 6 grams of MLN, 1 to 2 inches of bile duct, and 15 to 30 grams of caecal contents were cultured from each of 74 marketed pigs.

3. Pig environment: The test pens were cleaned and disinfected. Each floor of the 10 test pens was swabbed before the pigs entered.

Four materials were sampled in each pen on 7 sampling days: water from the automatic watering cup, fecal matter on the floor, feed material from the mouth-contact surface of the feeder lid, and feed from the self-feeder. Two sterile swabs were used to collect each of the first 3 materials.

Ten swab samples were taken on the truck bed after it was cleaned and disinfected. Before loading the swine for slaughter, 2 fecal samples of approximately 30 grams were collected from the floor of each test pen. During trucking, a partition on a single-bed truck separated the 2 groups of
swine. Ten fecal samples and 10 swab samples were obtained from each side of the partition of the truck after the swine had been hauled and on the truck 8 hours.

The dehairing machine and equipment along the dressing rail were swabbed before and again after slaughter of the test animals. A total of 1,928 samples was collected. Isolation and identification were made at the Regional Diagnostic Laboratory, Peoria, Illinois. Isolations were made by a previously described procedure but with the following additions: After 24 hours' incubation, the tetrathionate broth was streaked to 3 rather than 1 brilliant green plate. Incubation of the tetrathionate enrichment was continued for another 24 hours and, if the original plates were negative, 2 more brilliant green plates were streaked from the 48-hour enrichment.

Tetrathionate broth was added to swabs or specimens not later than 3 hours after collection. All isolates were serotyped at the Animal Health Division Diagnostic Services Laboratory, Ames, Iowa.

Most probable numbers (MPN) of salmonella organisms per 100 grams of meat meal were also determined at the Diagnostic Services Laboratory. A sample for MPN was collected from each of the 10 sacks of positive meat meal on April 1, 1971. The sample was obtained from 4 or more pounds of meat meal left in the sack for this purpose. The multiple-tube technique for coliforms was utilized.

RESULTS

All 40 samples of nonheated meat meal were positive for salmonellae. However, 23 of the 40 were negative on the first plating to brilliant green agar. Colonies appeared only after the enrichment broth was incubated for 48 hours and then streaked to the plating media. Eighteen serotypes were isolated and their frequency noted (Table 1). The 40 samples of heated meat meal were negative. The MPN of salmonella organisms in nonheated meat meal are in Table 2.

The 310 pre-trial rectal-swab samples were negative. The 10 pre-trial floor-swab samples from test pens did not reveal salmonellae.

One pig on negative meat meal was very unthrifty. Unfortunately it was killed on February 9, 1971, and incinerated without post mortem. The pig was deleted from weight-gains and feed-consumption data.

The 350 rectal swabs from pigs fed contaminated meat meal were negative as well as the water, floor, and feeder-lid samples (Table 3). Of 70 feed samples containing contaminated meat meal, 3 (4.3%) were positive. *S. montevideo*, *S. anatum*, and *S. senftenberg* were isolated. Two of the 3 positive feed samples were negative on the first plating.

No isolations were made from the 347 rectal swabs from the pigs on negative meat meal ration and from their environment (Table 3). On April 1, 1971, 38 pigs on positive meat meal ration and 36 pigs on negative meat meal ration were trucked 160 miles to slaughter. Most of the 25 head not shipped were retained for breeding purposes. A few were under 200 pounds. Before loading at 4:00 p.m. on April 1, 1971, rectal swabs were taken from the 74 head to be slaughtered. The same animals were again sampled 8 hours later on the truck at the packing plant. The swine were held on the truck until 5:45 a.m., April 2, 1971, and were killed at 7:00 a.m. They were the first animals to pass through the plant that day. The swine did not contact the holding facilities except the alley to the kill floor.

There were no isolations from the samples collected immediately before or after trucking. Samples taken from 8 swine during slaughter were positive (Table 4). The
8 positive swine were from the group on positive meat meal and were distributed among the 5 pens (Table 5). All positive MLN and caeca revealed salmonella colonies on the first plating.

The 40 before-and-after samples from the dehairing machine and equipment along the dressing rail were negative.

Measurements of growth and feed conversion are recorded in Table 6.

**DISCUSSION**

The number of salmonella organisms per unit weight of feed was relatively low. The MPN average was only 7 salmonella organisms per 100 grams of meat meal. The extreme MPN findings were not included in the calculations; 2 organisms in the second aliquot from sample 3 were used. The MPN average was 208 organisms per 100 grams using 1,609 organisms from the first aliquot of sample 3 and eliminating the extreme findings. There did not seem to be a homogeneous distribution of salmonellae in sample 3. The absence of growth on brilliant green agar plates indicated low numbers. Of the 40 positive meat meal samples, 23 had no salmonella colonies on the first plating. The level of salmonellae was further diluted by meat meal being only 4.2% and 2.5% of the ration. In addition, the feed with positive meat meal had only a 4.3% (3/70) contamination rate using the more sensitive isolation procedure. Two of the 3 positive feed samples were negative on the first plating. Other ingredients in the ration have been proven relatively free of salmonellae. The 3 serotypes found in feed were the same as those found in meat meal (Table 1). All 70 samples of feed with negative meat meal were negative.

Feed with low levels of salmonella organisms apparently resulted in 8 of the 38 (21.1%) animals being positive at slaughter. The 3 serotypes isolated from swine were atypical serotype 3,10,15:e,h-1,6, *S. bredeney*, and *S. montevideo*. These same 3 serotypes were also found in the meat meal part of the ration. Atypical serotype 3,10,15:e,h-1,6 is quite rare. To our knowledge, this is the first time an organism with this antigenic formula has been found under natural conditions. The control animals on negative meat meal with the same history and similar environment were negative at slaughter. Positive animals occurred in each of the 5 pens rather than clustered in 1 or 2 pens. This distribution of positive animals could be expected from ingesting contaminated feed. Other studies have found a relationship between contaminated feed and positive animals at slaughter.9,14

Low levels of salmonella organisms in the feed established infection and a carrier state in some animals. Six of 38 (15.8%) animals on positive feed had positive MLN. *S. bredeney* and 3,10,15: e,h-1,6 were isolated from the MLN, and the meat meal samples. The control animals had negative MLN. There was a significant difference in the infection rates between the 2 groups. The difference is significant at 95% level with a chi-square value of 4.25. Since only 3 to 6 grams of MLN were cultured per pig, a large sample size may well have revealed the majority of the animals on positive meat meal to be infected. Groves et al.6,7 reported that market swine slaughtered in abattoirs in southwestern Ontario are frequently infected with salmonellae. In 9-month study, salmonellae were isolated from the MLN of 20.3% of the swine examined in 5 abattoirs. All salmonella organisms are potentially pathogenic and may cause overt disease under favorable conditions.

Terminal heating at the blender eliminated salmonellae from the meat meal. All 40 samples of the heated meat meal were negative, while 40 samples of the nonheated material from the same lot were positive. Negative meat meal was
associated with negative feed (0/70) and negative swine (0/36). Positive meat meal was related to positive feed (3/70) and positive swine (8/38). These results are consistent with W. Edel et al. They concluded that salmonella infection occurred in pigs fed contaminated meal. Their findings indicated salmonella infection can be prevented by pelleting the meal. Environment (flies, dust) and starting with salmonella-free pigs were also important in maintaining pigs free of salmonellae. Since the study reported in this paper was conducted during winter, flies were in very low numbers. Dust was present, but no positive animals were identified in the control group across the alley. The low number of salmonella organisms in the feed may have reduced the chance of spread by dust particles.

The 2 groups of swine did not show a significant difference in their performances (daily weight gain and feed conversion — Table 6). Lack of stress or disease conditions probably did not provide a favorable environment for the relatively few organisms to invade and multiply extensively in the host. Additional studies under various farm conditions and time-of-year may suggest a direct relationship between low numbers of salmonellae in feed and performance. Infected animals held for breeding purposes may show measurable losses. Controlled studies would indicate whether infected animals develop disease problems, have smaller litters, produce less milk, or have more disease in their offspring.

The second plating of meat meal and feed samples from tetrathionate broth revealed additional positive samples. Twenty-three of the 40 meat meal samples were negative on the first plating from tetrathionate broth, but they were positive on the second plating. These 23 samples would have been negative using the recommended procedures. Two of the 3 feed samples were found positive on the second plating. The recommended procedure requires incubation in tetrathionate broth only 24 hours and one plate per sample. The second plating may rarely show such a large percentage of additional positive samples. This may occur when culturing material at the low level that salmonellae were present in the samples.

The study revealed salmonella organisms were most frequently isolated from MLN. Isolations occurred less frequently from caecal contents, no isolations from rectal swabs. These findings support B. I. Groves et al., who reported that MLN culture was the most effective means for the isolation of salmonellae from swine. The study also supports H. W. Smith’s findings that MLN is the preferred source for the isolation of salmonellae from swine.

Rectal swabs did not identify animals in either group shedding salmonella organisms. All rectal swabs, as well as fecal swabs and fecal samples from the floor, were negative. Organisms may have been excreted in very low numbers but not detected by sampling or isolation technique used.

Contaminated meat meal was apparently the only source of salmonellae for the 74 test pigs. No isolations were made from the swine on negative meat meal, their feed, or environment. The same serotypes from swine on positive meat meal and their feed were isolated from meat meal.

**SUMMARY AND CONCLUSION**

Feed with low levels of salmonella organisms was found to be a source of salmonellae for 21.1% (8/38) of the swine. Infection in the MLN occurred in 15.8% (6/38) of the swine on positive feed.

Meat meal with low numbers of salmonellae was the apparent source of
infection. All 8 positive swine and the 3 positive feed samples had the same serotypes as the meat meal samples. The control group on negative meat meal was negative.

The results from this study indicate that salmonella-contaminated animal protein is an important source of salmonellosis in swine. This source may be eliminated by feeding negative animal protein. Heat treatment of rendered protein can eliminate salmonella contamination. A larger percentage of salmonella-negative swine may then be shipped to market or added to the breeding herd.
### TABLE 1. Distribution of Salmonella Serotypes in Nonheated Meat Meal, Feed, and Swine Fed the Meat Meal

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>MEAT MEAL</th>
<th>FEED</th>
<th>CAECUM</th>
<th>MLN</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. alachua</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. anatum</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. arkansas</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. bareilly</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. binza</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. bredensey</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>S. derby</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. cimabueettel</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. infantis</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. kentucky</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. manila</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. melanagridis</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. montevideo</td>
<td>32</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. oranienburg</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. portsmouth</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. saint-paul</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. senftenberg</td>
<td>6</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3, 10, 15:a, h-1, 6*</td>
<td>1</td>
<td></td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

**TOTAL ISOLATIONS** 68 3 2 6
**TOTAL SAMPLES POSITIVE** 40 3 2 6
**TOTAL SAMPLES** 40 70 38 38

*This atypical serotype differs from S. anatum in that it has the additional O factor 15.*
TABLE 2. MPN of Salmonella Organisms per 100 Grams of Nonheated Meat Meal

<table>
<thead>
<tr>
<th>Sample</th>
<th>MPN</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>&lt;0.5-7</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>5-46</td>
</tr>
<tr>
<td>3</td>
<td>&gt;1609*</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>&lt;0.5-7</td>
</tr>
<tr>
<td>6</td>
<td>1609</td>
<td>640-5800</td>
</tr>
<tr>
<td>7</td>
<td>.5</td>
<td>0.5-13</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>7-70</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>&lt;0.5-13</td>
</tr>
</tbody>
</table>

*This sample was positive for salmonellae in all dilutions and, thus exceeded the highest tabulated value. The second aliquot from sample 3 had a value of 2.
TABLE 3. Recovery of Salmonellae from Two Groups of Swine, and Their Environment During Growing and Finishing

<table>
<thead>
<tr>
<th></th>
<th>50 Swine on Salmonella-</th>
<th>50 Swine on Salmonella-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive Meat Meal Ration</td>
<td>Negative Meat Meal Ration</td>
</tr>
<tr>
<td>Rectal Swabs</td>
<td>0/350*</td>
<td>0/347**</td>
</tr>
<tr>
<td>Water</td>
<td>0/35</td>
<td>0/35</td>
</tr>
<tr>
<td>Floor</td>
<td>0/35</td>
<td>0/35</td>
</tr>
<tr>
<td>Feeder Lid</td>
<td>0/35</td>
<td>0/35</td>
</tr>
<tr>
<td>Swine Ration</td>
<td>3/70</td>
<td>0/70</td>
</tr>
</tbody>
</table>

*Positive Samples/Total Samples

**The dead pig not available for last 3 samplings
<table>
<thead>
<tr>
<th>Pen Contamination On Farm</th>
<th>0/10*</th>
<th>0/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Truck Before Loading</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Truckbed After Transport:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swabs</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Fecal Sample</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Rectal Swabs Before Loading</td>
<td>0/38</td>
<td>0/36</td>
</tr>
<tr>
<td>Rectal Swabs After Arrival</td>
<td>0/38</td>
<td>0/36</td>
</tr>
<tr>
<td>Bile Ducts</td>
<td>0/38</td>
<td>0/36</td>
</tr>
<tr>
<td>MLN</td>
<td>6/38</td>
<td>0/36</td>
</tr>
<tr>
<td>Caecum</td>
<td>2/38</td>
<td>0/36</td>
</tr>
</tbody>
</table>

*Positive Samples/Total Samples
### TABLE 5. Salmonella Isolations From 38 Swine on Positive Meat Meal Ration

<table>
<thead>
<tr>
<th>Pen No.</th>
<th>Swine</th>
<th>Location Positive</th>
<th>Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/6*</td>
<td>Caecum</td>
<td>S. bredeney</td>
</tr>
<tr>
<td>2</td>
<td>3/8</td>
<td>MLN</td>
<td>S. bredeney</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MLN</td>
<td>3,10,15:e, h-1,6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MLN</td>
<td>3,10,15:e, h-1,6</td>
</tr>
<tr>
<td>3</td>
<td>1/8</td>
<td>MLN</td>
<td>S. bredeney</td>
</tr>
<tr>
<td>4</td>
<td>1/8</td>
<td>Caecum</td>
<td>S. montevideo</td>
</tr>
<tr>
<td>5</td>
<td>2/8</td>
<td>MLN</td>
<td>S. bredeney</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MLN</td>
<td>S. bredeney</td>
</tr>
</tbody>
</table>

8/38

*Swine Positive/Total Swine Slaughtered
TABLE 6. Measurements of Growth and Feed Conversion for 103 Days During Growing and Finishing Period

<table>
<thead>
<tr>
<th></th>
<th>50 Pigs On Salmonella-Positive Meat Meal</th>
<th>49 Pigs On Salmonella-Negative Meat Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning Weight (12-16-70)</td>
<td>2,816 lbs. (Av. 56.3)</td>
<td>2,755 lbs. (Av. 56.2)</td>
</tr>
<tr>
<td>Final Weight (3-30-71)</td>
<td>10,870 lbs. (Av. 217.4)</td>
<td>10,701 lbs. (Av. 218.4)</td>
</tr>
<tr>
<td>Total Days</td>
<td>5,150</td>
<td>5,047</td>
</tr>
<tr>
<td>Total Feed</td>
<td>26,615 lbs.</td>
<td>26,770 lbs.</td>
</tr>
<tr>
<td>Average Daily Gain</td>
<td>1.56 lbs.</td>
<td>1.57 lbs.</td>
</tr>
<tr>
<td>Average Daily Feed</td>
<td>5.17 lbs.</td>
<td>5.30 lbs.</td>
</tr>
<tr>
<td>Feed/Pound of Gain</td>
<td>3.30 lbs.</td>
<td>3.37 lbs.</td>
</tr>
</tbody>
</table>
REFERENCES


STATUES OF THE COOPERATIVE STATE-FEDERAL SALMONELLA PROGRAM

by
John W. Walker, D.V.M., M.P.A.*
Saul T. Wilson, Jr., D.V.M., M.P.H.**
Claude J. Pfow, D.V.M.***
J. N. Allred, D.V.M., M.P.V.M.****

As the United States farm population declines year by year, the number of producing farms declines accordingly. For example, the farm population dwindled from over 30 million persons in 1940 to around 12 million by 1966 and less than 10 million (9,712,000) by 1970. The gap left by the declining farm population has been filled in recent years by the phenomenon in the United States society referred to as agri-business. It is not my purpose here to discuss the pros or cons of agri-business, but simply to remind you of its existence and the fact that agri-businesses have not only social and economic impacts but also influenced the program arena and strategies selected in combating or eradicating animal diseases.

Utilizing up-to-date sophisticated animal science technology in highly concentrated animal production centers, these organizations have revolutionized the production of food animals. Concurrent with the evolvement of these centers, such as modern egg factories and feeding lots, new disease problems arise which are associated with intensive concentration of food producing animal populations. One of the disease conditions frequently seen as an animal population problem is salmonellosis. There are over 1,300 Salmonella species, any one of which is potentially capable of producing salmonellosis in warm blooded or cold blooded animals. However, in the United States a relatively limited number of Salmonella serotypes are regularly isolated from domestic animal species or their environments.

For example, from 1967 to 1971 inclusive, the top six most frequently isolated Salmonella serotypes constituted 80, 64, 84, 81, and 63 percent of all serotypes isolated from species of horses, chickens, cattle, sheep, and turkeys respectively.
Two serotypes, *S. typhimurium* and *S. heidelberg*, were in the top six of all species and *S. saint-paul* occurred in the top six in four of the five species. Therefore, the control or even the eradication of all Salmonella serotypes from a selected animal or poultry population may involve the problem of one to a few serotypes rather than all 1,300 serotypes.

However, it is not my intention to discuss the eradication of salmonellosis from domestic animals today but to report on the Cooperative State-Federal Salmonella Program aimed at breaking only one link in the domestic animal Salmonella cycle; namely, the elimination of Salmonella from animal and marine protein intended for use in animal feeds.

The interest in Salmonella control is growing. A few countries in Northern Europe have regulations to control Salmonella in domestic animals as well as in feed ingredients. Many experiment stations throughout the world are testing various methods of prevention and treatment for salmonellosis. The increased public concern for environmental protection adds emphasis to the importance of programs designed to control and prevent salmonellosis in both humans and animals.

The Cooperative State-Federal Salmonella Program is designed to prevent and minimize Salmonella contamination in feed supplements of animal and marine origin while working to eliminate it from these two ingredients of livestock and poultry feeds. During fiscal year 1971, the Cooperative State-Federal Salmonella Program had several significant major achievements. These include:

1. Prepared and distributed the Temporary Uniform Methods and Rules for Salmonella Control in Marine Processing Plants.
2. Revised and updated laboratory procedures for the isolation of Salmonella organisms from animal feeds and feed ingredients.
3. Conducted a four-day seminar for Salmonella Program specialists at the National Animal Disease Laboratory, Ames, Iowa.
4. Conducted two five-day training schools in laboratory techniques for the isolation of Salmonella. A total of 30 industry, 1 State, and 1 Federal personnel attended.
5. States began official recognition of Phase III "Approved" plants.

The accomplishment of this objective is greatly dependent upon a management operated Salmonella control program being developed by each rendering plant and each industrial fish plant that is participating in the program. The plan of work calls for the program to be conducted on an individual plant basis in the following three phases: Phase I "Evaluation", Phase II "Cleanup", and Phase III "Approved".

From a program standpoint, each plant may move through each program phase as rapidly as test results for Salmonella are obtained if the product is not contaminated. If a plant is producing a Salmonella contaminated product, it is the plant management's responsibility to correct the problem in the plant. It is the responsibility of State or Federal inspectors to give technical recommendations to the plant management for correcting the problem.

A most important aspect in any rendering plant Salmonella control program is the plant's own inplant self-monitoring product testing program which gives a measure of the total Salmonella control in the plant.

**Measuring Program Progress**

From a State wide standpoint, the criteria used to measure program progress
are three:

(1) The number of plants that produce a clean product. We now call these plants Phase III “Approved” plants.

(2) Prevalence of Salmonella in the samples tested.

(3) Ability to maintain clean or Phase III “Approved” plants.

**Phase III “Approved” Plants Recognized**

Based on fiscal year 1971 inspections and tests, there are 10 plants in Phase I “Evaluation”, 602 plants in Phase II “Cleanup”, and 148 plants in Phase III “Approved”. This is the first full year of recognizing plants in Phase III “Approved” status. Program statistics, though not dramatic, are encouraging and do reflect well for the industry and the cooperating agencies, even though, the Salmonella program received a low priority again this year and many States did not conduct 3 inspections for 100 percent of their plants.

During fiscal year 1971 and into fiscal year 1972, increased program emphasis is being placed on Phase II “Cleanup” as a means of getting into the final or Phase III of the program. This is the Phase that requires going the extra mile. Now we start seeing the “unusual” case that seems to defy all known tactics. Now we must spend more time and energy looking for new and finer techniques. For the most part, the easy plants have been approved, but there remains the difficult ones. But each will be solved and with each solution we will feel greater accomplishment.

**Prevalence of Salmonella Reduced**

Statistics in Table 1 summarizes the results of Salmonella testing conducted for program purposes and the status of the program at the close of fiscal year 1971. Even though there was an overall decrease in the number of routine samples tested, environmental samples stayed at the same level as last year and both indicate a slight decrease in contamination rate. This is the second year that a 1 percent decrease in the contamination rate for the finished product was obtained. On the other hand, Table 2 shows a steady rise in the number and percent negative plants over the last three fiscal years.

There has been a decrease of 133 plants in the program during the year. The reasons for deletions from the program probably reflect attrition from the industry and inability of some plants to make the necessary improvements to meet the requirements of the program.

The serotypes identified from Salmonella cultures isolated from animal and marine by-products are shown in Appendix 1 and 2. We note that 9 of the top 10 were also in the top 10 last year and make up 50 percent of the cultures serotyped.

The listing 9998 in 7th place is the code for samples not typed due to loss of the culture.

**Ability to Maintain Approved Plants to be Tested**

Since fiscal year 1971 was the first year to officially recognize Phase III “Approved” plants in most States, fiscal year 1972 will provide an opportunity to show if this progress can be maintained under the third criteria for measuring program progress.

The rendering industry through its National Association, the National Renderers Association, has set a goal of achieving 75 percent of the rendering plants
in the United States as in Phase III "Approved" by the end of fiscal year 1975. In order to achieve this, the Salmonella program will need to be given a higher work priority in many States. A review of program statistics shows that those States which make the most consistent efforts to meet the SUMR of 3 inspections in negative plants and an average of 4½ inspections in contaminated plants have the best record of getting the contaminated plants to produce a negative product.

Beginning in fiscal year 1972 (this fiscal year) the Phase III “Approved” plants will be inspected and tested twice yearly with the resources saved being applied in the contaminated plants. This is expected to pay dividends in fiscal year 1972 because it will allow more manpower and laboratory resources to be used in cleaning up the Phase II plants.

The program is very rapidly reaching the peak of application, and we are hopeful that fiscal year 1972 will show an accomplishment of 22 percent or 200 plants reaching Phase III “Approved” from 148 and the prevalence of Salmonella in all samples tested reduced from 15 percent in fiscal year 1971 to 13 percent in fiscal year 1972.

Our serotyping laboratories, in addition to work done for the Cooperative State-Federal Salmonella Program identified 1,451 isolates from chickens; 1,776 from turkeys; 504 from cattle, 535 from swine; 1,603 from other domestic species and their feed and environment during fiscal year 1971 for local diagnostic laboratories.
Table 1. Number of samples tested for Salmonella in FYs 1969, 1970, and 1971 by type of sample, number and percent positive

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Fiscal Year 1969</th>
<th>Fiscal Year 1970</th>
<th>Fiscal Year 1971</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples Tested</td>
<td>Samples Tested</td>
<td>Samples Tested</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Total</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>Positive</td>
<td>Number</td>
</tr>
<tr>
<td>Environmental</td>
<td>7,821</td>
<td>1,353</td>
<td>3,337</td>
</tr>
<tr>
<td>In-Line</td>
<td>17.3</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>Routine Program</td>
<td>22,824</td>
<td>3,835</td>
<td>24,329</td>
</tr>
<tr>
<td></td>
<td>16.8</td>
<td>15.9</td>
<td>14.78</td>
</tr>
<tr>
<td>TOTAL</td>
<td>30,645</td>
<td>27,666</td>
<td>23,071</td>
</tr>
</tbody>
</table>

Table 2. Number and percent of plants that met the Salmonella Uniform Methods and Rules definition for a negative plant based upon the last three inspections at close of Fiscal Years 1969, 1970, and 1971.

<table>
<thead>
<tr>
<th>Type of Plant</th>
<th>Fiscal Year 1969</th>
<th>Fiscal Year 1970</th>
<th>Fiscal Year 1971</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plants</td>
<td>Plants</td>
<td>Plants</td>
</tr>
<tr>
<td></td>
<td>Total Represented</td>
<td>Negative* Number</td>
<td>Represented</td>
</tr>
<tr>
<td></td>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
</tr>
<tr>
<td>Protein Blender</td>
<td>11</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Livestock S1.</td>
<td>286</td>
<td>60</td>
<td>380</td>
</tr>
<tr>
<td>Poultry S1.</td>
<td>22</td>
<td>5</td>
<td>38</td>
</tr>
<tr>
<td>Independent Ren.</td>
<td>342</td>
<td>96</td>
<td>377</td>
</tr>
<tr>
<td>Feather Meal</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Marine Product</td>
<td>63</td>
<td>35</td>
<td>81</td>
</tr>
<tr>
<td>TOTAL</td>
<td>728</td>
<td>197</td>
<td>899</td>
</tr>
</tbody>
</table>

*A negative plant is one that has had three consecutive negative tests of 10 sample units (total 30 negative sample units) of an official sample of finished product. The sample units are collected from the shipping or storage area at an interval of not less than 30 days apart within a 12 month period.

**This compares with 148 plants that had been recommended for Phase III "Approved." Probably most of the others could be approved if they institute a self-monitoring inplant weekly testing program.
### FIGURE 1.

**PREVALENCE OF SALMONELLA IN FINISHED PRODUCT SAMPLES**

**A. SAMPLES COLLECTED IN ALL 766 PLANTS**

\[
\frac{\text{NUMBER SAMPLES POSITIVE}}{\text{TOTAL SAMPLES TESTED}} = \frac{2,909}{19,669} = 15\%
\]

**B. SAMPLES COLLECTED IN 466 POSITIVE PLANTS**

\[
\frac{\text{NUMBER SAMPLES POSITIVE}}{\text{TOTAL SAMPLES TESTED}} = \frac{2,909}{13,302} = 22\%
\]

\(^1\)ACTIVE DURING FISCAL YEAR 1971
The number of finished product samples tested and the number and percent positive for FY 1971 is presented in Fig. 2 and for FY 1970 in Fig. 3.

FIGURE 2.

Salmonella Program

<table>
<thead>
<tr>
<th>TYPE PLANT AND NO.</th>
<th>PERCENT POSITIVE SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLENDER [20]PLANTS</td>
<td><em>(540 Samples Tested)</em> 41%</td>
</tr>
<tr>
<td>LIVESTOCK SL. [341]</td>
<td><em>(8,241)</em> 16%</td>
</tr>
<tr>
<td>POULTRY SL. [42]</td>
<td><em>(1,145)</em> 11%</td>
</tr>
<tr>
<td>INDEPENDENT [306]</td>
<td><em>(8,547)</em> 14%</td>
</tr>
<tr>
<td>FEATHER MEAL [8]</td>
<td><em>(154)</em> 1%</td>
</tr>
<tr>
<td>MARINE [49]</td>
<td><em>(1,042)</em> 10%</td>
</tr>
</tbody>
</table>

ACTIVE DURING FISCAL YEAR 1971

Total samples tested: 19,669 (15%pos.)
Total plants represented: 766

SOURCE: STATE ANNUAL SUMMARY REPORTS

Finished product samples only

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
### TEST RESULTS BY TYPE & NUMBER PLANTS - FY 1970*

<table>
<thead>
<tr>
<th>Type</th>
<th>Plant and No.</th>
<th>Percent Positive Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLENDER</td>
<td>(16) Plants</td>
<td>52% (433 Samples Tested)</td>
</tr>
<tr>
<td>LIVESTOCK SL.</td>
<td>[378]</td>
<td>16% (10,390)</td>
</tr>
<tr>
<td>POULTRY SL.</td>
<td>[41]</td>
<td>16% (1,078)</td>
</tr>
<tr>
<td>INDEPENDENT</td>
<td>[352]</td>
<td>16% (10,421)</td>
</tr>
<tr>
<td>FEATHER MEAL</td>
<td>[8]</td>
<td>10% (281)</td>
</tr>
<tr>
<td>MARINE</td>
<td>[77]</td>
<td>8% (1,726)</td>
</tr>
</tbody>
</table>

Total samples tested: 24,329 (16% pos.)
Total plants represented: 872

*Finished product samples only

Source: State Annual Summary Reports
FIGURE 4.

SAMPLES OF ANIMAL AND MARINE PROTEIN INGREDIENTS TESTED POSITIVE
By Quarters

QUARTER: 1st 2nd 3rd 4th

FY 1968
25.7%
18.1%
11.8%

FY 1969
20.0%
17.7%
14.8%
14.5%

FY 1970
15.7%
17.9%
18.3%
15.4%

FY 1971
10.2%
15.7%
13.8%
11.4%

COOPERATIVE STATE-FEDERAL SALMONELLA PROGRAM
U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
The drop in number of cultures serotyped is primarily a reflection of shift in program emphasis and budget restrictions. Fig. 5.

FIGURE 5.

SALMONELLA SEROTYPING

ANH Division and Cooperating Laboratories

CULTURES SEROTYPED
THOUSANDS

15

10

5


U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
FIGURE 6.

SALMONELLA SEROTYPING BY ANH LABORATORIES

CULTURES SEROTYPED
THOUSANDS

6
5
4
3
2
1

FY '63 '64 '65 '66 '67 '68 '69 '70 '71

AMES
ATLANTA
PHOENIX
ORONO

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
### STATE-FEDERAL SALMONELLA PROGRAM

**APPENDIX I**

**SALMONELLA SEROTYPES ISOLATED FROM ANIMAL AND MARINE BYPRODUCTS DURING FISCAL YEAR 1971 (LISTED ALPHABETICALLY)**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Plant Category</th>
<th>Percent of Total Serotyped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td></td>
<td>11 12 13 14 20 30 Total</td>
</tr>
<tr>
<td>abony</td>
<td>1</td>
<td>1 1 0.04</td>
</tr>
<tr>
<td>almadi</td>
<td>6</td>
<td>6 0.29</td>
</tr>
<tr>
<td>alachua</td>
<td>20</td>
<td>22 1.08</td>
</tr>
<tr>
<td>alagbon</td>
<td>1</td>
<td>1 0.04</td>
</tr>
<tr>
<td>albany</td>
<td>1</td>
<td>1 0.04</td>
</tr>
<tr>
<td>amsterdam</td>
<td>2</td>
<td>1 1 0.19</td>
</tr>
<tr>
<td>anatum</td>
<td>97</td>
<td>4 2 136 6.71</td>
</tr>
<tr>
<td>arkansas</td>
<td>1</td>
<td>1 0.04</td>
</tr>
<tr>
<td>assen</td>
<td>1</td>
<td>3 0.14</td>
</tr>
<tr>
<td>baragwanath</td>
<td>6</td>
<td>1 1 0.04</td>
</tr>
<tr>
<td>bareilly</td>
<td>10</td>
<td>3 20 0.98</td>
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<tr>
<td>bareilly var 14</td>
<td>5</td>
<td>5 0.24</td>
</tr>
<tr>
<td>bere</td>
<td>2</td>
<td>2 0.09</td>
</tr>
<tr>
<td>binza</td>
<td>40</td>
<td>3 1 80 3.94</td>
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<td>blockley</td>
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<td>1 0.04</td>
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<td>bornum</td>
<td>2</td>
<td>1 2 7 0.34</td>
</tr>
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<td>bredeney</td>
<td>15</td>
<td>1 6 3 76 3.75</td>
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<td>1 1 15 0.74</td>
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<tr>
<td>cerro</td>
<td>17</td>
<td>1 2 1 41 2.02</td>
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<td>cubana</td>
<td>13</td>
<td>4 1 19 45 2.22</td>
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<tr>
<td>derby</td>
<td>15</td>
<td>37 52 2.56</td>
</tr>
<tr>
<td>drypool</td>
<td>11</td>
<td>2 3 34 1.67</td>
</tr>
<tr>
<td>eimsbuettel</td>
<td>51</td>
<td>67 1 3 129 6.36</td>
</tr>
<tr>
<td>enteritidis</td>
<td>4</td>
<td>4 0.19</td>
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<tr>
<td>fanti</td>
<td>1</td>
<td>2 0.14</td>
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<tr>
<td>give</td>
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<td>16 1 2 20 0.98</td>
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<td>2 0.09</td>
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<td>20</td>
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<td>irenea</td>
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<td>1 0.04</td>
</tr>
<tr>
<td>java</td>
<td>4</td>
<td>4 0.19</td>
</tr>
<tr>
<td>jericho</td>
<td>2</td>
<td>2 0.09</td>
</tr>
</tbody>
</table>
### Appendix 1 Continued

2 - Salmonella Serotypes Isolated from Animal and Marine Byproducts During Fiscal Year 1971 (Listed Alphabetically)

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Plant Category</th>
<th>Percent of Total Serotyped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Salmonella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>johannesburg</td>
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<td>4</td>
</tr>
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<td>19</td>
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<td>kottbus</td>
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<td>lexington</td>
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<td>oranienburg</td>
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<td>putten</td>
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<tr>
<td>schwarzengrund</td>
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</tr>
<tr>
<td>tennessee</td>
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</table>
### APPENDIX II

**SALMONELLA SEROTYPES ISOLATED FROM ANIMAL AND MARINE BYPRODUCTS DURING FISCAL YEAR 1971 (LISTED IN DESCENDING FREQUENCY)**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Plant Category</th>
<th>Percent of Total Serotyped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>senftenberg</td>
<td>105 79 1 1 26 18</td>
<td>230 11.35</td>
</tr>
<tr>
<td>anatum</td>
<td>97 33 4 2 136 6.71</td>
<td></td>
</tr>
<tr>
<td>einesbuettel</td>
<td>51 67 1 7 3</td>
<td>129 6.36</td>
</tr>
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APPENDIX I CONTINUED

3 - SALMONELLA SEROTYPES ISOLATED FROM ANIMAL AND MARINE BYPRODUCTS DURING FISCAL YEAR 1971 (LISTED ALPHABETICALLY)

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Total Isolates Serotyped 844 895 63 8 110 106 2026 99.41

PLANT CATEGORIES

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### APPENDIX II CONTINUED

#### 3 - SALMONELLA SEROTYPES ISOLATED FROM ANIMAL AND MARINE BYPRODUCTS DURING FISCAL YEAR 1971 (LISTED IN DESCENDING FREQUENCY)

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#### PLANT CATEGORIES

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The Salmonella Committee has reviewed the Uniform Methods and Rules for the elimination of Salmonella in animal by-products intended for use in animal feeds and feels revision is necessary. A proposed revision is included in this report for study by a sub-committee appointed by the chairman. Final action on this proposed revision will be taken at the 1972 committee meeting in Miami.

**PROPOSED UNIFORM METHODS AND RULES FOR THE ELIMINATION OF SALMONELLA IN ANIMAL BY-PRODUCTS INTENDED FOR USE IN ANIMAL FEEDS**

**Part I: Definitions**

A. *Animal Products* — Blood meal, meat by-products, meat meal, meat and bone meal, meat meal tankage, whale meal, animal liver meal, animal liver and glandular meal, extracted animal liver meal, animal by-product — processed, poultry by-product meal, poultry hatchery by-products, dried meat solubles, poultry parts, poultry by-products, hydrolyzed poultry feathers, fleshings hydrolysate, whole eviscerated chickens, hair hydrolyzed, feather meal hydrolyzed, or blended mixtures thereof, to be used in animal feeds.

B. The definition for products listed in paragraph A of this part shall be those published in the current edition of the Association of Feed Control Officials Manual.

C. *Lot* — A lot of animal product shall be the amount of product for a single shipment of a day's production, whichever is greater.

*D. Official Sample Unit* — One hundred (100) grams of animal product collected by a State or Federal inspector. An official sample unit shall be collected in the manner described under randomization in Part I so as to be representative of 1/10 of the contents of the total lot. Each sample unit shall be individually packaged and identified.

*E. Official Sample (Sampling)* — Ten (10) official sample units collected by a State or Federal inspector. The official samples shall be collected in the manner described under randomization in Part I so as to be representative of the contents of the total lot.

**F. Randomization** — True randomization in the case of sampling for salmonella in feed or rendered product insures that each portion of the feed or rendered product being samples has a known probability of appearing in the sample. This means that if we are sampling from 100 tons of feed that each small portion of the feed has a known chance of being sampled. This is necessary in order to have the highest possible chance of detecting contamination if present. This is due to the tendency of contamination to cluster rather than to be truly random in occurrence. In practice, we cannot...
obtain a truly random sample. However, we can come close by spreading the samples out evenly throughout the feed or rendered product which is of interest. When ten (10) official sample units are collected, each sample unit should be collected in a manner so as to be representative of 1/10 of the lot being sampled.

*G. Plant Sample — A random sample of a finished product, ready for shipment, collected by plant personnel in such a manner it represents the contents of a total lot.

*H. Positive Sample Unit — An official sample unit of an official sample from which Salmonella is recovered by an official laboratory.

*I. Negative Sample Unit — An official sample unit of an official sample from which no Salmonella is recovered by an official laboratory.

*J. Salmonella Test — The laboratory examination of an official sample unit of animal product, collected as outlined in D above, and tested by the procedures and methods recommended by the USAHA and the U.S. Department of Agriculture for cooperative program activities. (ARS 91-68)

*K. Rendering Establishment — An establishment that cooks and may otherwise process animal products as defined in A (this part) for use in animal feeds, whether or not operated in conjunction with a slaughtering or processing plant.

*L. Blending Establishment — An establishment that grinds, blends, mixes, or further processes animal, marine, or animal and marine protein for the purpose of making such proteins suitable for use in animal feeds. A blending establishment does not cook animal products.

*M. Plant Salmonella Control Program (Self monitoring) — The sum of methods and procedures employed by an establishment designed to prevent, detect and eliminate Salmonella contamination in its finished product, as outlined in the Recommended Sanitation Guidelines, and includes a test for Salmonella of a plant sample representative of not less than one of the lots produced each week.

**N. Phase I "Evaluation" — A program phase to designate a rendering or blending establishment undergoing official inspections and Salmonella tests of official samples to determine if the plant is producing Salmonella negative or Salmonella positive animal protein.

**O. Phase II "Cleanup" — A program phase to designate a rendering or blending establishment producing Salmonella positive animal protein but is participating in the Cooperative State-Federal Salmonella Program as described here in Part II (B).

**P. Phase III "Approved" — A program phase to designate a rendering or blending establishment that has met and/or is maintaining the requirements for this designation as described here in Part II (C).

Q. Finished Product — Rendered material, ready for shipment, collected in the shipping or storage area, and will undergo no further processing in plant where collected.
R. *Negative* — Salmonella not detected in the finished product when sampled at the rate and examined according to the procedures specified for the program.

**S. Official Inspection** — A plant inspection conducted by a State or Federal inspector.

**Part II: Recommended Procedures**

A. *Phase I “Evaluation”*

1. Objectives:
   a. Determine the presence or absence of Salmonella in detectable amounts in the finished product produced by each plant in the State.
   b. Determine the plants that are operating in compliance with the Recommended Sanitation Guidelines.
   c. Determine the plants that are not operating in compliance with the Recommended Sanitation Guidelines.

2. Classification of Plants:
   a. Plants shall be classified as positive or negative based upon the results of laboratory examination of official samples.
   *b. Positive classification* — A positive classification shall result from one or more positive official sample units in an official sample. Plant is placed in Phase II “Plant Improvement.”
   *c. Negative classification* — A negative classification shall result from a negative test of all official sample units in an official sample. Plants determined to have a negative classification remain in Phase II and may be designated as Phase III “Approved” or Phase II “Cleanup” as soon as qualified.

3. Procedure:
   Conduct not less than three official inspections of each plant at an interval of not less than 30 days apart and submit 10 official sample units collected during these inspections to an official laboratory for bacteriological examination. The sample units shall be collected during a period of not less than 60 days nor more than one year.

B. *Phase II “Plant Improvement”*

1. Objectives:
   a. Reduce the number of positive plants.
   b. Increase the number of negative plants that remain negative.

2. Procedures:
   a. Positive Plants
      (1) Conduct an extensive epidemiological study of each positive plant in an effort to identify the source or sources of contamination or potential contamination.
      (2) Make recommendations to management to eliminate areas of contamination or potential contamination.
      (3) Make recommendations to management for bringing operations into compliance with the Sanitation Guidelines.
(4) Assist plant in developing a plant Salmonella control program as related to Salmonella contamination.

*b. Negative Plants
(1) Official inspections and official samplings shall be conducted at intervals of not more than 120 days. Samples shall be collected in such a manner that 30 official sample units are collected each 12 month period and not more than 10 official sample units are collected on any one inspection.

(2) Make recommendations to management to eliminate areas of potential contamination.

(3) Make recommendations to management to bring operations into compliance with the Sanitation Guidelines.

*(4) Assist plant in developing a Salmonella quality control program as related to Salmonella contamination.

C. Phase III "Approved"
1. Objectives:
   a. To give recognition to plants that operate in compliance with the Sanitation Guidelines and demonstrate the capability of consistently producing a product negative for Salmonella when sampled and examined by the procedures recommended by the USAHA and the U.S. Department of Agriculture for cooperative program activities.
   b. To encourage management to attempt to maintain the status of negative plants.

2. Procedures for qualification:
   A plant may qualify for designation as an approved establishment provided:
   *a. It has had negative tests of 30 consecutive official sample units collected in not less than 60 days and not more than 10 official sample units were collected on any one inspection.
   b. It is operating in compliance with the Recommended Sanitation Guidelines (ARS 91-47), as verified by a written inspection report by a State or Federal inspector.
   *c. It has established a plant Salmonella control program which meets the approval of the cooperating State and Federal Animal Health officials.
   *d. The management applies for Phase III "Approved" status and approval is granted by the cooperating State and Federal Animal Health officials.

*3. Procedure for maintenance:
   a. A plant may maintain its approved status each fiscal year provided two official inspections are conducted and not less than 20 consecutive negative official sample units of 100 grams each are collected during a 12-month period and not more than 10 official sample units are collected on any one inspection. Each official inspection and sampling not less than 90 days apart.

* Rewritten
b. In the event a Phase III "Approved" plant has a positive test, official sample unit, the following procedures shall be followed:

(1) Follow Paragraph B-2, this Part, for positive plants in Phase II "Plant Improvement." To retain Phase III "Approved" status:

(2) Within 90 days, 1 official sampling consisting of 10 negative official sample units weighing not less than 100 grams and selected at random from each 1/10 section of a lot.

*c. The plant maintains a Salmonella Quality Control Program which meets the approval of the cooperating State and Federal Animal Health officials. Inspector examines self monitoring records (culture results)

Uniform Methods and Rules for the elimination of Salmonella in meals derived from raw materials of marine origin were prepared and approved by the Committee as an addendum to the present Uniform Methods and Rules for the elimination of Salmonella in animal by-products intended for use in animal feeds. The Committee felt this was necessary as the present Uniform Methods and Rules are not applicable to all aspects of marine meal plants. They are as follows:

UNIFORM METHODS AND RULES FOR ELIMINATION OF SALMONELLA IN MEALS DERIVED FROM RAW MATERIALS OF MARINE ORIGIN

Part 1: Definitions:

A. Marine Products: Fish Meal, Crab Meal, Shrimp Meal, Fish Liver and Glandular Meal, Fish Protein Concentrate-Feed Grade, or blended mixture thereof, to be used in animal feeds.

B. The definition for products listed in Paragaph A of this part shall be those published in current edition of the Association of Feed Control Officials Manual.

C. Lot: A lot of marine product shall be the amount of product for a single shipment, or a day’s production, whichever is greater.

D. Official Sample: A representative sample of a finished product, ready for shipment, collected by a State or Federal inspector, in such a manner that it represents the contents of a total lot (the recommended sample size is given in Paragraph F).

E. Plant Sample: Same as D., above, except collected by plant personnel.

F. Recommended Sample Size: The recommended sample shall consist of ten sample units (sub-samples) weighing not less than 100 grams each, representative of each 1/10 section of the lot. Each sample unit shall be individually packaged and identified.

G. Sampling Procedure By State or Federal Inspectors: State or Federal inspectors are to collect one Official Sample as defined in F., above, on each of the three or more yearly inspection visits made to the plant.

H. Positive Sample: A sample unit of an Official Sample from which Salmonella is recovered by an official laboratory.

* Rewritten
I. Negative Sample: A sample unit of an Official Sample from which no Salmonella is recovered by an official laboratory.

J. Salmonella Tested: The laboratory examination of an Official Sample collected as in D. and F. and G., above, and tested by the procedure of the U.S. Department of Agriculture designated as ARS 91-68.

K. Marine Product Producing Establishment: An establishment that processes marine products into meals as defined in A., above, for use in animal feeds.

Chairman: A. A. Erdmann, Madison, Wisc.
Co-Chairman: W. E. Lyle, Madison, Wisc.

Concern was expressed to the Salmonella Committee by Industry representatives that not all participating State and Federal laboratories were following the minimum standards as prescribed in ARS 91-68. In view of this, the Committee recommends the Animal Health Division Regional Poultry Epidemiologists, in cooperation with appropriate state officials, make an effort to determine the uniformity of Salmonella testing by participating State or Federal laboratories as described in ARS 91-68.
The Animal Health Division has compiled Salmonellae serotyping statistics from Agricultural sources from the period of Fiscal year 1967 - 1971 as requested by this committee. Similar statistics will be published in future years for comparison purposes. They are detailed as follows:

**MOST FREQUENT SALMONELLA SEROTYPES ISOLATED FROM ANIMAL AND MARINE PROTEINS FY 1967 – 1971**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. senftenberg</td>
<td>1,123</td>
<td>10.07</td>
</tr>
<tr>
<td>S. montevideo</td>
<td>1,050</td>
<td>9.41</td>
</tr>
<tr>
<td>S. eimbsuettel</td>
<td>963</td>
<td>8.63</td>
</tr>
<tr>
<td>S. anatum</td>
<td>728</td>
<td>6.53</td>
</tr>
<tr>
<td>S. oranienburg</td>
<td>527</td>
<td>4.72</td>
</tr>
<tr>
<td>S. derby</td>
<td>438</td>
<td>3.92</td>
</tr>
<tr>
<td>S. infantis</td>
<td>437</td>
<td>3.91</td>
</tr>
<tr>
<td>S. binza</td>
<td>398</td>
<td>3.57</td>
</tr>
<tr>
<td>S. bredeney</td>
<td>397</td>
<td>3.56</td>
</tr>
<tr>
<td>S. thomasville</td>
<td>357</td>
<td>3.20</td>
</tr>
<tr>
<td>S. tennessee</td>
<td>338</td>
<td>3.03</td>
</tr>
<tr>
<td>S. cerro</td>
<td>275</td>
<td>2.46</td>
</tr>
<tr>
<td>S. schwarzengrund</td>
<td>275</td>
<td>2.46</td>
</tr>
<tr>
<td>S. worthington</td>
<td>257</td>
<td>2.30</td>
</tr>
<tr>
<td>S. livingston</td>
<td>246</td>
<td>2.20</td>
</tr>
<tr>
<td>S. cubana</td>
<td>217</td>
<td>1.94</td>
</tr>
<tr>
<td>S. minnesota</td>
<td>217</td>
<td>1.94</td>
</tr>
<tr>
<td>S. bareilly</td>
<td>211</td>
<td>1.89</td>
</tr>
<tr>
<td>S. kentucky</td>
<td>182</td>
<td>1.63</td>
</tr>
<tr>
<td>S. typhimurium*</td>
<td>181</td>
<td>1.62</td>
</tr>
</tbody>
</table>

**Subtotal** 8,817 79%

**Total** 11,148

*Includes var. copenhagen

From ANH Form 9-10
REPORT OF THE COMMITTEE

NUMBER OF DIFFERENT SALMONELLA SEROTYPES ISOLATED FROM SELECTED SOURCES
BY 1967 - 1971

<table>
<thead>
<tr>
<th>Animal Type</th>
<th>No. of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHICKENS</td>
<td>25</td>
</tr>
<tr>
<td>TURKEYS</td>
<td>58</td>
</tr>
<tr>
<td>SHEEP</td>
<td>53</td>
</tr>
<tr>
<td>CATTLE</td>
<td>35</td>
</tr>
<tr>
<td>ANIMAL AND MARINE BYPRODUCTS</td>
<td>108</td>
</tr>
</tbody>
</table>

ANIMAL SPECIES DATA FROM ANH FORM 10-3
ANIMAL AND MARINE PRODUCTS DATA FROM ANH FORM 9-10

NUMBER OF SALMONELLA ISOLATES FROM FOOD ANIMAL AND ALL OTHER SOURCES SEROTYPED FOR DIAGNOSTIC PURPOSES - BY 1967-1971

<table>
<thead>
<tr>
<th>ANIMALS</th>
<th>No. of Isolates</th>
<th>Percent of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHICKENS</td>
<td>8,048</td>
<td>20.9</td>
</tr>
<tr>
<td>TURKEYS</td>
<td>8,127</td>
<td>21.2</td>
</tr>
<tr>
<td>CATTLE</td>
<td>1,704</td>
<td>4.4</td>
</tr>
<tr>
<td>SHEEP</td>
<td>3,179</td>
<td>8.3</td>
</tr>
<tr>
<td>SHEEP</td>
<td>94</td>
<td>.2</td>
</tr>
<tr>
<td>ALL OTHER SOURCES</td>
<td>17,231</td>
<td>45.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>38,143</td>
<td>100.0</td>
</tr>
</tbody>
</table>

From ANH Form 10-3
SALMONELLA

THE NUMBER OF SALMONELLA ISOLATES FROM COMPANION ANIMALS SEROTyped FOR DIAGNOSTIC PURPOSES - FY 1967-1971

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>NO. OF ISOLATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HORSE</td>
<td>297</td>
</tr>
<tr>
<td>DOG</td>
<td>159</td>
</tr>
<tr>
<td>CAT</td>
<td>56</td>
</tr>
</tbody>
</table>

TOTAL 512
From ANH Form 10-3

SEROTYPES OF MOST FREQUENTLY REPORTED SALMONELLA ISOLATES FROM HORSES FY 1967-1971

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>NUMBER</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. TYPHIMURIUM*</td>
<td>190</td>
<td>61.68</td>
</tr>
<tr>
<td>S. HEIDELBERG</td>
<td>21</td>
<td>6.81</td>
</tr>
<tr>
<td>S. NEWPORT</td>
<td>12</td>
<td>3.89</td>
</tr>
<tr>
<td>S. SAINT-PAUL</td>
<td>9</td>
<td>2.92</td>
</tr>
<tr>
<td>S. ORANIEBURG</td>
<td>7</td>
<td>2.27</td>
</tr>
<tr>
<td>S. ANATUM</td>
<td>7</td>
<td>2.27</td>
</tr>
</tbody>
</table>

SUBLTOTAL 246 60%

TOTAL 308

*Includes S. TYPHIMURIUM VAR. COPENHAGEN

From ANH Form 10-3
REPORT OF THE COMMITTEE

**MOST FREQUENTLY REPORTED SALMONELLA ISOLATES FROM CHICKENS** FY 1967-1971

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>NUMBER</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. TYPHIMURIUM</td>
<td>1,409</td>
<td>17.47</td>
</tr>
<tr>
<td>S. HEIDELBERG</td>
<td>1,333</td>
<td>16.53</td>
</tr>
<tr>
<td>S. TIBORON</td>
<td>720</td>
<td>8.92</td>
</tr>
<tr>
<td>S. INFANTIS</td>
<td>701</td>
<td>8.69</td>
</tr>
<tr>
<td>S. BLOCKLEY</td>
<td>601</td>
<td>7.45</td>
</tr>
<tr>
<td>S. SAINT-PAUL</td>
<td>405</td>
<td>5.02</td>
</tr>
<tr>
<td><strong>SUBTOTAL</strong></td>
<td>5,169</td>
<td>64 %</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>8,064</td>
<td></td>
</tr>
</tbody>
</table>

INCLUDES S. TYPHIMURIUM VAR. COPENHAGEN

**SEROTYPES OF MOST FREQUENTLY REPORTED SALMONELLA ISOLATIONS FROM CATTLE** FY 1967-1971

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>NUMBER</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. TYPHIMURIUM</td>
<td>1,972</td>
<td>63.05</td>
</tr>
<tr>
<td>S. DUBLIN</td>
<td>147</td>
<td>8.64</td>
</tr>
<tr>
<td>S. NEWPORT</td>
<td>128</td>
<td>7.52</td>
</tr>
<tr>
<td>S. SAINT-PAUL</td>
<td>46</td>
<td>2.70</td>
</tr>
<tr>
<td>S. MARY</td>
<td>44</td>
<td>2.58</td>
</tr>
<tr>
<td>S. HEIDELBERG</td>
<td>43</td>
<td>2.52</td>
</tr>
<tr>
<td><strong>SUBTOTAL</strong></td>
<td>1,460</td>
<td>85 %</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>1,730</td>
<td></td>
</tr>
</tbody>
</table>

INCLUDES S. TYPHIMURIUM VAR. COPENHAGEN

*From A'H Form 10-3*
<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arizona 26:30</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Arizona 26:29,30</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>S. newport</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Untypable Group B</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>82</strong></td>
<td><strong>81%</strong></td>
</tr>
</tbody>
</table>

Total 101

*Includes S. typhimurium var. Copenhagen

From AH Form 10-3
REPORT OF THE COMMITTEE

TEN MOST FREQUENTLY REPORTED SALMONELLA ISOLATES FROM HAN IN THE UNITED STATES
FY 1967 - 1970

<table>
<thead>
<tr>
<th>SEROTYPES</th>
<th>NUMBER</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. TYPHIMURIUM*</td>
<td>22,555</td>
<td>26.97</td>
</tr>
<tr>
<td>S. ENTERIDITIS</td>
<td>7,999</td>
<td>8.82</td>
</tr>
<tr>
<td>S. HEIDELBERG</td>
<td>6,101</td>
<td>7.16</td>
</tr>
<tr>
<td>S. NEWPORT</td>
<td>5,822</td>
<td>6.94</td>
</tr>
<tr>
<td>S. INFANTS</td>
<td>4,235</td>
<td>4.97</td>
</tr>
<tr>
<td>S. SAINT-PAUL</td>
<td>4,193</td>
<td>4.92</td>
</tr>
<tr>
<td>S. THOMPSON</td>
<td>3,185</td>
<td>3.75</td>
</tr>
<tr>
<td>S. TYPHI</td>
<td>2,381</td>
<td>2.79</td>
</tr>
<tr>
<td>S. BLODGEY</td>
<td>2,171</td>
<td>2.55</td>
</tr>
<tr>
<td>S. JAVIANA</td>
<td>1,573</td>
<td>1.84</td>
</tr>
</tbody>
</table>

Subtotal       | 60,135 | 71 %    |

Total          | 85,092 |

From Salmonella Surveillance Reports,
Center for Disease Control, USPHS

TEN MOST FREQUENTLY REPORTED SALMONELLA ISOLATES FROM TURKEYS FY 1967-1971

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>NUMBER</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. HEIDELBERG</td>
<td>1,880</td>
<td>23.28</td>
</tr>
<tr>
<td>S. SAINT-PAUL</td>
<td>1,195</td>
<td>14.79</td>
</tr>
<tr>
<td>S. TYPHIMURIUM*</td>
<td>686</td>
<td>8.49</td>
</tr>
<tr>
<td>S. ANATRA</td>
<td>474</td>
<td>5.86</td>
</tr>
<tr>
<td>S. SAN-DIEGO</td>
<td>460</td>
<td>5.57</td>
</tr>
<tr>
<td>S. SENTIENTBERG</td>
<td>399</td>
<td>4.94</td>
</tr>
</tbody>
</table>

Subtotal       | 5,084  | 63 %    |

Total          | 8,075  |

*Includes S. TYPHIMURIUM VAR. COPENHAGEN

From Table 19-3
The Committee has reviewed the report of official program inspections and urge that many states need to increase the frequency of inspections to maintain minimum program standards. The following is a listing of inspections by states:

### FISCAL YEAR 1971 --- RENDERING PLANT INSPECTIONS

**PER CENT OF EXPECTED INSPECTIONS**

<table>
<thead>
<tr>
<th>EASTERN AREA</th>
<th>ACCOMPLISHED AT</th>
<th>ACCOMPLISHED AT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>THREE INSP./YR*</td>
<td>FOUR &amp; 1/2 INSP./YR*</td>
</tr>
<tr>
<td>Connecticut</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Kentucky</td>
<td>160</td>
<td>110</td>
</tr>
<tr>
<td>Maine (NH)</td>
<td>Ma. (177) - NH (67)</td>
<td>Ma. (118) - NH (50)</td>
</tr>
<tr>
<td>Mass. (RI)</td>
<td>Mass. (166) - RI (166)</td>
<td>Mass. (105) - RI (111)</td>
</tr>
<tr>
<td>Michigan</td>
<td>97</td>
<td>66</td>
</tr>
<tr>
<td>New Jersey</td>
<td>222</td>
<td>149</td>
</tr>
<tr>
<td>New York</td>
<td>148</td>
<td>109</td>
</tr>
<tr>
<td>Ohio</td>
<td>137</td>
<td>92</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>58</td>
<td>39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NORTH CENTRAL AREA</th>
<th>ACCOMPLISHED AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illinois</td>
<td>202</td>
</tr>
<tr>
<td>Indiana</td>
<td>101</td>
</tr>
<tr>
<td>Des Moines, Iowa</td>
<td>89</td>
</tr>
<tr>
<td>Kansas</td>
<td>89</td>
</tr>
<tr>
<td>Minnesota</td>
<td>312</td>
</tr>
<tr>
<td>Missouri</td>
<td>111</td>
</tr>
<tr>
<td>Montana</td>
<td>93</td>
</tr>
<tr>
<td>Nebraska</td>
<td>119</td>
</tr>
<tr>
<td>North Dakota</td>
<td>125</td>
</tr>
<tr>
<td>South Dakota</td>
<td>143</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>94</td>
</tr>
<tr>
<td>Wyoming</td>
<td>133</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SOUTH CENTRAL AREA</th>
<th>ACCOMPLISHED AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>194</td>
</tr>
<tr>
<td>Arkansas</td>
<td>102</td>
</tr>
<tr>
<td>Delaware</td>
<td>33</td>
</tr>
<tr>
<td>Florida</td>
<td>133</td>
</tr>
<tr>
<td>Georgia</td>
<td>98</td>
</tr>
<tr>
<td>Louisiana</td>
<td>118</td>
</tr>
<tr>
<td>Maryland</td>
<td>188</td>
</tr>
<tr>
<td>Mississippi</td>
<td>50</td>
</tr>
<tr>
<td>North Carolina</td>
<td>63</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>50</td>
</tr>
<tr>
<td>South Carolina</td>
<td>140</td>
</tr>
<tr>
<td>Tennessee</td>
<td>58</td>
</tr>
<tr>
<td>Virginia</td>
<td>139</td>
</tr>
</tbody>
</table>

(Continued on next page)
REPORT OF THE COMMITTEE

FISCAL YEAR 1971 -- RENDERING PLANT INSPECTIONS
PER CENT OF EXPECTED INSPECTIONS

<table>
<thead>
<tr>
<th>WESTERN AREA</th>
<th>ACCOMPLISHED AT THREE INS.P./YR*</th>
<th>ACCOMPLISHED AT FOUR &amp; 1/2 INS.P./YR**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaska</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Arizona</td>
<td>206</td>
<td>137</td>
</tr>
<tr>
<td>California</td>
<td>114</td>
<td>76</td>
</tr>
<tr>
<td>Colorado</td>
<td>77</td>
<td>51</td>
</tr>
<tr>
<td>Hawaii</td>
<td>100</td>
<td>67</td>
</tr>
<tr>
<td>Idaho</td>
<td>158</td>
<td>105</td>
</tr>
<tr>
<td>New Mexico</td>
<td>42</td>
<td>28</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>103</td>
<td>69</td>
</tr>
<tr>
<td>Oregon</td>
<td>126</td>
<td>84</td>
</tr>
<tr>
<td>Texas, Austin</td>
<td>53</td>
<td>35</td>
</tr>
<tr>
<td>Utah</td>
<td>100</td>
<td>61</td>
</tr>
<tr>
<td>Washington</td>
<td>119</td>
<td>79</td>
</tr>
<tr>
<td>Nevada</td>
<td>167</td>
<td>115</td>
</tr>
</tbody>
</table>

* All States with values of less than 100 did not make the equivalent of 3 inspections per plant.

** All States with values of less than 100 did not make the equivalent of 4.5 inspections per plant.
The Salmonellosis committee recommends that animal and fish by-products firms, particularly those in Phase III of the Cooperative State-Federal Salmonella Program, consider voluntary use of a rail car inspection system for those empty cars spotted for the firm's loading of animal or fish protein. A short checklist or inspection form could be used by the firm to record the condition of the car at the time of loading. A system such as this would provide a record of trends of conditions of carrier cars and this information on a systematic basis could serve as a gauge to evaluate progress made due to the mutual efforts of the railroads, the by-products firms, and the government agencies involved. An example of such a form that could be utilized is as follows:

<table>
<thead>
<tr>
<th>Car#</th>
<th>Boxcar</th>
<th>Hopper</th>
<th>Plant</th>
<th>Date: Requested</th>
<th>Placed</th>
<th>Loaded:</th>
<th>Bag</th>
<th>Bulk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SANITATION CONDITION**

1. Presence of insects? Yes No
2. Presence of residue? Yes No
3. Residue sample retained? Yes No
4. Car fumigated? Yes No
5. Car generally clean? Yes No
6. Car interior dry? Yes No

**PHYSICAL CONDITION**

1. Car Accepted? If not, give reasons.
2. General physical condition?
3. Floor - construction - condition.
5. Endwalls - construction - condition.
7. Roof - construction - condition.
8. Does car leak?
9. Repairs made? Detail and give materials used, their cost, hours labor used, and its cost.
10. Detail what had to be done (other than repairs) to prepare car for shipment. (Use reverse side if necessary.)

General remarks: (Use reverse side if necessary.)

Inspected by: __________________________
Date: __________________________
SHOULD TUBERCULOSIS BE ERADICATED FROM ALL SPECIES

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Michigan State University

In 1960, W. H. Feldman stated “It is obvious that eradication of tuberculosis, whether in man or animal, can be achieved only after the detection of all infected hosts. Persons with active tuberculosis should be isolated, animals found to be infected must be destroyed; and means should be employed to prevent the resurgence of the disease from obscure or hidden sources. The possible existence of latent avian tuberculosis infections in swine, sheep, and other species should be kept in mind. Latent infections may become activated and provide sources of contagion to other susceptible animals.”

At the present, efforts toward eradication of tuberculosis is primarily if not entirely, limited to Mycobacterium tuberculosis in man and M. bovis in cattle. Both programs are working successfully. In fact, so successful that many people believe that the disease in cattle is eradicated and the incidence in man is so low, the programs of eradication now in effect should be reduced materially.

It is not the intention of this paper to discuss present eradication programs but to present reasons why these programs or other programs which may be evolved should be applied to the eradication of tuberculosis in other species of animals and should include the eradication of tuberculosis caused by M. avium and possibly M. intracellulare. It is also the intent of this paper to present reasons why such programs should elicit the joint efforts of animal disease control agencies and public health agencies.

The disease in cattle caused by M. bovis was a major public health problem when this organism was transmitted to man in milk from infected cows. In my generation, most people of age 21 either had tuberculosis or had had tuberculosis. A large percentage of the cases were due to M. bovis. The eradication program of tuberculosis in cattle, started in 1917, and the pasteurization of milk both played an important role in reducing M. bovis infection in man. Only a few cases of M. bovis infection in man are reported in the United States in recent years. In our research laboratory in the past two years, we have identified the causative agent from three cases of human tuberculosis as M. bovis. In two cases the cultures were sent from a hospital laboratory in Michigan for identification and in the third case, body fluids were submitted. There may be more cases of M. bovis infection in man that are not being detected but the number of cases would still be very low. Certainly M. bovis infections in man are no longer a public health problem. However, areas of infection in other species of animals exist that are potential sources of infection to man and cattle. These reservoirs of infection must be eliminated if eradication is to be complete.

An example of one of the reservoirs is a roadside deer park in Michigan. Towar et al. in a period of six months removed 52 tuberculin positive deer from a herd of 220. From some of the twenty-three deer which had generalized tuberculosis, M.
bovis was isolated. Most of these had extensive lung lesions and undoubtedly were discharging *M. bovis* from the nose and mouth. Visitors bought grain at the park to hand-feed the deer. Did any of these people exposed to *M. bovis* develop tuberculosis? No data are available.

Two hundred or more deer from the same park were sold to deer parks in other states as well as Michigan. There are no regulations concerning the testing of such animals and there are no restrictions in shipping these animals intrastate or interstate.

Zoological parks frequently have animals infected with tuberculosis caused by *M. tuberculosis*, *M. bovis*, and *M. avium*. An eland herd in a city zoo was badly infected with *M. bovis*. Two animals were brought to our research laboratory for post-mortem. Both had generalized disease with marked lung involvement and *M. bovis* was isolated. Animal from this herd were sold or traded for other animals in other zoos. There are no legal restrictions if they were born at the zoo. How many cases of *M. bovis* infection in the caretakers and zoo visitors came from these elands and other infected animals? No data are available.

In each situation a reservoir of infection exists. Should these infected deer parks and zoos be permitted to have infected animals when eradication programs in operation on man and cattle are approaching completion?

In 1970, 889,427 swine were retained at slaughter for tuberculosis. This represents 1.09% of all swine slaughtered in federal-inspected plants in the United States. Probably only a relatively small number of these swine were infected with *M. Bovis*. The incidence of *M. Bovis* infection in swine has fallen with the marked reduction of *M. Bovis* infected cattle because cattle were the primary source of the organism.

Two organisms, namely *M. avium* and *M. intracellulare* are responsible for most of the tuberculous lesions in swine. Over a period of six months, our laboratory obtained lesions from swine slaughtered in Detroit packing plants. Fifteen cases were due to *M. intracellulare* (Runyon Group III), three were due to *M. avium* and 25 were due to mycobacteria that produced only microscopic lesions in chickens. If the latter group were classified as *M. avium* than the ratio of *M. avium* to *M. intracellulare* would be 1.8 to 1.

The swine received at the Detroit packing plants came from Illinois, Ohio, Indiana, Missouri and Michigan. The swine thus represented a cross-section of the swine producing area of the United States. Although the number of cases tested is small, the ratio of 2 cases due to *M. avium* to one case of *M. intracellulare* may be a fair representation.

Of the 889,427 swine retained because of lesions typical of tuberculosis, only 4350 were condemned. The remainder (99.48%) passed through inspection without restrictions after removal of parts containing lesions.

The swine producers do not consider the losses due to tuberculosis of economic value. If tuberculosis in swine due to *M. avium* and possibly *M. intracellulare* is not an economic problem to swine producers then the only avenue to eradication is through health hazard to man.

In a field study of swine tuberculosis, Dr. George Winegar, a member of our tuberculosis research group, found 27 herds of swine in Michigan infected with *M. avium*. At each farm, an infected flock of chickens was found. On one farm, in a flock of 120 chickens, 16 of 27 were tuberculin-positive and 6 out of 8 of these had generalized tuberculosis. Other farm animals were tuberculin-positive. Two of 12
swine, one of 3 horses, one of 3 goats and one of 3 calves were tuberculin-positive. In a flock of 70 sheep, 17 were tuberculin tested and 10 were positive. Two sheep were brought to the laboratory for post-mortem examination. Both had lesions. Five of 42 sparrows caught in the chicken house had lesions. *M. avium* was isolated. In the family of five, the father and son were positive to avian tuberculin but negative to mammalian tuberculin.

At two other farms, three people reacted to avian tuberculin at each farm. At another farm, one person reacted to avian tuberculin.

Under these circumstances, one would assume because none of these people reacted to mammalian tuberculin that they were infected by *M. avium* even though the tuberculin test is not that specific.

It is difficult to know the real incidence of tuberculosis in chickens in the United States. The only information available is the incidence of tuberculosis in mature birds slaughtered in plants under federal inspection. In 1970, 129,656 (.08%) chickens were condemned for tuberculosis. In the west-north central states, 0.3% of the mature birds were condemned for tuberculosis.

Tuberculosis in chickens is largely confined today to small farm flocks. The number of such flocks that are infected with tuberculosis is purely conjectural. Our experience with flocks on farms with swine would indicate that the frequency of infection may be relatively high.

Shortly after the discovery of the etiological cause of tuberculosis in chickens in 1892 by Maffucci4, Kruse5 claimed to have isolated *M. avium* from human tissues. Koch in 19016 in his characteristically finality of judgement stated that avian tuberculosis differed widely from human tuberculosis and need not be considered as a source of tuberculosis in man.

The literature is sprinkled with reports of avian tuberculosis in man but in most instances the identification of the cultures was incomplete. Tuberculosis authorities believe that avian tuberculosis in man is a rarity. Feldman7 in 1938 states “When one considers the countless thousands of instances in which the type of tubercle bacillus in human infections have been determined since the time of Koch, it may seem strange that the avian tubercle bacillus has been demonstrated so rarely. The explanation can only be that human beings have a formidable resistance to this particular form of the tubercle bacillus. The opportunities for infection are numerous and, if the avian tubercle bacillus were virulent in the accepted sense for human beings, it would seem reasonable to expect a far greater number of proved cases. Apparently, therefore, it is only the exceptional individual who becomes infected with the avian tubercle bacillus.”

Since 1960, identification of the avian tubercle bacillus has been by animal inoculation of guinea pigs, rabbits and chickens and/or serotyping. A literature review reveals 34 articles on avian and atypical mycobacteria tuberculosis in man8-41. Approximately 150 cases are reported. Karlson8 and Jahn10 report the isolation of avian tubercle bacillus in association of silicosis in man. Engbaeck26 in Denmark reports that three members of a family of five died of avian tuberculosis within a year. Marks and Birn22 report that 10 of 17 cultures isolated from man were *M. avian*. Bradbury and Young29 report on case of pulmonary tuberculosis due to *M. avium*. Kubin et al.32 report nine cases of avian infection with pulmonary, extra pulmonary and systemic disease. Schaefer34 serotyped 474 cultures obtained from human patients in North America, Netherlands, West...
SHOULD TUBERCULOSIS BE ERADICATED

Australia, Wales, Denmark and Czechoslovakia. Eighty cultures were *M. avium*. From North America, of the 297 strains, 29 were *M. avium*.

In the United States, few clinical laboratories run identification for *M. avium*. Most laboratories would more likely report a case of avian tuberculosis as due to a "atypical" or Group III mycobacterium.

We have no idea how many cases of avian tuberculosis might be found in the United States if examinations for Runyon Group organisms were extended beyond drug resistance to serotyping and/or animal inoculations. We do know that a surprisingly large number of cases are due to *M. intracellulare*. Inasmuch as *M. avium* and *M. intracellulare* are closely related, many of these organisms may be *M. avium*.

Runyon in 1971 states "*Mycobacterium avium* was previously considered to be a cause of disease in man only rarely. A recently published text states categorically that *M. avium* is completely avirulent for man. It is not well established however, that probably the commonest mycobacterial infection of man in many areas are with organisms of the Battey, *avium* and *scrofulaceum* species. Although, only a small percentage of the infected become diseased, Battey disease in our southeastern states, in parts of Australia and in several other countries is a serious and important medical problem. The mycobacteria involved may include serotypes of both *M. intracellulare* and *M. avium*.”

As previously stated, 885,427 carcasses of swine with tubercular lesions were passed without restriction, except for removal of parts with tubercular lesions. Are the tissues of these carcasses free of tubercle bacilli?

In 1909, Rievel isolated tubercle bacilli from lymph nodes supposedly free of tubercle lesions from cattle and swine with generalized tuberculosis. In 1913, Nieberle extracted juice from muscle adjacent to lymph nodes of tuberculous cattle. The tubercle bacillus was found in the muscle juice of three of 50 cattle. The lymph nodes showed small tubercle lesions. Feldman found the tubercle bacillus in lymph nodes of swine free of lesions. Lymph nodes with lesions had been removed. Feldman states "This study should emphasize anew the importance of the problem of avian tuberculosis and should indicate that virulent tubercle bacilli may still be present in the carcasses of hogs even though all visible lesions were removed."

Pierrotti and Bracca in 1966 found lesions in the hepatic and cava veins of tuberculous cows. Tison et al. in 1966 isolated *M. bovis* frequently from glands and muscle of tuberculous cows. We have isolated *M. bovis* from the lymph nodes of a non-visible lesion reactor from a herd with a history of tuberculosis. We have frequently isolated Runyon Group III mycobacteria from lymph nodes of tuberculin-positive cattle with no gross lesions. These cattle passed inspection at slaughter without any restrictions.

Tubercle bacilli have been isolated from tuberculous swine. Nassal in 1965 found mycobacteria in the muscles of 79 of 100 swine with tuberculose mesenteric lymph nodes. Bergman and Gotze in 1966 isolated *M. avium* from skeletal muscles of three of 107 swine with tuberculous mesenteric lymph nodes. Seeger and Schack-Steffenhagen in 1969 isolated mycobacteria from liver, lymph nodes and muscle tissue of 333 swine with tuberculous mesenteric lymph nodes and 100 swine with no tuberculous lymph nodes.

In our laboratory, we found Runyon Group III mycobacteria in tissues of
tuberculin-positive swine with no gross lesions. These swine came from a herd heavily infected with *M. intracellulare*. The outbreak was a typical epizootic type. After elimination of many tuberculin-positive animals and activation of a sanitation program, the disease disappeared, with no reappearance for the past nine years. During our studies the disease was transmitted from one animal to another when uninfected animals were housed with infected animals. The uninfected animals became tuberculin-positive, lesions were detected at slaughter, and organisms isolated. Reznokov and Robinson in 1970 reported a similar outbreak in Australia. The writer has just returned from Kentucky where an extensive outbreak is in progress in swine.

Rossi in 1966 isolated *M. avium* from the pectoral muscle of 16 of 29 naturally infected chickens. Maglione in 1966 and Matyas and Sulcord in 1965 also found avian tubercle bacillus in muscle tissue of tuberculous chickens.

Petrovic et al. in 1965 isolated tubercle bacillus from 17.9% of eggs from tuberculous chickens. Isolations were made from shell surface, yolks and also reproductive organs. Fritzsche and Allan in 1965 examined 899 eggs for mycobacteria from 58 infected flocks. Thirty two (3.55%) yielded *M. avium* and 10 yielded atypical mycobacteria.

To conclude: the carcasses of tuberculous swine and cattle may carry mycobacteria in the muscles. Without question, such carcasses are a potential hazard in the uncooked stage.

The thermal death point of *M. tuberculosis* and *M. bovis* is below 142 F for 30 minutes which is minimum pasteurization temperature. Chapman and Speight isolated 13 atypical mycobacteria from 485 milk samples of short time high temperature pasteurization. Scammon et al. tested 20 strains of *M. avium* and *M. intracellulare* from swine and human sources and 10 known avian strains. These were tested at 60 C for exposures 10, 30, 60 and 120 minutes. All strains survived 10 minutes exposure and 20 percent survived 30 minutes. Harrington and Karls in 1966 tested both *M. avium* and *M. intracellulare* in milk at 62.8 C for 30 minutes and 7.1. C for 15 seconds for survival. *M. intracellulare* survived both temperatures. In our experiments in progress, a culture of *M. avium* and a culture of *M. intracellulare* were surviving for 60 minutes at temperatures above 142 F.

Are meat products heated to a temperature that destroys *M. tuberculosis, M. bovis, M. avium* and *M. intracellulare*?

The answer, if negative, is not to condemn meat processing, but to eliminate the mycobacteria from meat by eradication of tuberculosis and mycobacteriosis from all species of animals.

Tuberculosis and mycobacteriosis is not only due to *M. bovis, M. avium*, and possibly *M. intracellulare* in animals but also in man. To eradicate tuberculosis and mycobacteriosis in animals, the animal health agencies, both federal and state, need the support of human health agencies. Only by united effort can a national program be initiated.
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57. Chapman, John S. and Speight, Maro. Isolation of atypical mycobacteria from pasteurized...

THE STATUS OF THE STATE-FEDERAL TUBERCULOSIS ERADICATION PROGRAM

by
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J. D. Roswurm², D.V.M., M.P.H.

This report on bovine tuberculosis continues to suggest progress toward eradication – an objective that has been foremost in our minds since the cooperative State-Federal program was initiated.

While progress has been slow, it has been steady. We have good reason to be optimistic for the future. Our optimism is supported by proposals to strengthen the program through consideration for more realistic indemnities; greater emphasis on health requirements relating to imported animals; revision of the Uniform Methods and Rules; and increased submission of granulomatous lesions for laboratory examination for tuberculosis by Federal and State Meat and Poultry Inspection personnel.

Evidence that our attack must be continued with increased vigor is borne out by the fact that Mycobacterium bovis was confirmed in at least one herd in each of 16 States and Puerto Rico during fiscal year 1971. The map (figure 1) shows the States where M. bovis was confirmed during the past year. It also shows those States where M. bovis was confirmed in the 4-year period prior to fiscal year 1971. It will be noted that approximately 60 percent of the States were involved during the 5-year period. Considering the insidious nature of bovine tuberculosis, it is likely that the disease still exists in a majority of these States.

The graph (figure 2) shows the number of herds that were confirmed as having M. bovis and the number of herds highly suspicious but not actually confirmed. These data cover the years 1962 through 1971. It may be observed that there was a decline in the number of herds recorded for each successive year until 1971 when there was an upswing from a low of 50 in 1970 to 67 in 1971. Are we doing better in finding infected herds as compared to the prior year? Was the number for 1970 just a low year? Has there been an increase in the prevalence of bovine tuberculosis?

The decline in infected herds detected yearly from 1964 to 1968 was much faster than the decline in lesions reported on routine slaughter. The number of known tuberculous herds discovered may be a better measure of program activity than a gauge for the amount of disease that actually exists. It should be remembered that the report in 1968 showed a 61 percent decline in area testing between 1963 and 1968. The total cattle tested in FY 1971 was 3,783,709 – the lowest number for any one year since 1923. This may be having a substantial effect on the amount of bovine tuberculosis detected.

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With such low numbers of both infected herds and regular kill cases, some variation up or down may be of little significance. It does, however, remind us that we still have a job to do. As pointed out in recent status reports to this association, there may be as many as five to ten undisclosed *M. bovis* herds for every one that is known.

The line near the bottom of this graph shows the number of herds that were depopulated each year starting with the year 1964. Limited funds and lack of authority on the part of a few States to pay indemnity for exposed animals have been responsible for restricting progress in the depopulation of infected herds. Some of the herds which we are not able to depopulate have been infected for many years.

The location of the 67 herds either confirmed or suspicious of *M. bovis* are shown for each State. (figure 3) One might say that this is where we have the problems. We should remind ourselves, however, that finding infected herds depends on comprehensive surveillance and that the disease in any state is a potential hazard to other States. We should also recognize that there is a real concern at the present time for the herds that may be released from quarantine before all of the infected animals have been detected and slaughtered.

Turning our attention for the moment to the 56 confirmed *M. bovis* herds, we see (figure 4) that the number is divided about equally between those herds in which infection was reported for the first time in 1971 and those in which infection was also reported prior to fiscal year 1971. As stated earlier, some of the 27 herds with infection reported in more than one fiscal year have had infection for many years and are a threat to the entire livestock industry. They also contribute to the delay in reaching our objective eradication.

The 22 *M. bovis* confirmed herds located in 12 States and Puerto Rico that were depopulated during the year are spotted on the map (figure 5). It may be observed that there is quite a variation among the States in the use of herd depopulation as an aid to eradication. In some of the States involved, depopulation is limited or not used in herds of considerable size. Depopulation of these herds would take a considerable expenditure of State and/or Federal indemnities. The elimination of some of these infected units, small or large, especially those with long standing or spreading infection, would greatly benefit the program. While the depopulation cost would be sizeable, it could be a factor in drastically decreasing the long term program expenditures.

There has been a marked decrease in the number of lesion cases recorded as suspicious of tuberculosis on regular kill meat inspection this last year. These are the ANH 6-35 reports. Figures of 148 for fiscal year 1971 may be compared to 240 for 1970 and 295 for 1968. A look at the map (figure 6) for the 148 regular kill suspicious cases showing the State where the animal was slaughtered and the State to which the animal was traced reminds us that we must be on our guard countrywide. Practically every State is potentially involved with one or more of the 148 suspicious cases either as a point of slaughter, the point to which the animal may have been traced, or a State through which the animal may have traveled (11 animals from known quarantined herds not included).

The suspicious cases have been broken down by slaughter class (figure 7). The tracing success of these is illustrated by three bars; one each for feeders, adults, and
unknowns. The feeder group (54) consists of beef type animals definitely identified with the feedlot up to 2½ years of age, and other animals under 2 years of age. The adults (61) are the remaining animals reported as over 2 years of age. The unknown (24) are cases for which an age was not given. There were nine cases involving international movements shown on the prior illustration that were not included in the data for this chart.

It is obvious that considerable work must be done before our traceback program reaches a high degree of proficiency. Only 28 percent of the cases involving feeder cattle were reported as successful. Increased efforts in dealing with this class of cattle is imperative if we are to locate the remaining foci of disease. While there may be major problems associated with identification of feeder cattle, solutions to these problems would materially strengthen the eradication effort.

This may (figure 8) give another illustration in support of our concern for the number of undetected *M. bovis* herds that may exist to haunt us. The data shows that there were 45 regular kill lesion cases confirmed as *M. bovis*. Less than one half (21) of these were traced to additional infection. The fact that 50 or over 50 percent of the confirmed *M. bovis* regular kill cases were not associated with additional infection causes us to have considerable apprehension for the likelihood of finding all our infected herds in the very near future. Nine (38 percent) of the 24 cases that were not traced to additional infection involved feeder cattle.

To have assurance that we can eventually find all of the *M. bovis* herds, with slaughter inspection as our chief surveillance procedure, means that many suspicious cases must be investigated for every *M. bovis* confirmed case.

The number of lesion cases reported by meat inspection, regular kill, for fiscal year 1971 was 1,011 as compared to 846 for the prior year. This is encouraging. Let us consider, however, the ANH 6-35 case rate. There is a sizeable variation depicted for the rate of sample submission in Federal plants slaughtering over 100,000 cattle annually. The plants with this capacity and the rate of lesion submission is shown on the map (figure 9). It must be realized that there may be considerable difference in the class of cattle slaughtered by plants in the same locality. It would seem, however, that 34 establishments (41 percent) not reporting any suspected tuberculous lesions or thoracic granulomas is rather high. It is of interest to observe that four plants (5 percent) submitted specimens at a rate of 10 per 100,000 or more.

We generally expect to find tuberculosis at a greater rate in adult animals slaughtered than in younger animals. The rate of specimen submission was thus figured for Federal plants slaughtering over 20,000 cows annually. The rate of slaughter (figure 10) shows that in those plants slaughtering a relatively large number of cows, 32 plants (35 percent), there was no submission of lesions to be examined for tuberculosis. Yet in another plant lesions were furnished at the rate of 5.1 per 10,000 slaughtered. More studies are necessary to determine the reasons for these big variations and the possible solutions to this apparent problem.

It is recognized that recurring contact between field personnel engaged in tuberculosis eradication and meat inspection personnel results in a significant increase in submission of lesions for differential laboratory diagnosis. In many instances, it is a case of getting people involved and developing a better understanding of the situation.

The expedite the eradication of bovine tuberculosis it might be well for us to ask
the question, is it more money that is needed or more efficiency in the use of the money?

"If we are not a part of the solution, we are a part of the problem." Special studies are continuing in an effort to improve our performance.

During fiscal year 1971, a field trial was begun to compare the specificity and sensitivity of four tuberculins in cattle. The first phase of the trial was designed to compare the amount of cross-sensitivity found using ARS contract tuberculin, beef broth tuberculin, and two purified protein derivatives (PPD's) of *M. bovis* origin in 27 institutional dairy herds scattered across the country. The herds were selected because each had a history of sensitivity to tuberculin without evidence of *M. bovis* infection.

The field work is completed on the first phase, but only preliminary results are available at this time. Of the 4,120 mature cattle tested, 545 were classified as deviators and 242 as suspects by the investigator, Dr. Lloyd Konyha. Each animal received only one tuberculin, which was selected by a method of randomization.

ARS contract tuberculin produced 7.7 percent suspects and 14.7 percent deviators. Beef broth tuberculin showed 5.3 percent suspects and 21.9 percent deviators. The two PPD's produced 5.9 and 4.4 percent suspects respectively and 9.1 and 7.1 percent deviators each.

During the trial in the institutional dairies, the suspects were retested with the avian-mammalian comparative test. Several infected herds have also been tested by these procedures. The results suggest that the cervical comparative tuberculin test, when used to retest caudal fold suspects, will help differentiate on a herd basis between *M. bovis* infected animals and animals with cross-sensitivity.

Infection has been detected in two herds with a mixture of *M. bovis* infection and cross-sensitivity by the use of the comparative test. It is anticipated that this test will be very useful in the hands of experienced epidemiologists to solve difficult diagnostic problems.

Currently, arrangements are being made to evaluate the sensitivity of ARS contract tuberculin, one PPD and Band 24, the new tuberculin under study at Michigan State, in tuberculous cattle in Mexico.

This report deals with tuberculosis eradication in the bovine. To reach the ultimate broad objective of tuberculosis eradication, a wider viewpoint must be adopted.

Tuberculosis in some form involves many species of animals. Thus, it is essential that the interspecies relationship of tuberculosis be considered in the broad concept of tuberculosis eradication.

Dr. William H. Feldman¹, a long-time member and friend of this association and who served with distinction as a member of the Tuberculosis Committee, made the statement in 1958: "A united and continuing attack on tuberculosis in all species is the only way to achieve the final conquest of this disease.

"The existence of bovine, human, avian, and vole types of tubercle bacilli, each of which has certain definite distinguishing characteristics, does not imply that any of these types are strictly host specific. If they were, the problem of preventing, suppressing, and eliminating the disease caused by each type of organism would be simplified. Instead, we are confronted with a disease of inconsistencies and one that is subject to remarkable variation.

Tuberculosis is unique in its variable symptomatology, its unpredictable
pathogenesis, and the inconsistent gross and microscopic tissue changes.

"Especially important is the fact that each of the four types of tubercle bacilli is capable of infecting, even fatally, species of animals other than its natural host."

In speaking of eradication; why do we still have people asking the age-old question, "Can tuberculosis be eradicated?" Perhaps they have not heard Chapin's2 dictum made more than three quarters of a century ago, "If we can prevent the spread of contagion at all, we can prevent it entirely."

In closing, may we be stimulated by the words of Feldman3 in his paper, "Yesterday's Triumph: Today's Problems":

"The task, never ending, is a formidable one. The final eradication or suppression of major infectious diseases will require the resources of many medical and allied disciplines, represented by dedicated persons who have a concern for humanity and a zest for conquest. Given scientists with tenaciousness of purpose, imaginative intuition, and a nod from Lady Luck, the potential benefits of biomedical research that can accrue to our civilization are immeasurable.

"The essence of the approach necessary to succeed is expressed approximately in a line from Tennyson's *Ulysses*, which reads, "To strive, to seek, to find, and not to yield.""

REFERENCES

FIGURE 1.

*Tuberculosis Eradication*

**TIME INTERVAL SINCE LAST REACTOR CONFIRMED M. BOVIS**

*JULY 1, 1971*

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**US DEPARTMENT OF AGRICULTURE**

**AGRICULTURAL RESEARCH SERVICE**
FIGURE 2.

_Tuberculosis Eradication_

**LESION HERDS - F.Y. 1962 through 1971 and those DEPOPULATED with INDEMNITY**

<table>
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<th>F.Y.</th>
<th>NO. HERDS</th>
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U.S. DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE
FIGURE 3.

*Tuberculosis Eradication*

**LESION HERDS - 1971**

- 56 *M. BOVIS* (confirmed)
- 11 SUSPICIOUS

U.S. DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE
**Tuberculosis Eradication**

**M. BOVIS (CONFIRMED) HERDS**

**FISCAL YEAR 1971**

* 29 NO INFECTION REPORTED PRIOR TO F.Y. 1971

• 27 INFECTION ALSO REPORTED IN A PRIOR F.Y.

56

U.S. DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE
FIGURE 5

Tuberculosis Eradication

M. BOVIS (CONFIRMED) HERDS
FISCAL YEAR 1971

22 △ HERDS DEPOPULATED WITH INDEMNITY FOR EXPOSED ANIMALS
34 ● HERDS NOT DEPOPULATED
56

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AGRICULTURAL RESEARCH SERVICE
FIGURE 6.

*Tuberculosis Eradication*

TB TRACEBACK—REGULAR KILL SUSPICIOUS AND CONFIRMED M. BOVIS

148 CASES FISCAL YEAR 1971

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ANIMAL HEALTH DIVISION
AGRICULTURAL RESEARCH SERVICE
**Tuberculosis Eradication**

139 SUSPICIOUS LESION CASES*(reg. kill)
BY SLAUGHTER CLASS- FISCAL YEAR 1971

- FEEDERS 54
- ADULTS 61
- UNKNOWN 24

*DOES NOT INCLUDE ANIMALS FROM KNOWN QUARANTINED HERDS, OR IMPORT ANIMALS
FIGURE 8.

*Tuberculosis Eradication*

24 LESION CASES *CONFIRMED M. BOVIS*

NOT TRACED TO ADDITIONAL INFECTION

FISCAL YEAR 1971

*REGULAR KILL

〇 PLACE WHERE SLAUGHTERED

〇 POSSIBLE SOURCE

U.S. DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE
FIGURE 9.

*Tuberculosis Eradication*

ANH 6-35 CASE RATE IN FEDERAL PLANTS
SLAUGHTERING OVER 100,000 CATTLE *

FISCAL YEAR 1971

RATE PER 100,000 SLAUGHTERED

<table>
<thead>
<tr>
<th></th>
<th>NO. PLANTS</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>34</td>
<td>41</td>
</tr>
<tr>
<td>0.1-9.9</td>
<td>45</td>
<td>54</td>
</tr>
<tr>
<td>10.0-39.9</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

U.S. DEPARTMENT OF AGRICULTURE

Agricultural Research Service
Tuberculosis Eradication

ANH 6-35 CASE RATE IN FEDERAL PLANTS SLAUGHTERING OVER 20,000 COWS

FISCAL YEAR 1971

RATE PER 10,000 SLAUGHTERED

\begin{itemize}
  \item \textbf{NONE} \hspace{1cm} 32 \hspace{1cm} 35
  \item \textbf{0.1-2.9} \hspace{1cm} 54 \hspace{1cm} 59
  \item \textbf{3.0-5.9} \hspace{1cm} 6 \hspace{1cm} 6
\end{itemize}

\textbf{U.S. DEPARTMENT OF AGRICULTURE}

\textbf{AGRICULTURAL RESEARCH SERVICE}
REPORT OF THE COMMITTEE ON
TUBERCULOSIS AND PARATUBERCULOSIS

Chairman, D. S. Ingraham, Harrisburg, Pa.
Co-Chairman: J. G. Flint, St. Paul, Minn.


Uniform Methods and Rules

One of the major tasks of your committee this year was the revision of the Uniform Methods and Rules for bovine tuberculosis eradication in cattle. The committee has defined the goal of tuberculosis eradication in cattle to mean *M. bovis* infection.

To have state and federal veterinarians participate in this project, initial drafts were written by the full committee at the last annual meeting and were revised at a sub-committee meeting which was held in Philadelphia in April 1971. Additional comments were received from tuberculosis epidemiologists of the ANH and many state and federal veterinarians. Your committee has therefore had much help from those who implement regulations to provide firm but practical guidelines to effect the eradication of bovine tuberculosis. This goal is in sight, and we hope that all states will use these Uniform Methods and Rules as minimum standards. Any policy or regulation that is less stringent could jeopardize the progress that has been made.

The committee therefore wishes to present the Uniform Methods and Rules for Bovine Tuberculosis Eradication as part of this committee report.

Proposed Increase in Federal Indemnity

The number of Red Flag herds has not been significantly reduced during the past few years. Some of these herds are a potential source of infection not only to herds in the state where they are located but also to others if the herd is released from quarantine even though the herd has passed the required number of tests. One of the factors which has prevented depopulation of some of these problem herds is a lack of sufficient state and federal funds.

Dr. H. Q. Sibley, Executive Director, Texas Animal Health Commission, and Dr. F. Henderson, Veterinarian in Charge, Louisiana, who spoke for Dr. F. B. Wheeler, State Veterinarian, Louisiana, presented to the committee factors which have prevented the depopulation of large beef herds in areas of east Texas and southwestern Louisiana. To date, these states have been unable to provide indemnities under the current matching indemnity provisions of the CFR to
depopulate these herds. Therefore, it is recommended that this association request the United States Department of Agriculture to amend the Department regulations for tuberculosis indemnity to eliminate the present requirement that State payments match Federal payments. It is further recommended that Federal indemnity be based on the difference between the appraised value and the salvage proceeds not to exceed $200 for a grade animal or $400 for a purebred animal.

**Interspecie Relationships**

For the past five years this committee has given an increasing amount of consideration to the interspecie relationship of tuberculosis. Recent papers that relate to this subject have called attention to the fact that tuberculosis with its broad aspects has considerable public health significance.

Roswurm and Ranney made the following statement in a paper presented at the recent meeting of the American Public Health Association. "Mycobacterial diseases of varied forms and virulence attack most species of animals including man. Neat categorizations which confine *M. bovis* to cattle, *M. tuberculosis* to man, and *M. avium* to birds are figments of the imagination." Feldman\(^1\) stated in 1965, "The final eradication or suppression of major infectious diseases will require the resources of many medical and allied disciplines, represented by dedicated persons who have a concern for humanity and a zest for conquest."

Dr. W. L. Mallmann, a member of our committee, presented a well documented paper on tuberculosis in various species at the American Public Health Association meeting earlier this month. This paper calls attention to many situations that focus attention on potential hazards associated with the transmission of tuberculosis between various species. He closes his paper with the following statement. "Tuberculosis and mycobacteriosis is not only due to *M. bovis*, *M. avium* and possibly *M. intracellulare* in animals but also in man. To eradicate tuberculosis and mycobacteriosis in animals, the animal health agencies, both Federal and State, need the support of human health agencies. Only by united effort can a National program be initiated."

We have been informed that the Tuberculosis Guidance Committee of the National Tuberculosis and Respiratory Disease Association is meeting early in December. Your committee has requested that the Chairman contact the NTRDA and suggest that one or two members of our committee present data on this subject to the Tuberculosis Guidance Committee. It is our hope that this may result in a coordinated wide-scale attack on tuberculosis in man and animals to better the health and welfare of this nation.

Dr. Rankin McIntyre, speaking for the Department of the Los Angeles County Veterinarian proposed the following:

1. In order to eradicate tuberculosis in all animals, request that the Tuberculosis Committee of the USAHA develop and publish uniform methods and rules for tuberculosis testing in all species.

2. If methods of tuberculosis testing are not currently known for certain species, request that the USAHA support the formation of a group of knowledgeable individuals who will develop methods and standards for each species.

The committee supported this general concept. A Subcommittee on Interspecie Relationships was appointed consisting of Dr. W. L. Mallmann and A. F. Ranney to make recommendations to the committee for the development of this concept.

Paratuberculosis

Dr. A. B. Larsen furnished the committee with current information concerning Johne's disease as follows:

Johne's disease or paratuberculosis control continues to be a problem. Current research on this disease at the National Animal Disease Laboratory at Ames, Iowa, consists of drug, vaccine and blood chemistry studies.

A johnin test to detect infected cattle has been developed. The test is based on changes in the number and kind of white cells following the administration of johnin intravenously. Smears are prepared from blood taken just before and six hours after the administration of johnin. The test is positive if the second smear has 50% or more increase in neutrophiles and a 25% or more decrease in lymphocytes. This change occurs in infected animals even in the absence of temperature increase.

It has been found that swine can be infected from exposure to infected cattle. Such swine may shed the organisms in their feces for variable amounts of time even in the absence of clinical signs.

The committee is aware of certain proposals to modify meat inspection procedures so that inspections will be made on a random sampling basis. The committee recommends that the ante mortem and post mortem carcass inspection be carried out on a 100% basis to provide for necessary surveillance for tuberculosis and other animal diseases.

Dr. Ian Lesslie, representing the Pan American Health organization of WHO, addressed the committee on the subject of comparative tests, tuberculin, and tuberculosis eradication programs in Latin American countries.

Dr. D. S. Ingraham, Chairman

UNIFORM METHODS AND RULES

BOVINE TUBERCULOSIS ERADICATION — MINIMUM STANDARDS

PART I — DEFINITIONS

2. Bovine Tuberculosis Eradication — eradication is the complete elimination of bovine tuberculosis from cattle in a State so that it does not appear unless introduced from another species or from outside the State.
3. Cattle — cattle refers to domestic bovine animals of all ages.
4. Natural Additions — animals born and raised in the herd.
5. Herd — a herd is a group of cattle maintained on common ground for any purpose, or two or more groups of cattle under common ownership or supervision geographically separated, but which have an interchange or movement of cattle without regard to health status (a group is construed to mean one or more animals).
6. Tuberculin — a product that is approved by and produced under license of the USDA for injection into cattle for the purpose of detecting bovine tuberculosis.
7. Official Tuberculin Test — a test for tuberculosis applied and reported by approved personnel in accordance with these Uniform Methods and Rules.
8. Comparative test — the injection of standardized mammalian and avian tuberculin at separate sites in the cervical area and a determination as to the probable presence of mammalian tuberculosis by comparing the responses of the two tuberculins.
9. Passed Herd — herd in which no animals were classified as reactors or suspects.
10. Annual test — tests conducted at intervals of not less than 10 months nor more than 14 months.

11. No Gross Lesion (NGL) Animal — an animal in which a lesion(s) of tuberculosis is not found during slaughter inspection. (An animal with skin lesions only will be considered in the same category as an NGL).

12. Surveillance — Surveillance is all measures used to detect the presence of tuberculosis in the cattle population.

13. Accredited Herd — An accredited herd is one which has passed at least two consecutive annual tuberculin tests and no other evidence of bovine tuberculosis has been disclosed and meets the standards of these Uniform Methods and Rules.

14. Modified Accredited Area — A State or portion thereof which is actively participating in the eradication of tuberculosis and which maintains its status in accordance with these Uniform Methods and Rules.

15. Accredited Free State — A State which maintains full compliance with these Uniform Methods and Rules and no evidence of bovine tuberculosis has been disclosed for 5 or more years.

16. Herd Depopulation — Removal of all cattle in the herd direct to slaughter prior to any restocking of the premises with cattle.

17. Direct to Salughter — Direct to slaughter shall mean the shipment of cattle from the premises of origin directly to a slaughter establishment without diversion to assembly points: such as auctions, public stockyards, and feedlots.

18. Quarantined Feedlot — A quarantined feedlot shall be a confined area under the direct supervision and control of the State Livestock Official who shall establish procedures for accounting of all animals entering or leaving such quarantined feedlot. The quarantined feedlot shall be maintained for finish feeding of animals in drylot with no provision for pasturing and grazing. All animals leaving such feedlot must move only direct to slaughter in accordance with established procedures for handling quarantined animals.

PART II — OFFICIAL TEST PROCEDURES

A. Authority To Test. State laws and/or regulations shall provide authority to apply a tuberculin test to any animal or herd at such times as may be deemed necessary by the cooperating State and Federal officials. These officials reserve the right to supervise any test conducted by an accredited veterinarian.

B. Restriction of Personnel To Apply Tuberculin Tests. Tuberculin tests shall be applied by a veterinarian employed in a full-time capacity by the State, USDA, or by an accredited veterinarian.

C. Caudal Fold Test. The official tuberculin test for routine use shall be the intradermic injection of 0.1 cc of tuberculin in the caudal fold.

D. Cervical Test. This test is limited to use in herds where bovine tuberculosis has been disclosed except:

1. When the comparative cervical test is used.
2. When special tests such as those applied to animals for export.

For retesting known Mycobacterium bovis herds, 0.2 cc of tuberculin shall be used and applied only by full-time employed state or federal regulatory veterinarians. The comparative and other cervical tests are to be used only as
specifically approved by the State-Federal cooperating officials.

E. **Requirements for Special Procedures in Infected Herds.** All cattle in herds from which tuberculous cattle originate and all cattle that are known to have associated with infected cattle shall be tested promptly. Cattle in feed lots known to be exposed to tuberculous cattle shall be quarantined and shipped under permit directly to slaughter. Disclosure of tuberculosis in any herd shall be followed by a complete epidemiological investigation. Every effort must be made to assure the immediate elimination of the disease from all species of domestic livestock and poultry on the premises.

F. **Tuberculin Test Interpretation.** Decisions will be based upon the professional judgment of the testing veterinarian in accordance with the policy established by the cooperating State and Federal officials. The injection site on each animal shall be palpated. Observation without palpation is not acceptable. The following are guidelines for classification of cattle tested with the caudal fold test:

1. **Reactor “R”** — Animals showing a circumscribed swelling 5mm. in diameter (3/16 of an inch) or a diffuse “swelling” twice as thick as the normal caudal fold or greater response to tuberculin on routine test should be classified as reactors unless in the professional judgment of the testing veterinarian suspect classification is justified.

2. **Suspect “S”** — Animals showing a response to tuberculin not classified as reactor with the exception noted below.

3. **Passed.**
   a. **Deviator “D”** — Animals showing a minimal response to tuberculin. This is usually designated as a pinpoint (PP) response.
   b. **Negative “N”** — Animals showing no response to tuberculin.

G. **Report of Tuberculin Tests.** — A report of all tuberculin tests, including the individual identification of each animal by eartag number or tattoo, age, sex, and breed, and a record of the size of the responses, shall be submitted in accordance with the requirements of the cooperating State and Federal officials.

**PART III — DISPOSITION OF TUBERCULIN RESPONSE ANIMALS**

**A. DISPOSITION OF REACTORS**

1. Reactors must remain on the premises where disclosed until a State or Federal permit has been obtained. Movement for immediate slaughter must be direct to a slaughter establishment where approved State or Federal inspection is maintained within 15 days of classification. Upon delivery to the slaughtering establishment, they shall be slaughtered as soon as practicable.

2. No animal classified as a reactor shall be retested.

**B. DISPOSITION OF SUSPECTS**

1. Suspects to the tuberculin test shall be quarantined to the herd where found or shipped under permit to slaughter in accordance with the State and Federal laws and regulations.

**C. DEVIATORS**

1. Record response for complete animal health history.
2. These animals shall not move from the herd, except for slaughter, until retested and negative.

**PART IV – QUARANTINE PROCEDURES**

1. All herds in which reactor animals are disclosed shall be quarantined. Exposed animals must remain on the premises where disclosed unless a State or Federal permit has been obtained. Movement for immediate slaughter must be direct to a slaughtering establishment where approved State or Federal inspection is maintained.

2. Sale of Feeder Calves from quarantined herds will be restricted. Feeder calves under 12 months of age that have passed a tuberculin test within 60 days may be permitted to move intrastate to a quarantined feedlot.

3. Herds in which *Mycobacterium bovis* infection has been disclosed shall remain under quarantine and must pass two tuberculin tests at intervals of at least 60 days and one additional test after six months. Minimum quarantine period shall be ten months from slaughter of lesion reactors. A case will be considered "*Mycobacterium bovis* infection" when a pathologic (granulomatous) lesion in cattle suspected of being tuberculosis is found unless a satisfactory examination at an accredited laboratory justifies a diagnosis other than bovine-type tuberculosis. Exception – lesions that occur only in the mesenteric lymph nodes.

4. Herds in which NGL reactor(s) only occur and no evidence of *Mycobacterium bovis* infection has been disclosed may be released from quarantine after a 60 day retest on the entire herd.

5. Suspects in herds where only suspect animals are disclosed shall be quarantined to the herd. They shall remain on the premises where disclosed unless a State or Federal permit has been obtained. Movement for immediate slaughter must be direct to a slaughtering establishment where approved State or Federal inspection is maintained. Suspects remaining on premises must pass a tuberculin test in not less than 60 days to be released from quarantine.

**PART V – SPECIAL RETESTS OF HIGH-RISK HERDS**

1. In herds where *Mycobacterium bovis* infection has been confirmed but the herd not depopulated, five annual tests on the entire herd followed by two tests at three year intervals shall be applied following the release of quarantine.

2. In herds with history of lesions suspicious of bovine tuberculosis (not confirmed), two complete annual herd tests shall be applied after release of quarantine; the first test to be applied approximately one year after release of quarantine.

3. In a newly assembled herd on a premises where a tuberculous herd has been depopulated, two annual herd tests shall be applied to all cattle; the first test to be applied approximately six months after assembly of the new herd. These tests shall be followed by two complete herd tests at three year intervals.

**PART VI – CLEANING AND DISINFECTION OF PREMISES**

1. Premises where tuberculous cattle have been maintained shall be thoroughly cleaned and disinfected with a disinfectant permitted by the Animal Health Division, USDA, and in a manner satisfactory to the cooperating State and
TUBERCULOSIS AND PARATUBERCULOSIS

Federal authorities.

PART VII – ORIGIN OF INFECTION

1. Tuberculosis found during slaughter inspection or otherwise in any bovine will be considered to have originated in the State where slaughtered or disclosed unless successful traceback procedures identify the source to another State.

PART VIII – IDENTIFICATION OF LIVESTOCK

1. All cattle tested shall be individually identified by official ear tag or other satisfactory means. Devices such as neck chains that are easily removed and transferred are not considered satisfactory.

2. The State shall* have and enforce dealer control laws and/or regulations that require dealers to maintain the identification of cattle and records of transactions for each animal purchased or sold.

3. Cattle moved in channels of trade within a State shall* be identified and recorded as to origin and destination at the first concentration point, (dealer, livestock auction, stockyard, etc.) as follows:
   a. Cattle over 2 years of age that are returned to farms or ranches, including feeding cattle, shall* be identified by official ear tag or by an official brand. If identified by brand, cattle must be accompanied by an official brand release.
   b. Cattle that are marketed for immediate slaughter shall* be identified by eartag, saletag, or official backtag. An official brand release will be acceptable identification for lots of animals of unmixed origin that are shipped directly to slaughter.
   c. Cattle without individual identification may be moved directly to and maintained in a quarantined feedlot under control of the State livestock sanitary official, provided they are inspected in the feedlot and are moved to slaughter under permit at the end of the feeding period.

*The word “shall*” will apply to those States which are to be considered for Accredited Free Status. The word “should” may be substituted for States with a modified accredited status only.

PART IX – ACCREDITED HERD PLAN

1. Animals to be tested – Testing of herds for accreditation or reaccreditation shall include all cattle over 24 months of age and any animals other than natural additions under 24 months of age. All natural additions shall be individually identified and recorded on the test report as members of the herd at the time of the annual test.

2. Additions – Herd additions must originate directly from one of the following:
   a. Accredited herd
   b. Herd in an Accredited Free State
   c. Herd in a Modified Accredited Area that has passed a herd test on all animals over 24 months of age within 12 months and the individual animals for addition were negative to the tuberculin test conducted within 60 days.
   d. Herd in a Modified Accredited Area not meeting requirements of a, b, and
c of this paragraph, individual animals for addition must pass a negative test within 60 days prior to entering the premises of the accredited herd and must be kept in isolation from all members of the accredited herd until negative to a test conducted after 60 days of date of entry.

Animals added under (b), (c), and (d) shall not receive accredited herd status for sale purposes until they have been members of the herd at least 60 days and are included in a herd retest.

3. Accreditation and reaccreditation- To qualify for accredited herd status, the herd must pass at least two consecutive annual tuberculin tests with no evidence of bovine tuberculosis disclosed. All animals must be bona fide members of the herd. Qualified herds may be issued a certificate by the local State and Federal officials. The accreditation period will be 12 months (365 days) from the anniversary date and not 12 months from the date of the reaccreditation test. To quality for reaccreditation the herd must pass an annual test within a period of 10 to 14 months of the anniversary date.

PART X – MODIFIED ACCREDITED AREA STATUS

1. TESTING & SLAUGHTER SURVEILLANCE
   An annual report shall be submitted for each State or appropriate subdivision at the close of each fiscal year to show the amount of testing and slaughter surveillance that has been conducted.

2. TRACE TESTING
   a. All cattle in herds of origin or cattle associated with those showing evidence of tuberculosis at time of slaughter must be immediately tested.
   b. The testing schedule of all reactor and suspect herds must be current.

3. REVOCATION OR SUSPENSION OF STATUS
   Disclosure of tuberculosis in the area and/or failure to take progressive steps to comply with these Uniform Methods and Rules to seek out and eliminate tuberculosis shall be cause for revocation or suspension of the modified accredited status.

PART XI – ACCREDITED FREE STATE

1. A State may be listed as Accredited Free if the State complies with all of the procedures in these Uniform Methods and Rules and no evidence of tuberculosis has been found for five or more years.

2. Disclosure of tuberculosis in an Accredited Free State will be sufficient justification for revocation or suspension of the Accredited Free Status. After all epidemiological studies have been completed and all exposed herds have been tested and it has been established that there has been no spread from the herd, the State may be considered for reinstatement of its Free Status.
EXPERIMENTAL MULTIPLE INFECTION OF ANIMALS WITH FOOT-AND-MOUTH DISEASE VIRUSES

G. E. Cottral and P. Gailiunas*

SUMMARY

Multiple foot-and-mouth disease virus (FMDV) infections with from two to seven of the recognized virus types and with two sub-types of type A were studied clinically and serologically in 25 cattle and a deer (Dama dama). Twelve cattle recovered from one type of FMDV infection were completely susceptible to a single re-infection with any of the other types tested. However, there was generally a cumulative effect towards resistance when the infections were continued in 8 cattle with additional types; the clinical response usually was less severe after infection with the third and succeeding FMDV types. Complete resistance to infection was found with two cattle to their fifth, one to its' fourth and one to its' seventh virus type. Neutralization test cross reactions usually correlated with subsequent partial or complete resistance to infections. The deer showed clinical signs and lesions after inoculation with type SAT-1, only a tongue lesion with type C and complete resistance to the other five types.

When a mixture of six types of FMDV was inoculated intramuscularly in two steers, each steer had a different, but only one virus type in all lesions and another virus type as a viremia. One of the steers became a carrier of the virus type found in the lesions (type SAT-1). With the exception of type O in one steer, the antibody response indicated that replication of all six types probably had occurred.

Five pairs of steers each recovered from infection with one of five different subtypes of type A, with the exception of one steer, became carriers of FMDV. After challenge exposure to the variant A22 subtype, all became carriers of A22, and only one steer of 10 failed to resist challenge.

INTRODUCTION

Multiple FMD infections are considered to be of three kinds: 1) reinfection with a different type after clinical recovery from the first infection, 2) superinfection with the same or a different subtype, and 3) mixed infection involving two or more types, subtypes or strains simultaneously. With reinfection or superinfection the original virus may or may not be present in the host.

In the early days of FMD research in Europe it was observed that cattle that had recently recovered from FMD usually would resist reinfection. But occasionally there were new outbreaks wherein nearly all of these recently recovered animals were again infected. Vallée and Carre in 1922 solved part of the problem by demonstrating the first two immunological types of FMD. The virus that was prevalent in France was designated type O because it was found in the Oise Valley. The virus that reinfected type O recovered animals was called type

* Dr. Gailiunas died Sept. 7, 1971.
A for Allemand, since the virus was obtained from East Prussia, Germany.\(^{21}\) A short time later (1926) Waldmann and Trautwein proved that there was a third FMD virus that could reinfect types O and A recovered cattle, which they designated type C (they wanted to rename the types A, B, C).\(^{22}\)

From 1934 to 1948 three new FMD viruses were found in Northern and Southern Rhodesia and later in other southern parts of Africa that would infect vaccinated cattle or reinfect cattle recovered from types O, A, or C infections. The British Animal Virus Research Institute, which is the FMDV World Reference Laboratory, named these new types, SAT-1, SAT-2, and SAT-3, for the Southern African Territories.\(^2\)

The seventh FMDV type was isolated in Pakistan in 1954 and it was designated Asia-1 by the British Laboratory anticipating that there may be other different Asian types found in the future.\(^2\) The Asia-1 and the three African types proved to be antigenically distinct from each other and from types O, A and C.\(^2\)

By mutation and antigenic drift influenced by passage through partially resistant or vaccinated animals, the viruses of FMD apparently have continually altered their structure so that new subtypes were formed. Currently there are 61 recognized subtypes of the seven FMDV types.\(^2\)

Experimental mixed FMD infections of cattle were reviewed and studied by Cunha et al.\(^9\). They inoculated types O, A and C at different sites in the tongue epithelium of each of 6 cattle. The original viruses were recovered from the respective tongue lesions, but, in 5 of the 6 cattle, type C was the only virus isolated from the secondary foot lesions. In the other animal both types O and A were found in the foot lesions. The serum antibodies were at high levels for type C, intermediate for A and low for O. More recently, Jain and Mehrota\(^{13}\) inoculated guinea pig's foot pads with mixtures of types O and A. They found that type A apparently predominated and produced antibodies at much higher levels than those for type O.

Several authors have reported finding more than one type of FMDV in an outbreak by complement fixation tests.\(^9,15\) Sutmoller et al.\(^{17}\) isolated types A and C FMDV from the oesophageal-pharyngeal (OP) fluids of carrier cattle in a Brazilian herd. However, only one virus type was found in individual animals.

Reinfection of the throat of cattle was reported by Fellowes and Sutmoller.\(^{10}\) While type A FMDV was still present in the throats of carrier steers, type O virus was inoculated. Thereafter only type O virus was found in the OP fluids.\(^{10}\)

Reinfection, using from 2 to 7 types of FMDV in succession, superinfection with a second subtype of type A, and mixed infection with six types were all employed in the present study to determine the clinical and serological response of cattle to multiple FMD infections.

**MATERIALS AND METHODS**

*Experimental animals.* The methods for management and feeding experimental animals in isolated rooms have been described previously.\(^4\) The biological safety precautions taken to prevent cross contamination also have been reported.\(^3\) Twenty-five Hereford cattle (one bull and 24 steers) were used and they were from 14 to 30 months old and averaged about 700 lbs. A European brown fallow deer*,

*The deer and two monkeys were obtained from the National Zoological Park, Washington, D.C.*
**EXPERIMENTAL MULTIPLE INFECTION WITH FMDV**

*Dama dama*, was a male about one year old. Control animals included a steer, two male Tamworth pigs about 4 months old, two female grade Corriedale sheep about a year old and two male grade Toggenburg goats about 6 months old. Accessory animals included two monkeys*, a Rhesus, *Macaca mulatta*, and a Capuchin, *Cebus capuchinus*, both young adult males.

**Routes of inoculation.** The various animals, unless otherwise noted in the results, were inoculated in the tongue epithelium. The virus dosage for this route was 1.0 ml, unless otherwise noted, and contained about $10^{6.0}$ FMDV bovine ID$_{50}$ units/ml. The dosage for the intramuscular route was 5.0 ml containing about $10^{6.0}$ bovine ID$_{50}$ units of FMDV, except as noted.

The aerosol used to expose the deer, a steer, 2 pigs, 2 sheep and 2 goats to FMDV A-119 was generated by a Hudson aerosol sprayer**. The original volume of fluid was 300 ml containing about $10^{5.7}$ bovine ID$_{50}$ units of FMDV/ml. This fluid was dispensed as a visible aerosol cloud in 15 minutes. The room air supply and exhaust was turned off during and for 45 minutes after aerosol production to permit maximum exposure of animals to the aerosol. The sprayer was located 6 feet above the floor and about 20 feet from the animals on the opposite side of the room. A millipore filter† was placed near the heads of the animals. Air was drawn through the filter for one hour, using an air pump outside of room connected by a rubber tube. The filter membrane was retrieved and ground in a mortar with 3.0 ml of tryptose phosphate broth. After centrifugation of the suspension of filter material at 800 X g, tenfold dilutions of the supernatant fluid were titrated for virus in suckling mice.

**Gradation of clinical response.** The severity of the clinical response of the animals to FMD was graded from 1+ to 4+. Small and relatively few tongue lesions, no foot lesions, fever absent or present only one or two days, very little salivation and nasal discharge, continued or a rapid return to eating and no loss of alertness were the general factors considered as a 1+ response. A response of 4+ involved extensive tongue, other oral and foot lesions, prolonged fever, profuse salivation and nasal discharge, lameness, cessation of eating and drinking for four or more days, general malaise and dullness. The 2+ and 3+ responses were subjectively judged between these two extremes. The urine pH during the acute stage also was used as a criterion for judging the severity of the clinical response in some of the animals.

**Collection and testing of samples.** The methods used for collection of blood samples and detection of viremia have been reported. The techniques for harvesting lesion material and processing for virus titrations have been reported. The use of the cup probang for collection of OP fluids and processing of the fluids with trichlorotrifluoroethane for virus titrations have been given, as have the tissue culture techniques for virus titrations by the CPE and PFU methods.

The technique and interpretation of results for the constant serum/variable virus dilutions neutralization test previously was reported. The 50 percent endpoint complement fixation test for typing FMDV was used. The technique and

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* Obtained from the National Zoological Park, Washington, D.C.
† Millipore Filter Corp., Bedford, Mass.
interpretation of the virus infection associated (VIA) antigen test has been reported.\textsuperscript{8}

Viruses. The viruses used for animals 1 to 10 and steers 25 and 26, and designated by subtype and strain were: \(O_1\) O-MII, \(A_{12}\) A-119, \(C_1\) C-149, SAT-1/2 S. Rhodesia-'37, SAT-2/1 N. Rhodesia-'48, SAT-3/1 S. Rhodesia-'34, Asia-1/1 Pakistan-1 '54; also for steers 1 and 2 — \(A_{11}\) Uruguay-'60 and \(A_{24}\) A-4691 Brazil-'64; for bull 10 — SAT-3/1 S. Rhodesia-'34 and \(C_3\) CANEFA-3 Argentina-'62; for steers 11 to 22 — \(A_1\) Bavaria-'42, \(A_3\) Mecklenburg-'44, \(A_4\) Wesdeutschland-'48, \(A_8\) Parma, Italy-'48, \(A_32\) Venezuela-'70 and \(A_{22}\) Iraq-'64; for steers 23 and 24 — SAT-1/4 S. Rhodesia-'58 and \(O_1\) CANEFA-2 Argentina-'61.

All viruses were prepared from bovine tongue epithelial lesions. Virus titrations were made in bovine kidney cell cultures, mice or cattle on all inoculums to verify virus dosage. Neutralization tests were used to verify virus types with type specific guinea pig antiserums. Subtype determinations were made with the complement fixation test.\textsuperscript{1}

**RESULTS**

The clinical responses of eight steers and a deer to consecutive inoculation in the tongue epithelium with from four to seven types of FMDV are given in Table 1. With the exception of the first three animals (2 steers and a deer), the cattle had typical clinical signs and tongue and foot lesions following inoculation of the first virus and all responded completely to the second virus. Usually a reduction in the clinical severity was not found until after the third, fourth or fifth clinical infections with FMD had occurred. The particular strains used of types SAT-2 and SAT-3 produced clinical responses that were slightly less severe than those of the other types of FMDV.

**Steers 1 and 2.** Blood samples from a Rhesus monkey at 48 hours postinoculation (HPI) and from a Capuchin at 72 HPI were used for the initial inoculation of steers 1 and 2, respectively. The monkeys had been inoculated with type A (strain A-119) FMDV for a susceptibility and viremia study.\textsuperscript{6} After inoculation, neither steer showed clinical evidence of FMD infection. However, 14 days after inoculation, serum from steer 1 gave a positive VIA reaction and an NI of 5.4 with A-119 virus. Serum from steer 2 gave a negative VIA reaction and an NI of 3.8. Both steers were resistant to challenge with A-119 virus (tongue inoculation). The antibody response of the two steers 15 days after challenge is given in Table 2 showing NI of 5.6 and 4.8, respectively, with type A FMDV. The serum VIA reactions after challenge did not change; positive for steer 1 and negative for steer 2.

Maximum clinical response (for the virus strains used), which indicated complete susceptibility, were found with steer 1 and 2 after inoculation with the second (type C) and the third (type SAT-2) FMD viruses (Table 1). Type C was given 29 days after initial exposure to type A (or 15 days after challenge with type A). Serum from steer 1 collected 36 days after type C inoculation, and just prior to inoculation of type SAT-2, gave a NI of 5.7 with type A, 5.4 with type C and 3.2 with type O virus, a cross reaction (Table 2). When the NI was 2.9 or more with a heterologous FMDV type it was considered to be a significant cross reaction.\textsuperscript{7} Values for NI of 0.1 and 2.6 have been found regularly with normal cattle serums.\textsuperscript{7}
Comparable serum from steer 2 gave NI of 5.7 with type A, 6.0 with type C, no definite cross reactions and a positive VIA reaction. Thus, this was the first time a positive VIA reaction was found with serums from steer 2; steer 1 serums were positive earlier. Serums collected 23 days after type SAT-2 inoculation from steer 1 showed NI cross reactions of 3.0 for SAT-3, 4.3 for Asia-1 and 3.0 for type 0 (Table 2). Comparable serum from steer 2 showed a cross reaction only with Asia-1 (NI 4.1).

Clinical responses less severe than would be expected with completely susceptible cattle were found after inoculation with the succeeding four types of FMDV (SAT-3, Asia-1, SAT-1 and 0).

The inoculation of SAT-3 virus in steer 2 seemed to augment the NI cross reaction with Asia-1 (4.7) and produce a cross reaction with SAT-1 virus (3.5), but a similar response was not found with steer 1 (Table 2). In steer 1 the NI for type A was raised almost a log. The inoculation of Asia-1 virus in steer 1 resulted in a cross reaction with SAT-1 (3.2) and a rise in the NI for type O (3.2). The inoculation of type SAT-1 raised the NI for types A and Asia-1 for steer 1 but not for steer 2. The inoculation of type O raised the NI for type C for steer 2, but not for steer 1.

No clinical evidence of infection was found after inoculation of steer 1 with the last virus, type O, and with the fifth virus, type Asia-1, with steer 2. Only tongue lesions at the inoculation sites were found on steer 1 after inoculation of Asia-1. Serums collected prior to inoculation with these respective viruses had NI of 3.1 with type O for steer 1 and a NI of 4.7 with type Asia-1 for steer 2 (and 4.0 for steer 1, partially resistant). Thus, while steers 1 and 2 received all 7 types of FMDV, they each were clinically ill from FMDV only five times. The hooves of steer 1 had four distinct ridges, which may have been associated with the FMD infections. The hoof ridges on steer 2 were less distinct.

With steer 1, the highest antibody levels (prior to challenge) for types A were found after inoculation of SAT-3 and SAT-1 viruses, for type C after SAT-2 inoculation and for type Asia-1 after SAT-1 inoculation. With steer 2, the type A antibody peak was reached after type C virus inoculation. For all other viruses in both steers the antibody peak (prior to challenge) was found after inoculation of each of the respective viruses (Table 2).

With the exception of type SAT-1, during the six months (184 days) required for inoculation of all 7 viruses, the serum antibodies for the viruses already used were maintained at fairly high levels (NI of 4.4 or more) in both steers. One year after inoculation of the first virus (six months after last virus) NI of 4.4 or more were found only with types A, C, Asia-1 and 0 with steer 1 and only with type A in steer 2. With minor fluctuations, the serum antibody levels continued to decay during the ensuing four years.

Five years after the first virus inoculation only type A antibodies for steer 1 were at a high level (NI 5.5). Type SAT-1 NI were below the level of significance with both steers, as were SAT-2 and SAT-3 NI with steer 2. The serums of both steers continued to give positive VIA reactions. At this time the first attempts were made to determine if the steers were carriers of FMDV. Two collections of OP fluids from both steers failed to infect tissue cultures.

At 5½ years after the first virus inoculation, the two steers were given a challenge inoculation of A22 (the original virus) intramuscularly. The steers showed no clinical evidence of FMD infection. Virus was not detected in blood samples
taken daily for a week. However, a control steer similarly inoculated had clinical
signs and lesions of FMD within 72 hours and viremia for 4 days.

The NI obtained with sera from the two steers before challenge at 5½ years
were not given in Table 2 because they were not significantly different from the NI
found at 5 years. Serums collected from the two steers at 7, 14, 21, 28 and 42 days
after challenge showed a rapid type A antibody rise; steer 1 NI rose from 5.5 to 6.6
and steer 2 NI soared from 3.7 to 6.8 (only the results with the 28th day sera are
given in Table 2). The NI for most of the other viruses each rose about one log.
However, the inoculation seemed to depress type Asia-1 antibody response in both
steers and did very little, if any, stimulation of type SAT-3 antibodies for steer 2.
The NI with steer 2 for type Asia-1 were: 3.3, 3.4, 3.0, 3.4, 2.8, and 2.8 for 0, 7,
14, 21, 28 and 42 days, respectively. Samples of OP fluids from both steers at 28
days after challenge were tested and FMDV was not found.

The next challenge, also at 5½ years (42 days after type A challenge), was an
intramuscular inoculation of a mixture of the other six types of FMDV (excluding
type A). This challenge produced no evidence of clinical infection in either steer.
Virus was not detected in blood samples taken daily for a week. Serums were
collected at 7, 14, 21 and 28 (the only one shown in Table 2) days after challenge.
There was a rapid rise in antibodies for the six virus types inoculated. The NI rose
about one log for most of the types and, with steer 2, about two logs for SAT-3 and
Asia-1. The highest antibody rise was with steer 1 and type SAT-1; the NI were:
3.9, 5.9, 6.8, 7.4 and 6.3 for 0, 7, 14, 21 and 28 days, respectively. Comparable NI
with steer 2 were: 3.6, 5.2, 5.7, 5.2 and 5.2. The NI with type A for the same days
were: with steer 1, 6.6, 6.3, 6.5, 6.3 and 6.1, and with steer 2, 6.8, 6.9, 6.8, 6.3 and
6.6, respectively. The VIA reactions were more strongly positive with the sera 21
and 28 days after this challenge than with sera collected before either challenge
and after the type A challenge. Samples of OP fluids from both steers at 28 days
after challenge were tested and FMDV was not found.

The controls for the challenge with six virus types developed clinical signs and
lesions of FMD within 72 hours. Additional information will be given later under
the designation, steers 25 and 26.

Serums from the two steers were collected 6 years after the first inoculation,
which was 74 days after challenge with the six virus types. The NI for the seven
virus types in their original inoculation sequence, beginning with type A, were: 6.0,
6.1, 5.1, 4.9, 5.0, 6.4 and 5.3 for steer 1 and 6.0, 4.6, 4.6, 4.2, 5.4, 5.4, and 5.1 for
steer 2, respectively (not listed in Table 2).

With serums that were collected from the two steers at 6½ and 7 years, the NI
were reduced so that only with type A were values above 5.0 found.

At 7 years after the first virus, which was 1½ years after the type A challenge,
the steers were re-challenged with subtype A11 intranasally in an attempt to induce
the carrier state. The steers showed no clinical evidence of FMDV infection and a
viremia could not be detected. Attempts to isolate FMDV from the OP fluids at 14,
21 and 28 days after inoculation failed. However, a bovine enterovirus was isolated
from OP fluids of both steers. Some of the sera collected 28 days after challenge
had lower NI values than before challenge. Type A antibody was not stimulated.

At 9 years the steers were given the final challenge with subtype A24 virus
intranasally. No clinical evidence of FMDV infection was noted and viremia was not
detected. However, type A virus was isolated from OP fluids collected from steer 1.
for as long as 7 days and from steer 2 for as long as 42 days after challenge (Table 3). Serum was collected from the two steers 28 days after challenge. The NI for types A, C and SAT-3 were raised by the challenge inoculation and the other types remained essentially unchanged for both steers. Only type A NI were above the 5.0 level. Steer 1 generally had higher antibody levels for the 7 viruses than did steer 2, but they finished close to the same level. The serum VIA reactions were still very strongly positive.

With steers 1 and 2, positive VIA reactions were continuously found for the five years after the first FMDV exposure. However, the reaction was weaker than at one year. The VIA antibodies came back to give their strongest reaction 28 days after challenge with the six virus types. At 9 years, the VIA reactions were again at lower levels, but after the final challenge with A24 the VIA reactions were stronger once more.

Controls for the second and some of the subsequent virus inoculations of steers 1 and 2 and the deer (3) were the first two virus inoculations of steers 4 through 9.

*Animal 3.* The deer initially was placed in a room with a steer, two pigs, two sheep and two goats for one hour exposure to an aerosol of FMDV A-119. The titer of FMDV in the air near the animals was about $10^{3.2}$ bovine ID$_{50}$ units/ml of the diluting fluid used to recover the virus from the filter pad.* After exposure the various species were placed in separate rooms. The controls (i.e. the steer, sheep, goats and pigs) all showed clinical signs and lesions of FMD infection and their sera had type A FMDV antibodies and positive VIA reactions. The clinical response of the pigs was less severe than that of the other animals.

The deer did not show clinical signs or lesions, FMDV antibodies were not found in the serum and the VIA test was negative. Fourteen days after exposure the deer resisted challenge with FMDV A-119 given intramuscularly. Fifteen days later the serum NI in virus sequence, beginning with type A, were: 3.4, 1.4, 1.3, 1.6, 1.3, 1.0 and 1.3, respectively (not given in Table 4). The VIA test was negative. At this time (29 days after aerosol exposure), the deer again resisted challenge with FMDV A-119, which was inoculated in tongue epithelium and coronary band of both front feet. The two pigs were susceptible to challenge with a similar dosage given by the same routes. Serum collected from the deer 23 days after the second challenge had a NI of 4.9 with type A FMDV and for the other types as given in Table 4. The VIA test with this serum was weakly positive.

At 23 days after the second type A challenge or 52 days after aerosol exposure, the deer was inoculated with $10^5$ bovine ID$_{50}$ units of type SAT-1 FMDV. Within 24 hours the deer had clinical signs and tongue lesions of FMD. Foot lesions were found at 48 hours after inoculation. Viremia was detected at 24, 48 and 72 hours after inoculation with titers of 2.8, 4.1 and 3.2 mouse LD$_{50}$/ml, respectively. The clinical severity was rated at only 2+ (Table 1). This was the only time the deer exhibited clinical signs of infection (salivation, fever, lameness) following inoculation with any of the FMDV types. Serums collected 22 days after this inoculation had NI of 5.5 with type A, 6.6 with type SAT-1, and cross reactions with types O and SAT-3. A strong positive VIA reaction was found with the serum.

Type O FMDV was inoculated 22 days after type SAT-1. No clinical signs or lesions of FMD were found. Serum collected 31 days later had NI of 5.5 with type

A, 6.6 with type SAT-1; 5.2 with type O and a cross reaction with type SAT-3 (Table 4).

Type C FMDV was inoculated 31 days after type O. No clinical signs of FMD infection were found. However, a small (5mm. in dia.) lesion was found on the tongue at one inoculation site about 72 hours after inoculation. Serum collected 36 days later had NI of 5.0 for type C, continued response to other types previously inoculated and cross reactions with types SAT-3 and Asia-1.

The deer did not have clinical signs or lesions following inoculation of the next three virus types, SAT-2, SAT-3 and Asia-1, which were inoculated at intervals of 36, 20 and 25 days, respectively. Serum collected 25 days after the last virus, Asia-1, had NI of 4.2 or more to all 7 types of FMDV. At 421 days after the first virus exposure or 216 days after the last virus inoculation, serum from the deer had NI with the respective viruses in sequence of inoculation as follows: 6.0, 6.2, 4.9, 4.9, 4.5, 4.3 and 4.5 (not given in Table 4).

Steers 4 and 5. Maximal clinical responses were observed with steers 4 and 5 following inoculation of the first 2 virus types, Asia-1 and A, and reduced clinical severity with the other viruses that produced clinical infection (Table 1). No evidence of clinical infection was found with steer 4 following inoculation of type SAT-3. Both steers were resistant to a final challenge with type Asia-1, the virus that started the sequence. Since the NI were similar for both steers, only those obtained with sera from steer 4 were included in Table 4. Significant NI cross reactions were found with types C, SAT-1, SAT-2, and SAT-3. With serum from steer 4, a NI of 3.4 with type SAT-3 was found before the steer received this virus and resisted clinical infection. Steer 5 also had a serum NI of 3.4 with type SAT-3, but clinical infection resulted after inoculation.

Steer 6. The clinical response of steer 6 to inoculation with the first four virus types (SAT-2, SAT-3, O and A) were considered nearly maximal reactions. No clinical evidence of infection was found after inoculation with the fifth virus type, Asia-1, nor after challenge with the first virus type SAT-2 (Table 1). A NI of 4.6 was found with Asia-1 virus for serum collected before the steer resisted clinical infection with this virus. Neutralization test cross reactions also were found with types C and SAT-1 after the steer’s third virus inoculation, type O (Table 4).

Steer 7. The first three types of FMDV (SAT-2, C and A) given to steer 7 resulted in typical severe clinical responses and the last two types (SAT-2 and SAT-3) produced a less severe illness without foot lesions. Unlike the other steers, steer 7 did not resist a challenge inoculation with the starting virus, SAT-2. However, the lesions were confined to the tongue inoculation sites. Neutralization test cross reactions were found with types A, SAT-1, SAT-3 and Asia-1 (Table 5).

Steer 8. Typical severe clinical responses in steer 8 were observed after inoculation of the first three virus types (SAT-3, Asia-1 and C) and less severe reactions with the third and fourth viruses (SAT-1 and SAT-2). No clinical evidence of FMD was found after challenge with the first virus (SAT-3). The NI results revealed cross reactions with types SAT-1, SAT-2 and A (Table 5).

Steer 9. The clinical responses of steer 9 to types SAT-1 and SAT-2 were nearly maximal reactions and with types SAT-3 and Asia-1 the responses were less severe. With the latter two viruses, foot lesions were not observed. The steer resisted challenge with the starting virus, SAT-1. With the sera, cross reactions were found with types Asia-1 and A, but not with SAT-3 (Table 5).
**Bull 10.** Only two viruses (SAT-3 and C) were used with bull 10 and nearly maximal reactions were found following inoculation of each virus. The tongue, feet and severity notations for both of the two viruses were, respectively: 1, 1, 3+ (Table 6). The type C virus was C3 CANEFA-3, not strain C-149 as was used with the preceding animals. The NI results revealed cross reactions with all the other types not used to inoculate the bull, A, O, SAT-1, SAT-2 and Asia-1 (Table 6).

**Steers 11 to 22.** These 12 steers were used in an experiment to determine if cattle recovered from infection with various different (5) subtypes of type A FMDV would be susceptible to the Middle East variant, subtype A22. When this variant subtype occurred in the field, the then current vaccines made with A10 and other subtypes of type A virus failed to protect cattle from infection.15

Each of the following subtypes were inoculated in the tongue of two steers: A1, A3, A4, A8, and A32. The clinical reactions of each of the steers was graded 4+ in severity and tongue lesions appeared within one day. Foot lesions were found after one day with steers 11 and 16, after two days with steers 12, 14, 15, 17, 18, 21 and 22 and after three days with steer 13. After recovery from the first virus infection, about 28 to 35 days postinoculation, (DPI) steers 11 (A1), 12 (A3), 15 (A4), 17 (A8) and the contact control, No. 19 were exposed for 7 days to steer 20, which had been inoculated intranasally with A22 virus three days before the exposure period began. Steers 12 (A1), 14 (A3), 16 (A4), 18 (A8), 21 (A32) and 22 (A32) housed in other isolated rooms were challenged by intranasal inoculation with A22 virus (Table 7).

Previously unexposed steer 20 was inoculated with 3.0 ml of a 10^{-2} dilution of A22 FMDV in each nostril. The total dosage was about 10^{7.5} mouse LD_{50} units of virus. Viremia was found at 2 and 3 DPI with titers of 10^{4.1} and 10^{4.5}, respectively. Clinical signs and lesions of the tongue and feet appeared on the third day and severity was graded 4+. On the third day steer 20 was moved into a room with steers 11, 13, 15, 17 and 19. Seven days later steer 20 was removed from the room. The titer of FMDV in the OP fluids of steer 20 in log_{10} PFU/ml was 2.0, 1.1, 1.9 and 2.0 at 7, 14, 21, and 28 DPI, respectively. (Table 8). Serums from steer 20 at 14 and 28 DPI had NI with A22 virus of 5.8 and 6.2, respectively (Table 7).

Steer 19, the previously unexposed contact control, had a fever and clinical signs of FMD three days after exposure. The beginning lesions of the tongue and feet became more advanced by the fourth day and the clinical severity was graded 4+. The virus titers of the OP fluids were slightly higher than those of steer 20 at 7 and 28 days (Table 8).

Steer 17, which had recovered from subtype A8 infection, had clinical signs, tongue and foot lesions of FMD six days after contact exposure to the A22 infected steers. The clinical severity was graded 2+. Tongue lesion tissue was harvested and the virus isolated from it was type A. Type A virus was also isolated from OP fluids collected at 7, 14, 21 and 28 days after exposure. Except for the 7th day, the virus titers were similar to those of inoculated steer 20 (Table 8).

None of the other steers developed clinical signs or lesions of FMD following exposure to subtype A22 virus by contact or by intranasal inoculation. The steers that were inoculated intranasally with FMDV subtype A22 received 1.0 ml of a 10^{-2} dilution of virus in each nostril. The total dosage was about 10^{7.0} mouse LD_{50} units of virus. Serums from all of the steers had high NI (5.7 to 7.6) 28 days after inoculation with their respective initial virus subtypes (Table 7). The O day
serum results with the heterologous subtype A22 virus were somewhat lower than those with the homologous virus, varying from NI 4.5 to 5.4. After exposure to subtype A22 the NI rose for this virus, varying from 4.9 to 7.0.

Following infection with the first virus, 11 of the 12 steers became carriers; virus was continually found in their OP fluids from 7 to 28 days (Table 8). Steer 11 was the exception, virus was not found after infection with subtype A1 virus. However, after exposure to A22 virus by contact, steer 11 became a carrier of A22 virus. Subtype determinations were not made with the viruses isolated 7 days after exposure to A22 virus. At 28 days after exposure to A22 virus all virus isolates were subtype A22. A decline in OP fluid virus titers at 14 days after inoculation or exposure was found with 17 of the 21 carrier states studied with the 12 steers. The lowest virus titer was found at 21 days in six cases and at 28 days in five cases.

**Steers 23 and 24.** These two steers were inoculated on the tongue epithelium with type SAT-1/4 virus as part of a serology and carrier study. Clinical signs and lesions of FMD were found within 24 hours and foot lesions appeared on the second day after inoculation. The clinical severity was graded 4+. Type SAT-1 virus was found in the OP fluids of both steers at 7 DPI (Table 9). At 14 and 21 DPI virus was found in the OP fluids of steer 24, but not in steer 23. At 27 DPI when the steers had recovered from the SAT-1/4 infection, they again had clinical signs of FMD and tongue and foot lesions were found as the result of an accidental infection. The clinical severity was graded 4+. Tongue tissue was harvested and virus was isolated, which proved to be type O. Serum samples were collected at 7, 14, 21, 27, 41, and 48 DPI. All showed relatively high NI with type SAT-1/4 virus. Serums from both steers had high NI with type O1 virus at 41 and 48 DPI. At 27 DPI, O1 virus NI at lower levels were found (3.3 and 4.7). The NI with O1 virus of 3.4 and 3.1 at 14 and 21 DPI with serums of steer 23 were thought to be cross reactions because they occurred before the probable time of infection with type O virus.

The accidental infection with type O virus was believed to have been caused by an air leak that was found in a door gasket so that infectious aerosols were drawn into the room because of the lower air pressure. Three steers in the acute clinical stage of infection with subtype O1 CANEFA-2 FMDV were moved through the adjacent corridor and autopsy room 5 days before steers 23 and 24 had clinical signs and lesions of FMDV type O.

**Steers 25 and 26.** These two steers were used to determine the response of cattle to the intramuscular inoculation of a mixture of six FMDV types and they also served as controls for the challenge of steers 1 and 2. The dosage of each type of virus was adjusted in accordance with its ability to produce gross lesions when inoculated via the intramuscular route as determined in a previous study. The total virus dosage was approximately $10^6$ bovine LD$_{50}$ units in a volume of 5.0 ml. On the second day after inoculation the two steers had viremia, fever, salivation and lesions of the tongue and feet. Material from each lesion of the mouth and feet was harvested when full development was reached on the second or third day.

Steer 25 had 6 individual oral lesions (5 on the tongue) and lesions of all 4 feet. The virus type isolated from all 10 lesion sites was SAT-1. At 7 and 110 DPI, SAT-1 virus was isolated from the esophageal-pharyngeal fluid of this steer. SAT-1 FMDV also was isolated from the blood of steer 25, at 2 and 3 DPI, with the highest titer at 2 DPI. However, at 4, 6 and 7 DPI, type SAT-2 was isolated from the blood. Viremia was not detected at 5 DPI.
In keeping with the lesions and initial viremia findings, serum samples collected at 7 and 14 DPI had the highest NI with SAT-1 virus (Table 10). At 21 and 28 DPI the NI were highest with SAT-2 virus. The NI response with types C, SAT-3, Asia-1 and O also were good, but at lower levels. Only with steer 25, was a cross reaction with type A virus evident (Table 10).

Steer 26 had only 2 individual tongue lesions and lesions on all 4 feet. The virus isolated from these 6 lesions was type C. Type C virus was isolated from the esophageal-pharyngeal fluid from this steer at 7 but not at 110 DPI. The viremia was the type SAT-2 throughout its course from 2 to 6 DPI. The highest titers were found at 3 and 4 DPI. Serum samples taken at 7, 14, 21, 28 and 74 DPI had consistently high NI with type C virus, and from 14 to 74 days with SAT-2 virus. These were the viruses found in the lesions and viremia, respectively. The other viruses were slower in showing a NI rise. The initial NI response to type O virus at 7, 14 and 21 DPI probably was a cross reaction, which diminished at 28 and 74 DPI. No cross reaction with type A was found with steer 26.

DISCUSSION

The viremic blood samples from the two monkeys used for the initial inoculations of steers 1 and 2 essentially performed as modified live virus vaccines; the steers did not show clinical signs or lesions and both were resistant to challenge with the homologous virus. Steer 1 had a good antibody response to type A virus and a positive VIA reaction, which indicated that a subclinical infection had occurred. Steer 2 had a poor antibody response and a negative VIA reaction, which was interpreted as only antigenic stimulation probably without virus replication.

The eight steers (1 and 2 and 4 through 9) that were inoculated with from 4 to 7 types of FMDV in various sequences all showed a reduction in the clinical severity of FMD beginning with the third, fourth or fifth virus type inoculated. Steer 4 was resistant to the fourth virus, steers 2 and 6 to the fifth and steer 1 to the seventh of the virus types inoculated. Thus, there was a cumulative or combined effect which reduced the severity or blocked clinical expression of infection. However, the inoculation of steer 2 with type O, the last virus, was an exception; the clinical severity was only slightly reduced.

The antibody levels (cross reactions), as indicated by the NI of the sera before some of the viruses were inoculated, generally correlate with the reduction in clinical severity or resistance to the particular virus types. The NI (cross reactions) with the deer’s serum before types O1, SAT-3 and Asia-1 were given could account for his complete resistance to inoculation with those types, and the lack of antibody to SAT-1 agrees with his susceptibility to this virus. However, the serological and clinical results with types C and SAT-2 do not agree. Also, the results with type A were unusual; the deer showed no clinical or serological evidence of type A infection following aerosol exposure, yet he was resistant to challenge with type A. The deer’s susceptibility to type SAT-1 may have some relationship to the fact that this strain of SAT-1 was originally isolated from a greater kudu while all other viruses were of cattle origin.

There was some evidence to support “the doctrine of original antigenic sin”.

The antibody level to the original virus was boosted by one or more of the succeeding viruses in animals 1, 2, 3, 6, 7, and 8, was only gradually increased in steer 4 and 5 and was gradually decreased in steer 9. However, when steers 1 and 2
were given the challenge inoculation containing all the virus types except the original, the antibody level for the original virus was not raised. This probably would have been a different result if the type A challenge had not been given just before the six virus challenge and the antibodies for type A were at the 5 year lower level.

The type A immunity resulting after recovery from infection with subtypes A₁, A₃, A₄, A₈ and A₃₂ was adequate to prevent clinical signs and lesions of FMD in 9 of 10 steers exposed to superinfection with subtype A₂₂, the Middle East variant. However, local immunity and the presence of the original FMDV did not prevent establishment of the A₂₂ virus as a throat infection; all 10 steers became carriers of subtype A₂₂, when previously 9 of the 10 steers were carriers of the first virus they received. A similar finding, but with type O virus superceding type A virus, was reported by Fellowes and Sutmoller.¹⁰ Likewise, the accidental reinfection of steers 23 and 24 resulted in type O superceding type SAT-1. Thus, apparently a second FMD virus may readily supercede the original virus as a throat infection.

Several unsuccessful attempts were made to find FMDV in the OP fluids of steers 1 and 2 five years after their virus inoculation and after their intramuscular challenge. They were challenged intranasally with subtype A₁₁ FMDV 7 years after their first exposure and this treatment also did not result in establishing a detectable carrier state. However, the subtype A₂₄ intranasal challenge 9 years after the first virus exposure was successful. While the virus was established in the throat of both steers for the first 7 days, only steer 2 would be considered a carrier, since FMDV was found in the OP fluids of this steer for as long as 42 days after challenge.

The mixed infection with six different types of FMDV given to steers 25 and 26 demonstrated that, with the exception of type O virus in steer 26, all six viruses probably were replicated somewhere in the body of the animals. However, only types SAT-1 and SAT-2 in one steer and only types C and SAT-2 in the other steer were isolated from the lesions, OP fluids and blood. The NI of the serums also was higher with these viruses than with the others.

The competition between viruses in a mixed infection could be called competitive avidity, and it seems to be an important factor in determining which will predominate. But this may be influenced greatly by the individual host and the virus dosage. Type C virus also was the predominant virus in the mixed infections reported by Cunha et al.⁹, and type O virus also appeared to be the least avid in other studies on mixed FMDV infections.¹²,¹³ However, as a throat infection, a second virus apparently may readily supercede the first virus.

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REFERENCES


Table 1. Clinical Response of Eight Steers and a Deer to Consecutive Inoculation with from Four to Seven Types of Foot-and-Mouth Disease Virus

<table>
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<tr>
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<td>SAT-2 C</td>
</tr>
<tr>
<td></td>
<td>1,2,3+</td>
</tr>
<tr>
<td>7</td>
<td>SAT-2 Asia-1 C SAT-1 SAT-2 SAT-3</td>
</tr>
<tr>
<td></td>
<td>1,2,3+</td>
</tr>
<tr>
<td>8</td>
<td>SAT-2 SAT-3 Asia-1 SAT-1 - -</td>
</tr>
<tr>
<td></td>
<td>1,3,4+</td>
</tr>
</tbody>
</table>

*F, S, T = tongue, feet, severity. The entries under T and F indicate the days after inoculation when tongue and foot lesions first appeared and the severity of the general clinical response was graded from 1+ to 4+.

** The inoculation interval between heterologous FMD virus types varied from 15 to 44 days.
Table 2. Neutralization Indexes of Serums From Steers 1 and 2 After Consecutive Inoculation of the Seven Types of Foot-and-Mouth Disease Virus and for Nine Years.

<table>
<thead>
<tr>
<th>Test Viruses</th>
<th>Serums in sequence of virus inoculations*</th>
<th>Steer No. 1</th>
<th>Steer No. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT-2</td>
<td>1.3, 1.1, 1.6, 1.9, 5.1, 5.0, 1.1, 1.5</td>
<td>4.7, 3.3, 3.0, 3.2, 3.1, 3.3, 3.0, 3.4</td>
<td>3.0, 3.0, 3.0, 3.0, 3.0, 3.0, 3.0, 3.0</td>
</tr>
<tr>
<td>SAT-3</td>
<td>1.3, 1.4, 0.7, 2.7, 3.0, 5.6, 0.4, 4.7</td>
<td>3.7, 3.8, 4.2, 3.6, 2.7, 3.2, 4.1, 3.9, 3.0, 3.8, 4.3</td>
<td>3.9, 4.0, 4.3, 4.2, 4.4, 4.6, 4.3, 4.3, 4.3, 4.4, 4.3</td>
</tr>
<tr>
<td>Acta-1</td>
<td>0.9, 1.2, 0.7, 2.0, 4.3, 4.0, 5.0, 5.6, 5.0</td>
<td>4.5, 4.6, 3.7, 4.6, 4.2, 4.3, 3.9, 5.2, 3.8, 4.3, 5.1, 5.0</td>
<td>5.0, 5.1, 5.0, 5.0, 5.0, 5.0, 5.0, 5.0, 5.0, 5.0</td>
</tr>
<tr>
<td>SAT-1-</td>
<td>1.3, 1.1, 1.6, 2.6, 2.3, 2.1, 3.2, 5.4, 3.7</td>
<td>3.9, 4.0, 3.7, 4.4, 4.1, 2.5, 3.9, 6.3, 4.5, 4.0, 4.3, 4.2, 4.4, 4.3</td>
<td>4.5, 4.6, 3.7, 4.6, 4.2, 4.3, 3.9, 5.2, 3.8, 4.3, 5.1, 5.0, 5.0, 5.0</td>
</tr>
<tr>
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<td>0.7, 1.8, 1.1, 3.2, 3.0, 2.2, 3.2, 3.1, 3.2</td>
<td>4.5, 4.6, 3.7, 4.6, 4.2, 4.3, 3.9, 5.2, 3.8, 4.3, 5.1, 5.0, 5.0, 5.0</td>
<td>4.5, 4.6, 3.7, 4.6, 4.2, 4.3, 3.9, 5.2, 3.8, 4.3, 5.1, 5.0, 5.0, 5.0</td>
</tr>
</tbody>
</table>

*Serums were collected and successive virus inoculations were made at the following intervals in days corresponding to the first 9 columns: 0, 14, 15, 36, 23, 25, 21, 22, 28. The Neutralization indexes (NI) to the right of the stepped lines are those obtained after the inoculation of the respective viruses. Thus, the sera have increasing virus type valencies. A NI of 2.9 or more is considered significant. Ch. A = Challenges with type A, which were made at 14 days, 5%, 7% and 9% years after first virus inoculation. Ch. 6 types = Challenge with a mixture of 6 FMDV types at 5% years (42 days after the type A challenge).

**No clinical signs after inoculation of type O FMDV for steer 1 and after type Asia-1 for steer 2.
**TABLE 3.** Titters of Type A Foot-and-Mouth Disease Virus in Oesophageal-Pharyngeal Fluids from Steers 1 and 2 After Intranasal Challenge Nine Years After Initial Virus Inoculations

<table>
<thead>
<tr>
<th>Days after challenge</th>
<th>Steer Nos. 1</th>
<th>Steer Nos. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>2.4*</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>0.6</td>
<td>2.6</td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>2.1</td>
</tr>
<tr>
<td>4</td>
<td>0.9</td>
<td>3.1</td>
</tr>
<tr>
<td>7</td>
<td>1.6</td>
<td>2.5</td>
</tr>
<tr>
<td>14</td>
<td>N</td>
<td>2.0</td>
</tr>
<tr>
<td>21</td>
<td>N</td>
<td>1.1</td>
</tr>
<tr>
<td>28</td>
<td>N</td>
<td>1.6</td>
</tr>
<tr>
<td>35</td>
<td>N</td>
<td>1.3</td>
</tr>
<tr>
<td>42</td>
<td>-</td>
<td>P</td>
</tr>
<tr>
<td>49</td>
<td>-</td>
<td>N</td>
</tr>
</tbody>
</table>

*Titers of FMDV in OP fluids treated with trichlorotrifluoroethane in log_{10} PFU/ml. N= negative, P= positive, - = not done.
Table 4. Neutralization Indexes of Serums From Animals 3, 4, and 6 After Consecutive Inoculations of Various Types of Foot-and-Mouth Disease Viruses

Serums in Sequence of Virus Inoculations*

<table>
<thead>
<tr>
<th>Test Viruses</th>
<th>none</th>
<th>Ch**</th>
<th>SAT-1</th>
<th>O</th>
<th>C</th>
<th>SAT-2</th>
<th>SAT-3</th>
<th>Asia</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
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<td>4.9</td>
<td>5.5</td>
<td>5.5</td>
<td>5.8</td>
<td>6.8</td>
<td>6.4</td>
<td>6.4</td>
</tr>
<tr>
<td>SAT-1</td>
<td>1.3</td>
<td>1.1</td>
<td>6.6</td>
<td>6.6</td>
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</tr>
<tr>
<td>0</td>
<td>0.9</td>
<td>1.4</td>
<td>3.4</td>
<td>5.2</td>
<td>5.7</td>
<td>5.3</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>C</td>
<td>1.0</td>
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<td>2.0</td>
<td>5.0</td>
<td>4.9</td>
<td>5.0</td>
<td>4.8</td>
</tr>
<tr>
<td>SAT-2</td>
<td>0.6</td>
<td>1.6</td>
<td>2.5</td>
<td>2.0</td>
<td>2.2</td>
<td>4.5</td>
<td>4.7</td>
<td>4.2</td>
</tr>
<tr>
<td>SAT-3</td>
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<td>2.0</td>
<td>2.9</td>
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<td>3.4</td>
<td>3.3</td>
<td>4.7</td>
<td>4.3</td>
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<td>1.8</td>
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<td>2.7</td>
<td>2.8</td>
<td>3.0</td>
<td>3.2</td>
<td>3.3</td>
<td>4.6</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Test Viruses</th>
<th>none</th>
<th>Asia</th>
<th>A</th>
<th>SAT-1</th>
<th>SAT-3</th>
<th>O</th>
<th>Asia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia-1</td>
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<td>5.4</td>
<td>5.7</td>
<td>5.6</td>
<td>5.8</td>
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<td>6.0</td>
<td>6.6</td>
<td>5.7</td>
<td>5.6</td>
<td>5.8</td>
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<tr>
<td>SAT-1</td>
<td>0.7</td>
<td>1.9</td>
<td>2.9</td>
<td>6.5</td>
<td>6.8</td>
<td>5.8</td>
<td>5.7</td>
</tr>
<tr>
<td>SAT-3</td>
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<td>2.9</td>
<td>2.5</td>
<td>3.4</td>
<td>4.4</td>
<td>4.7</td>
<td>3.9</td>
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<td>1.4</td>
<td>1.2</td>
<td>2.0</td>
<td>2.0</td>
<td>5.3</td>
<td>3.6</td>
</tr>
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<td>C</td>
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<td>2.7</td>
<td>3.0</td>
<td>2.3</td>
<td>2.6</td>
<td>3.9</td>
<td>3.1</td>
</tr>
<tr>
<td>SAT-2</td>
<td>1.4</td>
<td>3.1</td>
<td>3.2</td>
<td>3.0</td>
<td>3.1</td>
<td>4.1</td>
<td>2.9</td>
</tr>
</tbody>
</table>
TABLE 4. (Continued from preceding page)

<table>
<thead>
<tr>
<th>Test Viruses</th>
<th>none</th>
<th>SAT-2</th>
<th>SAT-3</th>
<th>O</th>
<th>A</th>
<th>Asia</th>
<th>SAT-2</th>
</tr>
</thead>
<tbody>
<tr>
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<td>3.5</td>
<td>3.8</td>
<td>5.3</td>
<td>3.8</td>
<td>4.0</td>
<td>5.4</td>
</tr>
<tr>
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<td>2.2</td>
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<td>4.2</td>
<td>4.6</td>
</tr>
<tr>
<td>O</td>
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<td>0.8</td>
<td>1.5</td>
<td>4.8</td>
<td>5.5</td>
<td>5.9</td>
<td>3.9</td>
</tr>
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<td>2.2</td>
<td>6.0</td>
<td>6.0</td>
<td>5.9</td>
</tr>
<tr>
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<td>3.2</td>
<td>4.1</td>
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<td>3.9</td>
<td>3.6</td>
<td>3.3</td>
</tr>
</tbody>
</table>

*Serums were collected and the succeeding heterologous viruses were inoculated at intervals that varied from 1½ to 4½ days. The neutralization indexes to the right of the stepped lines are those obtained after the inoculation of the respective viruses (i.e. the serums have increasing virus valencies). See Results for steer 5.

** Ch. A = challenge with type A. The NI listed were after 2nd. challenge. See results for NI after aerosol exposure and 1st. challenge of deer.

† No clinical signs or lesions after inoculation.
Table 5. Neutralization Indexes of Serums from Steers 7, 8, and 9 After Consecutive Inoculations of Various Types of Foot-and-Mouth Disease Viruses

Serums in Sequence of Virus Inoculations*

<table>
<thead>
<tr>
<th>Test</th>
<th>Viruses</th>
<th>none</th>
<th>SAT-2</th>
<th>C</th>
<th>A</th>
<th>SAT-1</th>
<th>SAT-3</th>
<th>SAT-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT-2</td>
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<td>4.9</td>
<td>4.7</td>
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<td>3.6</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.0</td>
<td>1.3</td>
<td>6.3</td>
<td>6.3</td>
<td>6.2</td>
<td>5.7</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.1</td>
<td>1.6</td>
<td>3.2</td>
<td>6.6</td>
<td>6.6</td>
<td>6.5</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>SAT-1</td>
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<td>1.6</td>
<td>3.3</td>
<td>3.9</td>
<td>6.1</td>
<td>5.7</td>
<td>5.1</td>
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</tr>
<tr>
<td>SAT-3</td>
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<td>1.2</td>
<td>2.1</td>
<td>2.2</td>
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<td>4.8</td>
<td>4.3</td>
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<td>2.0</td>
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<tr>
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<td>2.5</td>
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<table>
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<th>Test</th>
<th>Viruses</th>
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<th>Asia</th>
<th>C</th>
<th>SAT-1</th>
<th>SAT-2</th>
<th>SAT-3</th>
</tr>
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<tbody>
<tr>
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<td>3.9</td>
<td>5.0</td>
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</tr>
<tr>
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<td>2.1</td>
<td>2.1</td>
<td>5.5</td>
<td>6.1</td>
<td>5.3</td>
<td>5.6</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.0</td>
<td>1.3</td>
<td>2.2</td>
<td>6.2</td>
<td>5.8</td>
<td>6.0</td>
<td>5.9</td>
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</tr>
<tr>
<td>SAT-1</td>
<td>0.9</td>
<td>1.5</td>
<td>2.3</td>
<td>3.2</td>
<td>6.4</td>
<td>6.0</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
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<td>1.1</td>
<td>3.0</td>
<td>2.0</td>
<td>2.9</td>
<td>4.5</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>A</td>
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<td>2.3</td>
<td>1.0</td>
<td>3.0</td>
<td>2.0</td>
<td>1.8</td>
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<tr>
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<td>1.2</td>
<td>1.7</td>
<td>1.1</td>
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<td>1.3</td>
<td>1.6</td>
<td>1.4</td>
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</table>

(Continued on next page)
TABLE 5. (Continued from preceding page)

<table>
<thead>
<tr>
<th>Test Viruses</th>
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<th>SAT-1</th>
<th>SAT-2</th>
<th>SAT-3</th>
<th>Asia</th>
<th>SAT-1</th>
</tr>
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<td>SAT-2</td>
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<td>Asia-1</td>
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<td>4.4</td>
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<td>3.1</td>
<td>3.2</td>
<td>2.8</td>
<td>1.7</td>
</tr>
<tr>
<td>O</td>
<td>1.3</td>
<td>1.2</td>
<td>1.6</td>
<td>2.2</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>C</td>
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<td>1.1</td>
<td>2.3</td>
<td>1.2</td>
<td>2.7</td>
<td>2.8</td>
</tr>
</tbody>
</table>

* Serums were collected and the succeeding viruses were inoculated at intervals that varied from 17 to 29 days. The neutralization indexes to the right of the stepped lines are those obtained after the inoculation of the respective viruses (i.e. the serums have increasing virus valencies).
Table 6. Neutralization Indexes of Serums from Bull 10 After Inoculation with Types SAT-3 and C Foot-and-Mouth Disease Viruses

<table>
<thead>
<tr>
<th>Test Viruses</th>
<th>None</th>
<th>SAT-3</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT-3</td>
<td>1.5</td>
<td>4.2</td>
<td>4.3</td>
</tr>
<tr>
<td>C</td>
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</tr>
<tr>
<td>O</td>
<td>1.8</td>
<td>2.9</td>
<td>3.2</td>
</tr>
<tr>
<td>SAT-1</td>
<td>1.6</td>
<td>1.8</td>
<td>2.9</td>
</tr>
<tr>
<td>SAT-2</td>
<td>1.4</td>
<td>1.6</td>
<td>3.1</td>
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<tr>
<td>Asia-1</td>
<td>1.5</td>
<td>2.1</td>
<td>4.6</td>
</tr>
</tbody>
</table>

*Serums were taken 84 days after inoculation of SAT-3 and 21 days after type C (CAMEFA-3). Tongue and foot lesions appeared one day after inoculation and the clinical severity was graded 3+ for both virus infections.
Table 7. Neutralization Indexes of Serums From Steers After Inoculation With Subtypes of Type A and After Challenge with Middle East Variant A_{22} Foot-and-Mouth Disease Virus.

<table>
<thead>
<tr>
<th>Steer nos.</th>
<th>First virus</th>
<th>Days after homologous test viruses</th>
<th>Exposition method</th>
<th>Days after A_{22} test virus</th>
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<td></td>
<td></td>
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<td>28</td>
<td>A_{22} virus</td>
</tr>
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<td>6.4</td>
<td>Contact</td>
</tr>
<tr>
<td>12</td>
<td>A_{1}</td>
<td>6.2</td>
<td>6.4</td>
<td>Inoc.</td>
</tr>
<tr>
<td>13</td>
<td>A_{3}</td>
<td>6.0</td>
<td>7.6</td>
<td>Contact</td>
</tr>
<tr>
<td>14</td>
<td>A_{3}</td>
<td>5.9</td>
<td>7.6</td>
<td>Inoc.</td>
</tr>
<tr>
<td>15</td>
<td>A_{4}</td>
<td>6.3</td>
<td>6.1</td>
<td>Contact</td>
</tr>
<tr>
<td>16</td>
<td>A_{4}</td>
<td>6.1</td>
<td>6.3</td>
<td>Inoc.</td>
</tr>
<tr>
<td>17</td>
<td>A_{8}</td>
<td>5.5</td>
<td>5.7</td>
<td>Contact</td>
</tr>
<tr>
<td>18</td>
<td>A_{8}</td>
<td>5.8</td>
<td>6.0</td>
<td>Inoc.</td>
</tr>
<tr>
<td>19</td>
<td>A_{22} (control)</td>
<td></td>
<td></td>
<td>Contact</td>
</tr>
<tr>
<td>20</td>
<td>A_{22} (infected)</td>
<td></td>
<td></td>
<td>Inoc.</td>
</tr>
<tr>
<td>21</td>
<td>A_{32}</td>
<td>5.8</td>
<td>6.1</td>
<td>Inoc.</td>
</tr>
<tr>
<td>22</td>
<td>A_{32}</td>
<td>5.8</td>
<td>5.7</td>
<td>Inoc.</td>
</tr>
</tbody>
</table>

* After recovery from first virus infection about 28 to 35 days after inoculation, steers 11, 13, 15, 17 and the contact control, No. 19, were exposed for 7 days to steer No. 20, which was inoculated with A_{22} virus. Steers 12, 14, 16, 18, 21 and 22 were challenged by inoculation of A_{22} virus. The homologous virus results at 28 days are nearly comparable (in time) to the results at 0 day with the heterologous subtype A_{22} virus.

** Only steer No. 17 had clinical signs and lesions of FMD after challenge with A_{22} virus.
Table 8. Titers of Foot-and-Mouth Disease Viruses in Oesophageal-
Pharyngeal Fluids from Steers After Inoculation with Subtypes of
Type A and After Challenge with Middle East Variant A_{22} Virus.

<table>
<thead>
<tr>
<th>Steer Nos.</th>
<th>First virus</th>
<th>Days after first virus</th>
<th>Exposure Method</th>
<th>Days after A_{22} virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>A_{1}</td>
<td>N N N N</td>
<td>Contact</td>
<td>2.8 1.5 2.6 2.8</td>
</tr>
<tr>
<td>14</td>
<td>A_{2}</td>
<td>2.0 2.8 2.7</td>
<td>Inoc.</td>
<td>2.8 1.8 2.4 2.6</td>
</tr>
<tr>
<td>20</td>
<td>A_{3}</td>
<td>3.1 2.6 2.8 2.6</td>
<td>Contact</td>
<td>2.8 2.5 2.2 2.2</td>
</tr>
<tr>
<td>21</td>
<td>A_{4}</td>
<td>2.4 2.2 2.8 2.1</td>
<td>Contact</td>
<td>2.3 2.9 2.3 2.5</td>
</tr>
<tr>
<td>22</td>
<td>A_{5}</td>
<td>2.0 2.9 1.6 3.0</td>
<td>Inoc.</td>
<td>2.9 2.7 2.8 0.8</td>
</tr>
<tr>
<td>23</td>
<td>A_{6}</td>
<td>2.2 1.1 0.8 2.0</td>
<td>Contact</td>
<td>P** 1.2 1.1 2.0</td>
</tr>
<tr>
<td>24</td>
<td>A_{7}</td>
<td>2.6 2.4 1.1 0.8</td>
<td>Inoc.</td>
<td>0.9 1.4 1.5 1.8</td>
</tr>
<tr>
<td>25</td>
<td>A_{8}</td>
<td>- - - -</td>
<td>Contact</td>
<td>2.8 1.0 0.6 2.5</td>
</tr>
<tr>
<td>26</td>
<td>A_{9}</td>
<td>- - - -</td>
<td>Inoc.</td>
<td>2.0 1.1 1.9 2.0</td>
</tr>
<tr>
<td>27</td>
<td>A_{10}</td>
<td>1.4 1.6 2.1 1.9</td>
<td>Inoc.</td>
<td>2.9 2.2 2.8 2.0</td>
</tr>
<tr>
<td>28</td>
<td>A_{11}</td>
<td>2.4 1.6 1.7 2.5</td>
<td>Inoc.</td>
<td>2.4 1.9 2.5 2.2</td>
</tr>
</tbody>
</table>

* N = negative; P = positive CPE, but titer not found; - = does not apply.
The titers are \( \log_{10} \) PFU/ml.
See footnote to Table 7: for experimental plan.

** Only steer No. 17 had clinical signs and lesions of FMD after challenge.
Table 9. Neutralization Indexes of Serums and Titers of Foot-and-Mouth Disease Viruses in Oesophageal-Pharyngeal Fluids from two Steers After Inoculation with Subtype SAT-1/4 and After Accidental Reinfection with Subtype 01 Virus.

<table>
<thead>
<tr>
<th></th>
<th>Steer 23</th>
<th></th>
<th>Steer 24</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAT-1/4</td>
<td>01</td>
<td>OP fl.</td>
<td>SAT-1/4</td>
</tr>
<tr>
<td>DPI*</td>
<td>NI</td>
<td>NI</td>
<td>Titer</td>
<td>NI</td>
</tr>
<tr>
<td>7</td>
<td>5.4</td>
<td>2.7</td>
<td>2.8</td>
<td>5.5</td>
</tr>
<tr>
<td>14</td>
<td>6.8</td>
<td>3.4</td>
<td>N</td>
<td>5.6</td>
</tr>
<tr>
<td>21</td>
<td>6.6</td>
<td>3.1</td>
<td>N</td>
<td>6.4</td>
</tr>
<tr>
<td>27</td>
<td>6.0</td>
<td>3.3</td>
<td>-</td>
<td>6.3</td>
</tr>
<tr>
<td>41</td>
<td>6.8</td>
<td>6.2</td>
<td>-</td>
<td>5.6</td>
</tr>
<tr>
<td>48</td>
<td>6.5</td>
<td>6.4</td>
<td>1.1</td>
<td>5.5</td>
</tr>
</tbody>
</table>

* DPI = Days postinoculation of SAT-1/4 FMDV, NI= neutralization indexes, OP Fl.=oesophageal-pharyngeal fluid titers in tissue culture PFU/ml. Dotted line indicates that beginning clinical signs and lesions of a second FMDV infection (type 01 CANEFA-2) were first noted at 27 days. Subsequently advanced tongue and foot lesions were found and the severity was graded 4+. The initial SAT-1/4 infection was also graded 4+ and tongue and foot lesions were found one and two days after inoculation, respectively.
Table 10. Neutralization Indexes of Serums of Two Steers Inoculated Intramuscularly with a Mixture of Six Types of Foot-and-Mouth Disease Viruses (Type A not inoculated).

<table>
<thead>
<tr>
<th>Test</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>26*</th>
<th>25*</th>
<th>26**</th>
<th>25*</th>
<th>26**</th>
<th>25*</th>
<th>26**</th>
<th>25*</th>
<th>26**</th>
<th>25*</th>
<th>26**</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.1</td>
<td>1.9</td>
<td>3.8</td>
<td>2.7</td>
<td>3.3</td>
<td>2.6</td>
<td>3.6</td>
<td>2.6</td>
<td>2.6</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>3.8</td>
<td>3.9</td>
<td>3.3</td>
<td>3.3</td>
<td>3.7</td>
<td>2.9</td>
<td>4.2</td>
<td>2.2</td>
<td>3.4</td>
<td>2.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4.5</td>
<td>4.9</td>
<td>5.1</td>
<td>4.9</td>
<td>5.8</td>
<td>4.8</td>
<td>5.1</td>
<td>4.6</td>
<td>4.1</td>
<td>4.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT-1</td>
<td>5.5</td>
<td>4.1</td>
<td>7.1</td>
<td>3.8</td>
<td>6.4</td>
<td>3.7</td>
<td>6.3</td>
<td>4.6</td>
<td>5.4</td>
<td>5.8</td>
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<tr>
<td>SAT-2</td>
<td>2.6</td>
<td>3.2</td>
<td>6.6</td>
<td>6.6</td>
<td>6.6</td>
<td>6.4</td>
<td>6.4</td>
<td>5.9</td>
<td>5.0</td>
<td>4.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT-3</td>
<td>4.7</td>
<td>3.9</td>
<td>4.5</td>
<td>4.6</td>
<td>3.8</td>
<td>5.2</td>
<td>3.7</td>
<td>4.7</td>
<td>3.7</td>
<td>3.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia-1</td>
<td>4.8</td>
<td>2.9</td>
<td>3.9</td>
<td>4.4</td>
<td>4.2</td>
<td>4.4</td>
<td>5.1</td>
<td>3.7</td>
<td>3.6</td>
<td>3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Steer No. 25: Viruses isolated from all tongue and foot lesions and OP fluids were type SAT-1. Viremia on days 2 and 3 was type SAT-1, but was type SAT-2 on days 4, 6 and 7. Tongue and foot lesions were found on 3rd day, and the clinical severity was graded 4+.

** Steer No. 26: Viruses isolated from all tongue and foot lesions and OP fluid was type C. Viremia on days 2 through 6 was type SAT-2. Tongue and foot lesions were found, respectively, on 4th, and 3rd days, and the clinical severity was graded 4+.
AMERICAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS

FOURTEENTH ANNUAL MEETING
OF THE AMERICAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS

(Formerly
Conference of Veterinary Laboratory Diagnosticians)
AMERICAN ASSOCIATION OF
VETERINARY LABORATORY DIAGNOSTICIANS

CONTENTS

Veterinary Diagnostic Laboratory Management—by W. L. Sippel .......... 472
Veterinary Diagnostic Laboratory Records—by N. E. Hutton .......... 475
Personnel Training and Continuing Education—by W. E. Ketter .......... 481
Encephalomyelitis—Vomiting and Wasting Disease Complex of Swine—by
W. L. Mengeling ................................................................. 485
Diagnosis of TGE by FA: Evaluation of Accuracy on Field Specimens—by
J. W. Black ................................................................. 492
Reovirus Infection in Pigs—by J. P. McAdaragh, et al ............... 499
Specific Pathogen Free Breeding Turkeys—the Progress and Outlook—by
W. M. Dungan ................................................................. 506
Lactose Fermenting Salmonellae—A Dilemma for Diagnostic Laboratories?—by
N. W. Rokey and M. D. Mecca ............................................. 509
Sudden Death Syndrome in Feedlot Cattle—by H. D. Anthony .......... 515
Case Report Isolation of M. Paratuberculosis from a cow at Routine Slaughter
—by W. D. Richards and R. L. Muhm ........................................ 518
Assessing and Defining a State’s Veterinary Diagnostic Needs—by S. R.
Nusbaum, et al ................................................................. 523
Need for Personnel Safety and Health Programs in Veterinary Diagnostic
Laboratories—by V. A. Seaton ............................................. 532
Biological Security and Environmental Pollution—by J. F. Sullivan .... 536
Diagnostic Laboratories and Extension Veterinary Medicine—by H. K. Caley
Inter-Relationship of the Diagnostic Laboratories and Regulatory Medicine
—by G. B. Rea ................................................................. 545
Some Diagnostic and Health Problems at the San Diego Zoo—by L. A. Griner
Serum and Plasma Neuraminic Acid Levels in Clinically Healthy Cats and in
Cats Exhibiting Certain Diseases—by M. Inverso ......................... 554
Use of Tracheal Organ Cultures for Virus Propagation and Bioassay of Myco-
toxins—by W. M. Colwell .................................................. 561
Applications of Recommended Fluorescent Antibody Techniques for Viral
Diseases in Veterinary Diagnostic Laboratories—by E. A. Carbrey and
W. C. Stewart ................................................................. 563
Investigations of Epizootic Bovine Abortion in Colorado and South Dakota—
by D. E. Reed, et al ............................................................ 574

468
<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis of Mycotic Abortion in Cattle—by C. A. Kirkbride, et al</td>
<td>580</td>
</tr>
<tr>
<td>An Appraisal of Fetal Serology for the Diagnosis of Bovine Abortion and</td>
<td></td>
</tr>
<tr>
<td>Neonatal Anomalies in Diagnostic Laboratories—by C. M. Hibbs, R. G. White</td>
<td></td>
</tr>
<tr>
<td>and H. W. Leipold</td>
<td>595</td>
</tr>
<tr>
<td>Laboratory Techniques for Demonstrating Nebraska Calf Diarrhea Virus—</td>
<td></td>
</tr>
<tr>
<td>by C. A. Mebus, M. B. Rhodes, and E. L. Stair</td>
<td>599</td>
</tr>
<tr>
<td>Chlamydiosis in Calves—by A. K. Eugster</td>
<td>601</td>
</tr>
<tr>
<td>Health Problems in Intensive Veal Calf Production Units in New York</td>
<td></td>
</tr>
<tr>
<td>State—by A. L. Britt</td>
<td>603</td>
</tr>
<tr>
<td>A Survey of Fee Systems in Veterinary Medical Diagnostic Laboratories—</td>
<td></td>
</tr>
<tr>
<td>by L. G. Morehouse, K. K. Keahey, and V. A. Seaton</td>
<td>608</td>
</tr>
<tr>
<td>Protocol for the Complement-Fixation Test for Equine Piroplasmosis—</td>
<td></td>
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<tr>
<td>by A. Holbrook, et al</td>
<td>617</td>
</tr>
<tr>
<td>Recommended Standard Laboratory Techniques for Diagnosing Infectious</td>
<td></td>
</tr>
<tr>
<td>Bovine Rhinotracheitis, Bovine Virus Diarrhea and Shipping Fever (Para-</td>
<td></td>
</tr>
<tr>
<td>influenza-3)—by E. A. Carbrey, et al</td>
<td>629</td>
</tr>
<tr>
<td>Protocol for the Immunodiffusion (Coggins) Test for Equine Infectious</td>
<td></td>
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<tr>
<td>Anemia—by J. E. Pearson, et al</td>
<td>649</td>
</tr>
</tbody>
</table>
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K. K. Keahey
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Secretary-Treasurer
Of a laboratory director's duties, budgeting is second in importance only to recruiting and holding an excellent staff. If both of these duties are well done they will each make the other easier.

Budgeting for a New Laboratory

A. Building costs

The floor plan should be roughed in by the Director with the aid of architects and visits to other similar laboratories. The director should have a stronger input in the designing of a diagnostic laboratory than is usual for other college or institutional buildings due to the highly specialized nature of the laboratory's purpose and the importance of the working interrelationships between departments.

The cost per square foot for laboratories is known for an area and is rising fantasticallly but predictably. Our laboratory was constructed in 1968 for $33.00 per square foot and now would cost $42.00 per square foot an increase of about 7% per year. Plan enough room at the start as the building will soon be too small. Permanent equipment and expendable supply costs can be estimated quite closely by listing the items needed for each department and using catalogue prices.

B. Budget

Certain costs such as salaries for professional and sub-professional help will be known fairly accurately. This figure should be about 60-70% of the budget. Other categories as permanent equipment additions and operating expense funds will vary with needs but will amount to between $20-$35,000 per department, per year.

Budgeting for an Established Laboratory

This job amount to obtaining enough funds to enable the laboratory to be operated at a high level of competence.

If a laboratory is not able to operate this way it will not be able to keep excellent staff members as they will not be proud of their work. In my opinion pride of accomplishment is the secret of high morale, of outstanding work and is
the hallmark of a good laboratory. This emphasizes the importance of budgeting which should be done very carefully by the laboratory director.

One of the difficulties of budgeting is that budgets usually must be drawn up 2 to 3 years in advance. This leads to several difficulties such as estimation of needs for items of permanent equipment that far ahead, to say nothing of the cost of these items and other changes. One can only profit by past experience, use an escalation factor, or specify equipment not really intended for purchase merely to encumber the funds. Needs for staff in certain areas can increase rapidly as new developments take place. This may leave the laboratory short of needed personnel for one or two years or possibly more if the first request for these positions is not granted.

In some laboratories items ordered near the end of the fiscal year must be physically on hand at the end of the fiscal year or be paid for from the next year’s budget. This adds the problem of reliance on delivery date estimates of suppliers of scientific supplies and equipment, a notoriously hazardous practice.

Ordering many items near the end of the fiscal year is a common practice to “use up” remaining funds. This places a hardship on all people connected with placing the order, on through to delivery—especially if a deadline date is involved. This is a characteristic of poor planning and should not be necessary. However, I venture to say that a very high proportion of Directors present, including the speaker, have been guilty of this practice.

Control of Spending

In order to be able to prevent the above poor budgeting practice it is helpful to have a good bookkeeping service that provides monthly account totals. In these days of computer accounting this can often be accomplished on a daily basis if desired.

By careful attention to accounts on a regular basis, a fiscal awareness on the part of the Director is maintained and spending better controlled.

I feel that it is a good management technique to give each department head some idea of the amount of money he will have for his supplies and for permanent equipment. There is seldom enough money to do the things desired and providing each department head with a budget to live within makes them “cost conscious” and leads to wiser spending.

Retrospective Budget Analysis

Much can be learned by a study of budgets of years past. It is revealing to look back and see how the money was actually spent and for what. How much operating cost money was spent by individual departments and how much for permanent equipment. How much does it cost to heat, cool, light and maintain the laboratory? How much for maintenance contracts, for insurance? If these totals are examined, areas may be found where adjustments are indicated.

Probably all will agree that it is fiscal irresponsibility to spend unused funds for unnecessary supplies or equipment merely to maintain a budget at the same level as last year. In established laboratories making normal progress and with an alert staff this will not be a problem. Such an organization will always be moving forward and want to do more tests, with newly developed equipment,
Laboratory records are a most important part of any veterinary diagnostic laboratory program. The laboratory record serves primarily one purpose — it is a record of a transaction, or more specifically, it is the documentation of the laboratory procedures conducted in connection with the processing of animals or specimens submitted to the laboratory for examination and the results of these procedures. The second aspect of a laboratory record is that when it is needed, it must be retrieved rapidly.

There are four areas concerning laboratory records which will be discussed: records management, microfilm, legal considerations and the mechanics of handling laboratory fees.

RECORDS MANAGEMENT

There is a large field developing called Records Management and covers primarily four areas: origin or creation of records, routing of records, storage and protection of records and retrieval of data or information.

ORIGIN OR CREATION OF RECORDS

Most diagnostic laboratories keep a daybook which is usually the first known record of a "case" being entered into the laboratory and from this point all other records originate. In the daybook the accession number, the referring veterinarian, the owner's name and address, the species of animal or the type of specimen submitted and the laboratory diagnostician handling the case are recorded. Also, the date that the final report was sent is often recorded. This may provide the director with a rapid means of reviewing cases which may not have been completed.

ROUTING OF RECORDS

There are several methods used for routing records through the laboratory. In some cases a file card is given to the diagnostician in charge of the case which he retains until the final report is written. In other cases the accession sheet and accompanying reports such as bacteriology results, toxicological analyses, or chemistry findings are put in a laboratory folder and proceed, along with the laboratory specimens, to the various disciplines within the laboratory. The disposition of final reports is also an item for consideration. Some laboratories send a copy of the final report to both the veterinarian and the owner; and in other cases two copies are sent
"procedures" manual. All like items should be grouped by size, by type of record, by like retention schedules and by color. This grouping will help with proper camera functioning and efficiency of microfilming.

The condition of the records to be microfilmed should also be considered. Where feasible all illegible documents should be rewritten or retyped. Torn records should be mended, staples pulled out and creases removed.

In regard to the legal aspects of microfilm, some states, although a minority, do not accept microfilm as admissible evidence. Also, if a "film splice" is made it should be documented in writing as to the pertinent details of the splice.

One of the main reasons to use microfilm is space reduction. A five drawer letter-size file cabinet holds approximately 20,000 letter-size documents. A 100 foot roll of microfilm will hold approximately 2,400 letter-size document images and one file cabinet will hold approximately 800 rolls of microfilm so utilizing microfilm as a method of storage, a file cabinet will hold about two million documents.

Other reasons to use microfilm include records integrity, speed with which information may be stored, ease of filing, speed and ease of retrieval, ease of duplication, and security of records. Microfilming does not fit every laboratory and a thorough study should be made before it is undertaken. In some cases, the costs of microfilming may exceed the benefits of having the records on microfilm.

An example of microfilming costs is as follows: one-100 foot roll of film can hold 2,400 8½x11 pages; camera rental charge of $8.00 per day; a camera operator, at $20.00 per day, can film about 400 pages per hour or 3,200 pages per day; one roll of film costs approximately $6.00. Using these figures, the cost is calculated at about one cent per page.

There are many types of microfilm readers and some have an attachment which will provide both a visual display on a screen and a paper copy output.

LEGAL CONSIDERATION IN REGARD TO DIAGNOSTIC LABORATORY RECORDS

The most important aspect of laboratory records is the legal considerations. When a case is submitted to the laboratory, it is generally not known if it will be discussed in court. The best approach is to assume every case may become a legal matter.

In preparing this paper, several lawyers were asked to outline items that might be of value to diagnostic laboratory personnel, and the following remarks are based on these discussions. One of the points made was that a laboratory’s records are a reflection of the quality of the laboratory. Every laboratory director discusses this with his employees; however, the lawyers made the point that people sometimes get careless and all laboratory personnel should be constantly reminded of the importance of quality laboratory procedures and records.

A particular emphasis should be placed on completely documenting the case. The laboratory record should be large enough to hold all the pertinent information and not be over-crowded to the point of being illegible. Only facts should be recorded on the record and general comments of a personal nature are not appropriate. Also, when the final report is written, remarks which may indicate that the veterinarian to whom you are writing is a personal friend are not appropriate.
and also the practice (in case of two or more veterinarians in the practice) has a code so cases can be submitted under the doctor's name or the practice name. In other laboratories each veterinarian has a code, and if there is a three-man practice and each of the veterinarian's within the practice submits specimens, then each gets a separate bill at the end of the month. In another laboratory, a computer system uses each veterinarian's code and it is cross-referenced to a practice and then only the practice name is used for billing purposes. In all types of systems there may be problems. One that comes up is when two veterinarians within a practice dissolve partnership and establish their own practices or go with another practice, then the bills must be straightened out. This can usually be done easily with a manual system but sometimes with a computer system it can be quite difficult.

In other laboratories, when a case is submitted by the farmer, the farmer gets the bill; however, if the case is submitted by a veterinarian and the veterinarian wants the farmer to be billed, he gives the farmer's address to the laboratory whereas if the veterinarian does not want the farmer to be billed, the laboratory gets the farmer's name but not his address.

Since there are many types of laboratories such as state, state-federal, university, private and many other combinations, one question asked is, "who does the billing"? Although over 90% of all fees are collected via billing, there is some cash business. None of the laboratories contacted were considering a credit card billing procedure even though several small animal clinics and some veterinary college clinics are using credit card billing. One laboratory has nearly a cash-on-the-barrelhead policy and in their area it works very well. The billing in some cases is handled completely by the diagnostic laboratory. Some have a one-half time bookkeeper who handles everything — sends bills — receives receipts and maintains all books. In another case the laboratory sends a copy of the accession ticket to the university business office and they handle all the billing. Another laboratory sends a copy of their bills to the computing center where the bills are printed and files are maintained to keep track of the accounts receivable, but the bills go back to the laboratory where they are mailed out and all actual bookkeeping is handled within the laboratory. Some laboratory directors have commented that if bills are sent out with the final report and another bill is sent out at the end of the month, confusion may develop. Some laboratories send only a detailed bill at the end of the month. Another comment was that the books must be kept right up-to-date. Bad debts and complaints develop when the bills are late. All laboratories contacted indicated that bad debts have been nearly zero. Publishing fees can present a problem. If a farmer gets a bill of $10.00 for a necropsy and then a month later the veterinarian charges the farmer $13.00 for a similar necropsy that he took to the laboratory, the farmer may not consider the veterinarian's time involved and a misunderstanding may develop.

There are many different policies regarding who gets billed for laboratory services. Some laboratories bill everyone for everything. Others do not charge for public health services such as rabies and some have a policy that if a case has epidemiological significance, then there is no bill. An example might be that if specimens were submitted for Hog Cholera or Anaplasmosis examination, there would be no fees charged. However, if the tests or laboratory assistance was required for "purpose of sale" then a charge was made. Some laboratories utilize toxicological services from other areas such as the Department of Agriculture and then no charges are made for these services.
PERSONNEL TRAINING AND CONTINUING EDUCATION

by

W. E. Ketter, D.V.M.

Personnel training and continuing education are necessary elements in diagnostic laboratory management. Training makes it possible for laboratory supervisors to develop or improve the capabilities of their personnel and increase the competency of their laboratory, thus being able to offer new or better services to the livestock industry.

As a training officer in the Animal Health Division of the United States Department of Agriculture (USDA), experience has been acquired by developing and offering a variety of training courses to state and federal regulatory veterinarians and diagnostic laboratory personnel. Continued association with Diagnostic Services of the Animal Health Division at the National Animal Disease Laboratory in Ames, Iowa, has also allowed involvement in the personnel training and development of that group.

Training for laboratory personnel is available from many sources. A Laboratory supervisor should consider and evaluate each of these training sources and select training which will best meet the current needs of the organization. A laboratory can employ highly trained personnel if its budget will allow, however, many laboratories must employ less well trained personnel and rely on training to increase their capabilities and competency.

Diagnostic Services is located near Iowa State University and excellent use of this facility has been made for graduate training and special courses for veterinarians, other professional and non-professional employees. When training will benefit the laboratory programs of Diagnostic Services the employee is allowed to attend class during work hours and the fees are paid by the laboratory. The number of hours of course work and the number of students attending class depend on the diagnostic workload during the quarter. Personnel who have college training but lack an undergraduate degree are encouraged to complete their degree. In completing a degree the student is required only to pay the fees for those courses which do not benefit the laboratory programs and a non-standard tour of duty is worked while attending these courses.

Many non-university laboratories are also located near or on a university campus. These locations also allow for the extensive use of this type of training. Regulations under which your laboratory operates will determine whether the laboratory can pay the employees fees and whether work time will be allowed for class attendance.

If a junior college or area community college is located in the area, technicians and other non-professionals can be encouraged to enroll in night courses such as mathematics, chemistry, or microbiology. These courses should help laboratory employees to better understand their work and thus increase their work output.

Personnel of Diagnostic Services often attend training courses at the National Center for Disease Control (NCDC) to learn new diagnostic procedures or tech-
which would meet such needs.

Safety for laboratory personnel is as important as their technical training. Personnel in Diagnostic Services have had the opportunity to attend a course in defensive driving offered by the Iowa Highway Patrol, observe a demonstration and hear a lecture on mouth to mouth resuscitation offered by the Iowa Highway Patrol and to observe various films on laboratory safety. Similar courses and films may be available to personnel in other laboratories.

After personnel have been trained in an area it is just as important that they receive some type of refresher training periodically. This can be through attendance at seminars or scientific meetings. Attendance at these meetings allow personnel to maintain competency in the area and to visit with personnel from other laboratories to discuss their mutual problems. If attendance at these meetings require that a report be prepared or a seminar given, the other members of a laboratory staff will also benefit from the meeting.

If laboratory personnel learn a new procedure from a course or bench training and check tests are offered by the training institution, personnel should be encouraged to participate in these check tests. Check tests should not be conducted to embarrass laboratory personnel but should routinely provide an analysis for one's competency, reagents and equipment.

A diagnostician assigned to a laboratory should routinely be able to make field trips when they are warranted. A diagnostician may not get the full picture of a disease or its epidemiology from the animals or specimens and the history presented to the laboratory. Field trips give a diagnostician the opportunity to observe the herd or flock condition, the environment, sanitation, nutrition as well as obtain additional history of the case. Perhaps one of the greatest benefits of field trips is that of providing training to the practicing veterinarian involved in the case. Several diagnostic laboratories in the midwest have utilized field trips in this manner.

A diagnostic laboratory should have or have ready access to a good scientific library. A library can be a training aid or a method of continuing education for laboratory personnel. Money spent on a good usable library will be money well spent and it is one of the requirements of the AAVLD program for accrediting laboratories.

Diagnostic Services has just embarked on a new program of training in diagnostic laboratory medicine. At the present time this program is utilized to train replacement personnel for Diagnostic Services but if it is successful it could be followed by other laboratories or used to train personnel for diagnostic laboratories. The program is two years in length and the personnel are trained for employment in a specific diagnostic laboratory discipline. The first year consists of on the job training in all areas of Diagnostic Services, but primarily in the area of assignment, and limited classwork at Iowa State University.

During the second year the person is a full time student finishing the requirements of a Masters Degree which includes a thesis. The degree work will be individualized according to the predetermined laboratory assignment of the individual. This program, if successful in producing the desired results, would be of value to diagnostic laboratories throughout the country.

In summary, personnel training and continuing education are valuable for continued personnel development and laboratory competency. A progressive lab-
ENCEPHALOMYELITIS-VOMITING AND WASTING DISEASE COMPLEX OF SWINE

W. L. Mengeling, D.V.M., Ph. D.

INTRODUCTION

In 1962 Greig et al. reported the isolation of a previously unrecognized viral pathogen of swine from the brain of baby pigs with encephalomyelitis. The virus was found to agglutinate erythrocytes of several species of animals and was named hemagglutinating encephalomyelitis virus (HEV).

Recently a strain of HEV (designated strain 67N) was isolated from swine in the United States. Although the virus was recovered from apparently normal pigs, it was found to produce clinical illness in pigs infected experimentally. The latter observation suggests that HEV may also be associated with a naturally occurring disease of swine in this country.

The purpose of this report is to present information concerning: (1) properties of HEV, (2) characteristics of the associated disease, (3) incidence of infection, and (4) laboratory confirmation of clinical diagnoses.

PROPERTIES OF HEMAGGLUTINATING ENCEPHALOMYELITIS VIRUS

Examination of several strains of HEV by electron microscopy has revealed a coronavirus morphology. With negative staining, virions of strain 67N appear approximately circular in outline with club-shaped projections and a mean diameter of 120 nm. The membrane-bound portion of the virion is about 90 nm in diameter and the projections approximately 15 nm in length (Fig. 1). In thin sections of porcine kidney cells, the virus is observed within and budding from the wall of cytoplasmic vesicles (Fig. 2). The mean buoyant density of strain 67N is low i.e., 1.212, and thus similar to that of the prototype of the coronavirus group, namely, avian infectious bronchitis virus. Based on experimentation using inhibitors of deoxyribonucleic acid synthesis, Greig and Girard concluded that the genome of HEV is ribonucleic acid.

Strain 67N is poorly cytopathogenic; however, like other strains of HEV it induces the formation of syncytia in cell culture. Moreover, erythrocytes of several species are adsorbed to infected cells (Fig. 3). Based on the examination of infected cells using immunofluorescence microscopy, it appears that replication of the virus occurs within the cytoplasm (Fig. 4).

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The author acknowledges the technical assistance of Mrs. Lorene Vaughn and extends his appreciation to Mr. A. E. Ritchie and Dr. A. D. Boothe for supplying the photographs of negative staining and thin section electron microscopy of HEV.

485
24, 0-day-old, hysterectomy-derived, colostrum-deprived pigs of 7 litters (2 to 4 pigs of each litter). Two additional pigs from each of the 7 litters were kept as non-infected controls. Ten of the 24 principals became clinically ill following exposure to the virus. Of these 10, 6 developed clinical signs of vomiting and wasting disease of variable severity, and 4 developed a pneumonia confirmed at necropsy by macroscopic and microscopic examination. The remaining 14 principals and 14 controls remained clinically normal. The details of these experiments are presented elsewhere; however, from the aforementioned observations, it is apparent that strain 67N is a potential pathogen, notwithstanding a marked difference in susceptibility of pigs.

INCIDENCE OF INFECTION

A serologic survey conducted by Girard et al. in Canada with porcine serums collected prior to 1964 revealed a high incidence of infection with HEV. Of 116 adult pigs from 8 premises exposed to HEV-infected baby pigs, 111 (96%) had hemagglutination inhibiting (HI) antibody for HEV. Thirteen (16%) of 83 pigs from 8 premises where HEV was not known to occur had antibodies of HEV and 66 (31%) of 211 pigs that were 5 to 6 months of age with unknown histories had antibodies to HEV.

A survey done in England by Cartwright and Lucas with porcine serums collected from 1960 to 1969 indicated an incidence of infection from 10.6% to 82%, apparently depending on the area in which the pigs were raised and on the type of pig.

The incidence of infection in Northern Ireland in 1970 and 1971 was found to be approximately 50%, even though HEV has not as yet been isolated there.

In our laboratory, 152 serums collected in 1971 from sows and butcher hogs representing 39 farm-raised swine herds (1 to 5 samples per herd) were tested for HI antibody to HEV. All pigs were from central Iowa farm herds and all had antibody for HEV. One hundred and sixteen serums (76%) has an HI titer of 40 or greater. The presence of specific antibody was further confirmed by virus neutralization and precipitation tests (Fig. 5).

LABORATORY CONFIRMATION OF CLINICAL DIAGNOSES

During naturally occurring cases of both the encephalomyelitic and the vomiting and wasting form of the disease, HEV was isolated from the brain of infected piglets. To provide additional information concerning tissues that should be collected from infected pigs for virus isolation, the following experiment was conducted in our laboratory. Eleven hysterectomy-derived, colostrum-deprived pigs were infected with HEV and killed at various times thereafter. At necropsy, 35 tissues were collected from each of the pigs and examined for virus. The tissues were triturated in cell culture medium and the suspensions (approximately 10%) were inoculated onto porcine kidney cell cultures which in turn were examined for infected cells about 24hrs. later by immunofluorescence. The tissues examined were: liver, kidney, spleen, pancreas, adrenal, stomach (cardiac, fundic, and pyloric portions), duo-

LEGENDS

Fig. 1.—Two negatively stained virions of HEV. Diameter, including projections, is approximately 120 mu.

Fig. 2.—Ultrathin section of a portion of the cytoplasm of a porcine kidney cell infected with HEV. Note virus development by budding into cytoplasmic vesicles (arrows).

Fig. 3.—Porcine kidney cell culture infected with HEV. Chicken erythrocytes adsorbed to virus induced syncytium.

Fig. 4.—Immunofluorescence of porcine kidney cells infected with HEV. Note absence of nuclear fluorescence.

Fig. 5.—Double immunodiffusion of concentrated medium from HEV-infected porcine kidney cells and porcine anti-HEV serum. Antigen is in the 6 peripheral wells, antiserum is in the center well. Two lines of precipitation are evident.

Fig. 6.—Immunofluorescence of a cryostat section of nasal mucosa collected from a piglet 6 days after intranasal exposure to HEV.
Table 1. Isolation of Hemagglutinating Encephalomyelitis Virus from Tissues of Experimentally Infected Pigs

<table>
<thead>
<tr>
<th>Pig No. (days)</th>
<th>Age of pig when exposed to virus (days)</th>
<th>Necropsy post exposure (days)</th>
<th>Tissue examined&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Titer hemagglutination inhibiting antibody&lt;sup&gt;c&lt;/sup&gt;</th>
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<sup>a</sup>After exposure each pig was kept in a separate isolation cage. Pigs 1 through 5 were exposed by intranasal instillation of virus. Pig 1 was given 1 ml. of cell culture fluid containing approximately 10,000 infective units of virus. Pigs 2, 3, 4, and 5 were given 1 ml. of a 10% suspension of triturated nasal mucosa of the pig of the previous number, e.g., pig 2 was given a suspension of nasal mucosa of pig 1. Pigs 6 through 11 were placed in a chamber into which HEV was nebulized. The concentration of virus was low. Virus was not demonstrated in either of two samples representing 10 liters of air. However, the pigs became infected as evidenced by isolation of virus and production of antibody.

<sup>b</sup>+++ to + = Numerous to few infected cells observed by immunofluorescence; - = No infected cells were observed.

<sup>c</sup>Blood collected at necropsy.

<sup>d</sup>Virus was not isolated in cell culture but was present in the sample as evidenced by the fact that pig 3 was infected with HEV when exposed to the sample.
DIAGNOSIS OF TGE BY FA:
EVALUATION OF ACCURACY ON FIELD SPECIMENS

by
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INTRODUCTION

The differentiation of bacterial and viral gastroenteritis of neonatal swine has been a diagnostic problem of some importance for many years. Of the two etiologies, we must speak of colibacillosis and transmissible gastroenteritis (TGE), respectively, as the prime offenders for each category. Both diseases are of great economic importance and reach epidemic proportions during the winter months. For these reasons, a rapid, reliable and accurate laboratory method of differentiation is needed. While standard methods of diagnosis such as history, gross and subgross pathology and bacteriology are currently in use, they are occasionally subject to inconsistencies that might render them misleading.

The fluorescent antibody (FA) test for TGE has been investigated and reported previously. Much of this data, however, has been collected from experimental cases of TGE in specific pathogen free (SPF) pigs. The purpose of this report, therefore, is to assess the accuracy of the TGE-FA test on a substantial number of field cases using history, gross and subgross pathology, blood chemistry, bacteriology and sometimes TGE virus isolation as criteria.

MATERIALS AND METHODS

Production of TGE antiserum:
The hyperimmune TGE antiserum used for conjugate production was prepared by Dr. A. W. McIurkin* in a 250-300 pound SPF sow. The antigen used was cell culture fluid containing $10^6-10^7$ TCID50 of low cell culture passage Purdue TGE isolate per milliliter. The initial dose was 20-25 ml virus-oil adjuvant mixture in a proportion of 3 to 1, in 4-5 sites, subcutaneously. Three subsequent booster 2-3 weeks apart of 25-30 ml virus-cell culture debris administered in the anterior vena cava completed the hyperimmunizing schedule. The animal was exanguanated two weeks after the last booster.

Fractionation and labeling of antiserum:
TGE antiglobulin was prepared by three successive one-third saturated (NH4)2SO4 precipitations carried out in the cold. The purified globulin was redissolved in

*TGE project leader, NADL
one-half the original volume of cold saline on the last precipitation. The material was then passed through a Sephadex * G-25 gel filtration column to eliminate residual (NH₄)₂SO₄. After desalting, the total protein concentration of the preparation was determined by the Biuret method.

For conjugation, the globulin preparation was diluted to contain 0.5% protein. Carbonate-bicarbonate buffer pH 9.0 was added to make 10% of the final volume. Flurocine isothiocyanate ** (FITC) was added to this alkaline protein mixture in a ratio of 0.035 mg FITC to 1.0 mg protein. The reaction mixture was then allowed to stir in the cold for exactly 12 hours. At the end of the conjugation period, the mixture was passed through a Sephadex column (as in desalting) to remove the excess FITC.

Removal of nonspecific staining factors from the conjugate was accomplished by absorbing with acetone dried rabbit liver powder (Difco). This was rehydrated with 2.5 ml phosphate buffered saline (PBS) pH 7.2 per gram of powder to prevent loss of conjugate. The labeled antibody was then mixed with the rehydrated powder in a ratio of 1 gram of powder (dry weight) per 10 ml conjugate. This mixture was stirred in the cold overnight and centrifuged at high speed the next morning. The supernatent conjugate was clarified, if necessary, by filtration. Titration of the conjugate was performed in TGE infected swine testicle (ST) cells.

**FA staining procedure**

A 3-5 cm section of suspect small intestine was placed on a paper towel (to hold it firmly in place) and opened along the long axis. It was then laid out so that the mucosa faced upward, and all intestinal contents were rinsed off with a gentle stream of water. A section of the mucosal surface was then removed by gentle scraping with a small scalpel blade. The gut epithelium had a gray-white, mucoid appearance. The mucosa was then placed in the middle of a pre-cleaned glass slide. Another slide was then used to compress the smear to a uniform thickness between the two parallel slides. The top slide was then drawn off parallel to the bottom one with a quick, smooth motion. Intact villi and/or individual gut epithelial cells were, by this process, evenly distributed over two-thirds of the slide surface in a “monolayer” or tissue culture-like manner.

The smears were allowed to air dry (5-10 min.) and then fixed in acetone at room temperature for 10 minutes. After fixation, the slides were air dried and stained with TGE conjugate for 30 minutes at 37°C in a moist chamber. The slides were then drained of conjugate and flushed briefly in PBS pH 7.2 before rinsing for 10 minutes in carbonate-bicarbonate buffer pH 8.5.

After the alkaline buffer rinse, the slides were drained, briefly flushed in distilled water, drained again and then air dried. They were then mounted with glycerol-bicarbonate buffer (pH 8.5) medium. Reading was accomplished on a Leitz Ortholux FA microscope using a BG-38 and BG-12 primary and exciter filters and an OG-1 barrier filter. The 10X objective was used for scanning and the 25X for confirmation of positive cell morphology.

**TGE virus isolation attempts:**

A 20-30 cm section of suspect small intestine was opened longitudinally (as

* Pharmacia; Upsula, Sweden
** BBL
for FA staining) and all of the mucosa removed by scraping. The mucoid epithelium was resuspended to 20% in Hank's balanced salt solution (HBSS) with 300 units penicillin G, 300 mg streptomycin, 300 mg neomycin and 10 mg fungizone per milliliter and 2% fetal bovine serum (FBS). The preparation was then either sonified* in the cold for 2 minutes or freeze-thawed four times. Cell debris and particulate matter were centrifuged out at 3500 rpm for 30 minutes. The supernatent was then used to infect leighton tubes or slide-tray** cultures of ST cells. After 60 minutes infection, the homogenate was washed from the cell sheet and an overlay consisting of MEM (Earle’s salts), 0.5% lactalbumin hydrolysate (LAH), 10% FBS, antibiotics as previously mentioned and 1% Nobel agar (Difco) was added. At the end of 48 hours incubation, the agar was removed and the cell sheets were stained with TGE FA conjugate. Subpassages were not made.

**TGE serology:**

The microtiter (MTC) serum neutralization (SN) test as previously reported was used. The virus strain used was high passage level Purdue isolate. Lately, it was found that ST cells grew better in MTC plates treated with sulfuric acid instead of gelatin.

**RESULTS**

Staining intensity of the TGE-FA conjugate was quite good (Fig. 1) with specific fluorescence standing out in good contrast against a dull background. The specificity of the reaction was confirmed by using purified convalescent TGE antiglobulin in an FA-inhibition test where the fluorescence of TGE infected cells was diminished 50-75% as compared to a similar smear using hog cholera antiglobulin for a negative control. Positive staining did not always appear in the entire cytoplasm of infected cells, but occasionally localized toward the distal or absorptive surface. In this case, identification of positive cells by morphology was not always possible on low power. Occasionally, the 54X oil objective was necessary to confirm this type of cell. On low power, this type of partial localization could be seen as “Wisps” of fluorescence in the smear. Even on this type of slide, however, diligent searching could usually demonstrate cells of a characteristic “doughnut” or cytoplasmic fluorescence morphology. Negative smears were of a dull gray-green appearance and could, for the most part, not be confused with a positive.

If a smear could be made and the characteristic “honeycomb” appearance could be seen, the specimen was considered satisfactory. If, however, nothing but amorphous debris could be seen on the smear, the epithelium had undergone post-mortem autolysis and was unsatisfactory. Suspect guts could be kept in the cold for as long as a week without detectable loss of staining intensity. The intestines could even be frozen and thawed once with only a 20-25% loss of fluorescence. With multiple freeze-thawing, however, the positive cells lysed and released positive staining material that was deposited over the smear as a fine, fluorescent “dust”.

Table 1 shows the percentage correlation between standard methods of TGE diagnosis and TGE-FA test. Standard methods of diagnosis were considered to be case history, necropsy findings, subross pathology, blood chemistry, histopathology, alimentary canal pH values and bacteriologic results. Only those cases that were

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* Bronwill Biosonic II

** Lab-Tek
considered to be classical TGE by standard methods were used in this comparison. Of 43 cases showing a typical TGE picture, all 43 proved to be positive by FA. Eighteen cases proved to be classical bacterial enteritis and were negative by FA. When the individual animals in these cases were considered, 107 were TGE by standard methods and 28 were negative. Only 100 of these pigs, however, proved to be positive by FA and 35 negative. The standard method-FA test correlation was 100% for cases and 93.4% for pigs within these cases.

Only those pigs proving to be FA positive were cultured for TGE virus. One hundred pigs were assayed with 21 positive isolations resulting (Table II). Of the 21 isolations made, each was from a separate case, thus showing a response of 21 out of 43 cases positive. The percentage correlation between isolation and FA for TGE was 21% for individual pigs and 48.8% for cases.

Convalescent sera from sows or surviving pigs was only obtained from 16 cases, 12 of which proved to be positive by both TGE serology and FA (Table III). Individual animals could not be counted because they did not survive. The correlation between convalescent serology and FA was 100%.

TGE diagnosis by standard methods required approximately 24-48 hours for completion; virus isolation and TGE serology required 48 hours after receipt of convalescent serum and the FA test required 1%-2 hours from submission of specimen.

**DISCUSSION**

Other investigators have used frozen sections and impression smears of gut, lymph nodes and other tissues for TGE-FA examination. The reasons for adopting a compression smear for this procedure, however, were ease and rapidity of preparation with a minimum of equipment and a much greater sampling of suspect tissue per slide. An experienced technician can prepare one smear each from 4-6 pig intestines in 5-10 minutes. Each of the smears, if properly made, covers approximately 15-20 times the epithelium seen in a frozen, anular gut section. Even though the histopathology of TGE varies with the area of the jejunum, there appears to be little difference in FA smears made from different sections of the gut. Usually, one smear per pig was sufficient for diagnosis unless a borderline case was evident. In this event, one or two more smears would be made from other parts of the intestine in order to confirm or deny the earlier diagnosis.

In some cases, great numbers of leukocytes were seen to infiltrate the gut mucosa. These cells would tend to autofluoresce in the green region under neutral pH conditions and give a false positive appearance. This phenomena was effectively controlled by using the alkaline (pH 8.5) buffer rinse and mounting medium. At this pH, the secondary fluorescence of white cells is yellow to white, and therefore, easily differentiated from true positive material. The use of this buffer also tended to quench the normal background of negative tissue while leaving the specific fluorescence unaltered.

Table I demonstrates the apparent high degree of reliability of the FA test for TGE. Only those cases were counted during this time period that could definitely be diagnosed by standard methods as classical or "text-book" TGE. Many more cases, however, were diagnosed during this time that were unverifiable. The few pigs that were from known infected litters that were FA negative cannot at this time be explained. It is possible, however, that some of these deaths were due to other causes, or that the gut mucosa was so severely atrophic that no infected cells could be found. This emphasizes the fact that more than one pig from a litter or case should always be submitted. Even though some pigs were apparently missed by FA, all of the cases were accurately diagnosed. Those pigs in the earliest or degenerative
phase of the disease showed the strongest FA reactions. Animals that were severely atrophic or regenerating the gut epithelium showed less decisive staining.

Virus isolation was attempted on only those pigs that were FA positive. The poor showing of virus recovery (21%-50%) was apparently related to the stage of the disease when the isolation attempt was made. Without exception, the positive TGE isolations were made from pigs that were in the early degenerative phase of the disease and were delivered to the laboratory alive. No isolations were made from severely degenerate, moribund or dead pigs. This fact, coupled with the inconvenience (agar overlay) and length of the isolation would seem to render it of doubtful value to the average diagnostic facility.

TGE serology was undertaken on some cases solely as supportive data for the FA test. Even though it has a 100% correlation with standard methods, it is of little value to the immediate needs of the swine producer and practitioner. Its only use would be in the verification of an earlier diagnosis or determination of the immune status of sows to be used for future breeding.

Of the currently used or available technique for TGE diagnosis, the FA test appears to be the most reliable. Specimens for TGE diagnosis are submitted to laboratories in every conceivable form and state of preservation. Often only scanty or inaccurate histories and frozen small intestines are submitted. This rules out any data from an accurate postmortem examination. Under this wide range of conditions, the gut smear FA-test appears to be capable of a highly credible diagnosis within 2 hours of submission, and demonstrates the added advantages of ease and relative economy.

REFERENCES

TABLE I

<table>
<thead>
<tr>
<th></th>
<th>Number of cases</th>
<th>Number of pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive by Standard Methods</td>
<td>43(18)*</td>
<td>107(28)</td>
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<tr>
<td>Positive by FA-test</td>
<td>43(18)</td>
<td>100(35)</td>
</tr>
<tr>
<td>Correlation</td>
<td>100%</td>
<td>93.4%</td>
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*Numbers in parenthesis indicate negative responses.

TABLE II

<table>
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<tr>
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<th>Number of pigs</th>
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<tr>
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<td>43(0)</td>
<td>100(0)</td>
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<td>Positive by virus isolation</td>
<td>21(22)</td>
<td>21(79)</td>
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<tr>
<td>Correlation</td>
<td>48.8%</td>
<td>21.0%</td>
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TABLE III

CORRELATION OF TGE-FA TEST WITH CONVALESCENT SEROLOGY

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<tr>
<td>Positive by FA-test</td>
<td>12(4)</td>
</tr>
<tr>
<td>Positive by serology**</td>
<td>12(4)</td>
</tr>
<tr>
<td>Correlation</td>
<td>100%</td>
</tr>
</tbody>
</table>

*serum neutralization
**convalescent serum showing ≥16 (recip.) titer

Fig. 1. Positive TGE-FA mucosal smear. (250X)
REOVIRUS INFECTION IN PIGS

J.P. McAdaragh; M.S., M.G. Robl, D.V.M., Ph. D.
C. Phillips, B.S. and D.E. Reed, Ph. D.

INTRODUCTION

Reoviruses are a group of viruses found to infect both the respiratory and intestinal tract of mammals. The virus was first isolated from stools of healthy children and found to have some characteristics similar to other human enteroviruses. (4) Evidence for the presence of reovirus infection in pigs was presented by Spradbrow, who found that 71 per cent of the 200 pigs tested in Australia had antibodies against one or more of the three types of reovirus. (8) McFerran and Connors (3) and Kasza (2) last year reported almost simultaneously the isolation of reovirus from the feces of pigs in England. These viruses had characteristics similar to reovirus prototype I of humans.

The isolation of a reovirus in the U. S. from the lung and lymph nodes of a pig with a pneumatic condition was previously reported by Robl et al. (5) Animals were experimentally infected and contact transmission occurred.

Methods utilized in isolation of viruses from porcine specimens submitted to the S.D.S.U. Animal Disease Research and Diagnostic Laboratory are described. Biological, serological and morphological examination of some of these virus isolations indicate that they have similar properties to other viruses classified in the reovirus group.

Tissue Culture

Primary and secondary pig thyroid, adrenal and monkey kidney (Vero) cells were grown in Eagles minimum essential medium (MEM) with Earle's balance salt solution (B.S.S.) plus 10% fetal bovine serum (FBS) and maintained in Eagles (MEM) containing 2% FBS. Penicillin 150 units/ml streptomycin 100 mg/ml and kanamycin 100 mg/ml were added to both maintenance and growth media.

Preparation of Specimens

Specimens submitted as routine diagnostic accessions included fetuses and placentas from abortions, lungs and lymph nodes from pneumatic conditions and intestine and fecal material from enteric diseases.

A 10% suspension of each specimen was made using maintenance medium and centrifuged for 30 min. at 5000 rpm at 50°C. An inoculum of 0.2 ml of the super-

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Approved for publication by the Director, Agricultural Experiment Station, South Dakota State University as Journal Series No. 106.3.
natant was inoculated onto each monolayer. The cultures were incubated at 37°C in a roller drum for 10 days or until a cytopathic effect was noted. Cultures having no cytopathic effect were frozen and thawed and 0.2 ml of the supernatant was inoculated onto fresh monolayers and reincubated. All cultures were passed three times and tested for hemagglutinating activity before they were considered negative.

**Virus Properties**

Chloroform sensitivity test was performed using 0.05 ml reagent chloroform and 1 ml of virus. A virus control was established by adding 0.05 ml maintenance medium to 1 ml of virus. The mixtures were incubated at room temperature for 10 minutes with repeated shaking. All tubes were centrifuged at 500 rpm for 10 minutes. The supernatant was removed and virus titrations set up. The dilutions were inoculated onto Vero cells and incubated at 37°C for 10 days until CPE appeared. Acid sensitivity was demonstrated by adjusting the virus sample to pH 3 with 1.0 M acetic acid and allowing it to incubate at room temperature for one hour. The pH was then adjusted to pH 7 with 7.5% sodium bicarbonate. Monkey kidney (Vero) cells were inoculated with the dilutions and incubated for 10 days.

**Hemagglutination**

Fluids from infected tissue culture cells were tested for hemagglutination at room temperature using erythrocytes from rat, chick, guinea pig, sheep, human "O" and Rhesus monkey.

The antisera against human isolates of reovirus type I, II and III were obtained from Dr. Francis Forrester. 1 Sera to be tested for hemagglutination-inhibition were treated to remove possible non-specific inhibitors and hemagglutins using the method of Rosen. (6)

**Electron Microscopy**

Monolayers of pig adrenal cells were grown in petri dishes. 2 When monolayers were confluent the cells were inoculated with 0.5 ml of fluid containing the virus and incubated at 37°C until approximately 50% of the cells showed cytopathic effects. The cells were washed three times with phosphate buffered saline pH 7.2 and scraped free from the surface. The cells were allowed to sediment and supernatant replaced with osmium tetroxide (1% in phosphate buffer) for 20-30 minutes and dehydrated with an alcohol gradient, propylene oxide and embedded in epon. The sections were stained with uranyl acetate and lead citrate.

**Animal Inoculation**

The virus was inoculated intranasally in 6 SPF-origin pigs weighing 150 lbs. each. Two animals served as contact controls.

---

1 Dr. Francis Forrester, Communicable Disease Center, Atlanta, Georgia
2 Falcon Plastic Div., Bioquest, 5500 W. 83rd., Los Angeles, Cal. 90045
RESULTS

The virus usually produced a very slight and sometimes undetectable cytopathic effect (CPE) in thyroid cells incubated at 37°C for 10 days. When a blind passage was made the CPE could be detected at about 6 or 7 days and progressed until the 10th day at which time the cell layer was approximately 50% destroyed. In some cases the CPE did not appear until the 7th day of incubation of the 3rd passage.

Viruses studied were resistant to chloroform treatment and no reduction in infectivity titer occurred when treated at pH 3. Perinuclear cytoplasmic inclusions were evident when stained with May-Grunwald Giemsa stain. Electron microscopic studies indicated that the particles had cubic symmetry and were approximately 70 nanometers in diameter. The virus particles were arranged in a lattice arrangement in the cytoplasm. No particles were noted in the nucleus of infected cells.

All the viruses replicated in Vero cells where they produced CPE and hemagglutinating antigens typical of reoviruses.

The viruses did not hemagglutinate rat, chick, guinea pig, or sheep but did hemagglutinate human "O" red blood cells.

HI TITER USING SPECIFIC HUMAN
REOVIRUS ANTISERA

<table>
<thead>
<tr>
<th>Virus No.</th>
<th>Clinical Condition</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
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<td>71-2111</td>
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<td>±1:10</td>
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<td>71-2198</td>
<td>abortion</td>
<td>±1:10</td>
<td>&lt;1:10</td>
<td>+1:20</td>
</tr>
<tr>
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<td>abortion</td>
<td>±1:10</td>
<td>±1:10</td>
<td>±1:20</td>
</tr>
<tr>
<td>71-2829</td>
<td>Enteritis</td>
<td>±1:10</td>
<td>&lt;1:10</td>
<td>+1:20</td>
</tr>
<tr>
<td>71-3465</td>
<td>Enteritis</td>
<td>±1:10</td>
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</tr>
<tr>
<td>70-5887</td>
<td>Lung &amp; Lymph Node</td>
<td>±1:20</td>
<td>±1:10</td>
<td>+1:40</td>
</tr>
</tbody>
</table>

DISCUSSION

Kasza (2) found canine thyroid adenocarcinoma, primary swine lung and monkey kidney cell cultures showed the greatest degree of CPE when infected with reoviruses. Cell cultures obtained from pig thyroids appeared to be more satisfactory for the isolation of reoviruses from pigs than either primary pig kidney or primary pig adrenal cell cultures. The thyroid cell cultures were less susceptible to enterovirus infections.

The use of the fetal bovine sera in the media may be more suitable than sera from adult animals for the isolation of reoviruses. Rosén et al (7) indicated that adult sera may contain antibodies against reoviruses, and thus inhibit infection of cells in tissue culture.
The biochemical, morphological and perinuclear inclusions indicate these viruses are quite similar to the viruses described in the reovirus group. Hemagglutination-inhibition studies using antisera specific for the reovirus prototypes indicate the six virus isolates to be closely related to type III reovirus. There was some cross reaction to type I.

The significance of the reovirus isolations from cases of porcine disease syndromes submitted for diagnostic purposes is not known at the present time. However, a virus isolated from a field case in which 14 of 50 pigs died of pneumonia has been reported. (5) In that report, pigs derived from SPF sows were inoculated with the virus intranasally. The virus was reisolated from the inoculated animals, an antibody titer rise was demonstrated, and the development of pneumatic lesions were indicative of infection by this particular virus. This virus is described in this report as 7-5887. In the absence of mycoplasma, bacteria parasites and other viruses, it appeared that these pneumatic lesions were caused by the virus isolated from the field case of pneumonia.

Respiratory diseases require careful interpretation because of the numerous agents known to cause swine pneumonia. Many field cases of swine pneumonia are complicated by several of these agents. The role this virus plays in pneumonia in the field is not known, but this virus may cause much more severe lesions when it occurs in conjunction with other known agents that can cause respiratory disease of swine.

This porcine virus has been isolated from the kidney, lung and intestinal tract of swine and consequently, it may be present in the urine, nasal droplets and fecal material. The isolation of these porcine viruses may indicate they are spread by contact with infected animals. Previous experiments indicated that the virus was isolated from animals in contact with infected animals.

SUMMARY

Methods utilized at S.D.S.U. Animal Disease Research and Diagnostic Laboratory in the detection of viruses from porcine disease syndromes are described. Viruses isolated from several conditions have biological, serological and morphological characteristics that are similar to properties of other viruses in the reovirus group.

LITERATURE CITED

Fig. 1. Electronmicrograph showing a lattice of reovirus virus particles in the cytoplasm of an infected tissue culture cell. 44,000 X

Fig. 2. H & E section of lung tissue from case number 70-5887. Ascarid larvae shown in interstitial tissue of alveolii.
Fig. 3. Lung from pig inoculated 4 weeks previously with virus 70-5887. Showing involvement of the cardiac lobes of the lung.

Fig. 4. Lung from pig inoculated 4 weeks previously with virus 70-5887. Showing swollen and congested pulmonary lymph nodes.
Fig. 5. Photomicrograph of lung of a pig inoculated 2 weeks previously with virus isolated from field case 70-5887. Interstitial thickening of alveolar walls is evident along with some alveolar emphysema. H & H-100 X.

Fig. 6. Photomicrograph of lung of a pig inoculated 7 weeks previously with virus isolated from field case 70-5887. The alveoli had collapsed. The cellular reaction was composed primarily of lymphocytes and fibroblasts. A small amount of proteinaceous fluid and lymphocytes were observed in the bronchioles. H & H-100 X.
SPECIFIC PATHOGEN FREE BREEDING TURKEYS
THE PROGRESS AND OUTLOOK

W. M. Dungan, D. V. M.
Sonoma, California

In 1969 the "Dillon Beach Project" was initiated for the purpose of developing and maintaining specific pathogen free turkey breeders under commercial conditions. The project participants, operating under a Joint Memorandum of Understanding, include a basic breeder of turkeys, the California Department of Agriculture, the University of California, and the Animal Health Division and Veterinary sciences Research Division of USDA.

The intent of this project was to investigate and identify sources that have a role in the introduction, transmission and perpetuation of diseases in turkey flocks. The first objectives agreed upon were (1) to establish flocks free of Salmonella, Arizona hinshawii (paracolon) and Mycoplasma disease organisms, (2) to extend the free status to multiplier flocks, and (3) to extend the specific pathogen free concept to include other turkey diseases as it becomes feasible.

Whereas this was a cooperative venture, each of the participants was responsible for particular activities.

The turkey breeding farm involved furnished facilities for hatching, brooding and rearing; the eggs, pouls and breeder turkeys; and the feed, equipment and labor to maintain flocks in acceptable isolation and sanitary condition mutually agreed upon by the participants.

The State of California Department of Agriculture was responsible for laboratory facilities, including testing materials, diagnostic examinations, clerical records and consultation.

The University of California, through its Food Protection and Toxicology Center, participated in project planning and consultation.

The USDA Animal Health Division bears the primary responsibility for field operations. This agency has provided a full time veterinary epidemiologist and a part time supervisory veterinary epidemiologist, in addition to collaborating in the planning and execution of the project.

The USDA Veterinary Sciences Research Division provided consultation, guidance and overall research backup to the project.

A steering committee of representatives from each of the participants has met quarterly to review progress and plan future action. The Dillon Beach Project has been, and will be, a continuing type of activity, designed to provide information of value to the entire turkey industry.

Presented at the American Association of Veterinary Laboratory Diagnosticians 14th Annual Conference, Oklahoma City, Oklahoma, October 25, 1971.
In 1968, ten months before this program began, a basic male line of turkey breeding stock was transferred, as hatching eggs, from Oregon to the Dillon Beach facilities near the ocean. Previous salmonella isolations from this line included *S. worthington* in 1966, *S. san diego* in 1967, and *S. infantis* and another C Group salmonella in 1968. With this history, and in addition to the fact that the property had been a dairy farm at one time, it was conceivable that salmonella might be isolated at some future date on the new Dillon Beach site, although it had never before been used for turkey or poultry raising.

Because of certain features, this facility lends itself particularly well to disease prevention efforts. Newly constructed semi-environmental housing permits continuous confinement of turkeys of all ages, excludes free flying birds, and simplifies surveillance. Poults are brooded and grown in an area more than 1,000 feet from the breeder hen pens, with minimum traffic between the two areas. The hatchery is isolated from the turkey buildings. When the three areas (brooding, breeding and hatching) operate simultaneously, separate personnel are assigned to each. Special attention is given to the use of effective sanitation measures in egg handling, equipment operation, vehicle and personnel movement. Traffic is restricted entering the ranch and no domestic animals, dogs, cats or pet birds are allowed on the premises. Wildlife, including deer, rabbits, rodents and reptiles, are seen occasionally.

The only turkeys reared on the ranch are those hatched there for replacement stock. All eggs incubated and hatched at the ranch have been produced on the ranch with one exception, when in April 1970 a genetically desirable turkey from Wisconsin was introduced as hatching eggs. Prior to its importation, this line was surveyed at its source, using methods similar to the Dillon Beach monitoring, which I will describe later. Results were negative for *salmonella* and *Arizona hinshawii*.

All breeders located at Dillon Beach, including those in production before the project began, have been serologically tested for *Salmonella pullorum*, *S. gallinarum*, *S. typhimurium*, and *Mycoplasma gallisepticum* in accordance with NTIP requirements. Results have been negative.

In this attempt to develop and maintain *Salmonella-Arizona* negative turkeys, we used a surveillance system, sampling all common sources of contamination. The following table shows the number and type of samples cultured from three generations of turkeys during the first two years of monitoring. As indicated, the majority of the samples were from litter, dead germs, cull poults and feed. (All feed has been heat processed in pellet form as standard procedure).

Litter sampling encompasses the total area within a house. A grid pattern system is used, each grid averaging 25 square feet in size. Five samples, one each from five different points within the grid area, make up a composite litter sample. Each composite is labeled and recorded so that it is possible to return to the same area to confirm any suspicious or positive findings.

To date only one salmonella isolation has been made. This was *S. tennessee*, recovered from a feed sample in 1970. Subsequent and continuing attempts have failed to recover a salmonella from any source on the farm. Thus no evidence has been found that the positive feed sample has contaminated the birds or premises.

In summary, during the past two years at Dillon Beach some 80,000 hatching eggs, approximately 40,000 poults from 18 different hatches, 12,000 selected primary breeders, and about 2,000 tons of feed have been under routine and contin-
using surveillance. This monitoring suggests that the ranch and its population of turkeys are salmonella and *Arizona hinshawii* free, and it appears that these infections have been controlled under the existing conditions.

<table>
<thead>
<tr>
<th>Type of Material</th>
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<td>Environmental swabs</td>
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<tr>
<td>Dead germs</td>
<td>3245</td>
</tr>
<tr>
<td>Dead and live embryos</td>
<td>850</td>
</tr>
<tr>
<td>Cull poult's</td>
<td>1179</td>
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<tr>
<td>Hatcher and sexing debris (composites)</td>
<td>18</td>
</tr>
<tr>
<td>Poult mortality (first 2 weeks)</td>
<td>366</td>
</tr>
<tr>
<td>Feed (1 lb. per ton)</td>
<td>1438</td>
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<tr>
<td>Miscellaneous (rodents, wild birds, etc.)</td>
<td>114</td>
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LACTOSE FERMENTING SALMONELLA -
A DILEMMA FOR DIAGNOSTIC LABORATORIES?

Ned W. Rokey, D.V.M., B.S.
and
Michael D. Mecca, B.S.

INTRODUCTION

Most authorities agree that the genus *Salmonella* generally fails to ferment lactose. This characteristic among other biochemical and fermentation reactions is used to differentiate salmonellae from other lactose fermenting *Enterobacteriaceae*. Lactose sugar in combination with certain dye indicators is regularly incorporated into specially designed selective plating media and is specifically recommended in standard procedures for salmonellae isolation. Bismuth sulfite is the notable exception where dextrose is used in place of lactose.

A report published in 1969 reviewed a limited number of lactose positive salmonellae isolated from man. There is a paucity of published reports of lactose positive salmonellae in animals.

This paper reports the natural occurrence and the procedures for isolation of lactose positive salmonellae in domestic animals presented for laboratory examination.

METHODS AND PROCEDURES

Standard procedures for isolation and identification of salmonellae with modifications were used. Additional ancillary procedures used in this study included a salmonella fluorescent antibody system and culture techniques where lactose fermenting colonies were deliberately picked from initial selective plating media to study biochemical and fermentation reactions.

Source material was domestic animals presented for laboratory examination and diagnosis. Six separate tissues (lung, liver, gall bladder, prescapular and mesenteric lymph nodes, and joint fluid) were cultured from each animal when applicable.

From the Animal Disease Diagnostic Laboratory, University of Arizona Experiment Farm, Mesa, Arizona, where Dr. Rokey is pathologist in charge of the Laboratory and Mr. Mecca is research associate.

The authors thank Dr. H. Gilbert Crecelius, Chief, Laboratories Division, Arizona State Health Department, for serotyping salmonella cultures.

The authors acknowledge the assistance of Mrs. Marian Buckley in preparation of this manuscript.

Agriculture Experiment Station Technical Paper No. 1856.

This investigation was supported by funds from Regional Project 112 and State Research Project 134.
Approximately ten grams of tissue, depending on application and availability, was placed in selenite enrichment broth and incubated overnight. Broth cultures were streaked to selective plating media, usually Salmonella-Shigella (SS) agar; slides for fluorescent antibody (FA) evaluation were also prepared from broth cultures. When applicable, six colonies characteristic of genus *Salmonella* and six lactose fermenting colonies from each tissue were subcultured to Triple Sugar Iron (TSI) agar slants – a total of 72 subcultures for each individual animal when all tissues were studied. Responses to urea, KCN, citrate, sulfide, and indol were determined. Those isolates with positive FA response from enrichment broth and biochemic characteristics of salmonellae were subjected to carbohydrate fermentation studies. Group typing was carried out on isolates with typical salmonella fermentation responses as well as those where lactose fermentation was the only notable deviation from normal salmonellae behavior. Those isolates exhibiting positive salmonella group-typing were serotyped to species.*

RESULTS

Twenty-one separate lactose positive [lac (+)] salmonella isolates were recovered from domestic animals presented for laboratory examination during the period of December 1969 to October 1971. These isolates were recovered from 17 neonatal calves, 2 swine, a dog, and a chicken, and originated from 11 separate premises. Lactose positive strains were identified in three of the fourteen serotypes isolated during the period cited. (One additional lac (+) isolate identified as a group “B” salmonella has not yet been serotyped.)

Salmonella serotypes and the number of typical and lac (+) isolates are shown in Figure 1.

Initial isolation of lac (+) salmonellae from diagnostic accessions was made in December of 1969; subsequently, ten lac (+) salmonella isolates were made in both 1970 and 1971 (to date). These isolates were made principally from calves. High rates of morbidity and mortality occurred in neonatal calves on the premises where lac (+) strains were isolated.

The occurrence of lac (+) salmonellae and source of isolation are shown in Figure 2.

The number of animals with salmonella infections, both standard and lac (+) strains, is shown in Figure 3. Two additional neonatal calves had mixed infections of both standard and lac (+) salmonellae.

Lactose positive salmonellae only were isolated from 19 of 54 individual salmonella-infected animals, approximately 35 percent of the total. Forty-three percent (15 of 35) of the calves with salmonellosis was attributed to (+) salmonellae. See Figure 3.)

*Courtesy of Arizona State Department of Health, Division of Laboratories, Phoenix, Arizona; H. Gilbert Crecelius, PhD, Director.
**FIGURE 1. SALMONELLA SEROTYPES**
1969 through SEPTEMBER 1971

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>TOTAL ISOLATES</th>
<th>STANDARD SALMONELLA ISOL</th>
<th>LACTOSE (+) SALMONELLA ISOL</th>
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<tr>
<td><em>S. berta</em></td>
<td>7</td>
<td>7 100.0</td>
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<tr>
<td><em>S. derby</em></td>
<td>2</td>
<td>2 100.0</td>
<td>0 0.0</td>
</tr>
<tr>
<td><em>S. heidelberg</em></td>
<td>11</td>
<td>8 73.0</td>
<td>3 27.0</td>
</tr>
<tr>
<td><em>S. newport</em></td>
<td>8</td>
<td>8 100.0</td>
<td>0 0.0</td>
</tr>
<tr>
<td><em>S. poona</em></td>
<td>2</td>
<td>2 100.0</td>
<td>0 0.0</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>36</td>
<td>20 56.0</td>
<td>16 44.0</td>
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<tr>
<td><em>S. typhimurium</em> var. copenhagen</td>
<td>2</td>
<td>1 50.0</td>
<td>1 50.0</td>
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<tr>
<td>Group &quot;B&quot;</td>
<td>2</td>
<td>1 50.0</td>
<td>1 50.0</td>
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<tr>
<td>All Other*</td>
<td>8</td>
<td>8 100.0</td>
<td>0 0.0</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td><strong>78</strong></td>
<td><strong>57 73.0</strong></td>
<td><strong>21 27.0</strong></td>
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</tbody>
</table>

*S. anatum, blockley, havana, infantis, pomona, st paul, takeony, and group "D", 1 isolation from each.

**FIGURE 2. OCCURRENCE AND SOURCE OF LACTOSE POSITIVE SALMONELLEA**
1969 through September 1971

<table>
<thead>
<tr>
<th>YEAR</th>
<th>AVIAN</th>
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<th>CANINE</th>
<th>SWINE</th>
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<tr>
<td>1969</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1970</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1971</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>1</strong></td>
<td><strong>17</strong></td>
<td><strong>1</strong></td>
<td><strong>2</strong></td>
</tr>
</tbody>
</table>
LACTOSE FERMENTING SALMONELLAЕ—
A DILEMMA FOR DIAGNOSTIC LABORATORIES?

FIGURE 3. DETECTION OF LACTOSE POSITIVE SALMONELLAЕ
IN INFECTED ANIMALS

December 1969 through September 1971

<table>
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<th>SPECIES</th>
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DISCUSSION

Lactose fermenting salmonellae were first encountered in diagnostic accession material at this Laboratory in 1969. Initial interest in lac (+) salmonellae was stimulated when a wide discrepancy was noted between results of an experimental FA salmonella system6 and those obtained by standard cultural procedures5. A significant number of false positive FA tests was encountered that could not be supported by standard cultural procedures. Further investigation revealed that this discrepancy was not due to failure of the FA system but was due to failure to recognize that certain salmonella strains ferment lactose and that these strains produce pink colonies on SS agar. In accordance with standard procedures these lactose fermenting organisms were deliberately discarded as non-salmonellae. Further study of these organisms disclose I that the discrepancy was due to occurrence of lactose fermenting organisms which were eventually identified as aberrant salmonellae.

Salmonellosis was diagnosed in 54 animals in the study cited. It is significant that ancillary isolation procedures accounted for detecting salmonellosis in 19 (approximately 35 percent) of the total infected animals and that these infections would have gone undetected had standard procedures alone been used.

Following use of ancillary isolation procedures, an increasing number of lac (+) salmonellae has been isolated from animals presented for laboratory examination. This increase is probably due to efficacy of the ancillary system rather than recent emergence of lac (+) salmonellae. It might be concluded that lac (+) salmonellae have existed for some period of time but have not been detected by standard procedures currently in use.
Demonstration and identification of the number of lac (+) salmonella isolates cited in this report repudiates entire reliance previously placed on standard procedures generally in use for isolation and identification of salmonellae. Basic standard procedures, with the minor exceptions involving introduction of new differential media, have remained relatively unchanged for several years. Primary isolation procedures depend almost entirely on tinctorial characteristics of salmonella colonies on differential plating media. Tinctorial characteristics of lac (+) salmonellae on these media are inconsistent with those of the genus *Salmonella*. Lactose fermenting salmonellae isolated in this study regularly produced pink colonies on SS and MacConkey's; clear colonies on Brilliant Green; and black surface sheen colonies on Eosin Methylene Blue agar plates. Subculture to TSI agar slants produced an acid slant, acid butt, varying amounts of gas, and regularly failed to produce hydrogen sulfide (H₂S). These isolates - with the exception of lactose which was utilized with the essential rapidity of that of dextrose—had biochemic and fermentation reactions comparable to the genus *Salmonella*, including group typing.

Reactions of lac (+) salmonella isolates on TSI, as previously cited, are similarly inconsistent with those of the genus *Salmonella*. Lactose positive salmonellae typically produce H₂S in motility sulfide media in contrast to failure to produce H₂S on TSI slants. All other fermentation reactions, with the exception of lactose, were in general agreement with those of the genus *Salmonella*.

The exact nature of lac (+) characteristics is not clear. A 1969 report indicated that lac (+) characteristics could be transmitted to other members of the genus, notably *Salmonella typhi*, and that lactose utilization was due to a certain chromosomal transference; the exact method of tranference was unknown. Previous reports indicate that lac (+) tendencies were associated with phage transduction.

Results of limited studies with lac (+) isolates cited in this report revealed that some degree of permanency of lactose fermentation existed. Certain isolates retained lac (+) characteristics through at least eight subcultures. One isolate tested maintained lac (+) characteristics when inoculated into guinea pigs. The same lac (+) salmonella serotype was recovered from tissues cultured from both the inoculated and control animals. It would be remiss not to point out that some lac (+) salmonella isolates produced both conventional and lac (+) colonies on subculture. Replating of conventional colonies from these subcultures generally failed to reveal evidence of lactose utilization.

The genus *Salmonella* is considered pathogenic by definition. Results of experimental trials revealed that lac (+) salmonellae produced death in experimentally infected guinea pigs. Untreated contact controls confined with experimentally infected animals also contracted the disease and died. High rates of morbidity and mortality in neonatal calves on premises where the infection was detected lend additional support to the pathogenicity of lac (+) salmonellae.

**SUMMARY**

Demonstration of significant numbers of lactose positive salmonella isolates from domestic animals presented for laboratory examination poses a dilemma for
diagnostic laboratories. Lactose fermenting salmonella strains were isolated from 17 neonatal calves, 2 swine, a dog, and a chicken. Isolates were made during the period of December 1969 to October 1971. Affected animals originated from 11 separate premises.

These isolations were accomplished by use of ancillary methods consisting of a fluorescent antibody system and modification of standard cultural procedures. Ancillary methods accounted for approximately 35 percent of the salmonellae identified (19 of 54 salmonella-infected animals) which would presumably have gone undetected by standard methods of isolation.

High rates of morbidity and mortality in neonatal calves were associated with lac (+) salmonellae. Lactose positive salmonellae produced death in experimentally infected guinea pigs; untreated contact controls also contracted the disease and died.

The number of lactose fermenting salmonella isolated identified in this study places some doubt as to the entire reliance apparently placed on currently approved standard procedures for laboratory diagnosis of salmonellosis in domestic animals.

REFERENCES

SUDDEN DEATH SYNDROME IN FEEDLOT CATTLE

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Approximately two years ago death losses were reported occurring in fat cattle, weighing from 1,000 to 1,500 pounds, in a number of major feedlots in Kansas. The losses far exceeded what was commonly expected in this weight of cattle. The mortality became alarming in some lots that experienced daily losses to this syndrome. It was difficult to realize whether this was a condition new to the industry or whether it is one that had become more prominent.

Clinical signs have not been described due to the fact that animals dying from this particular syndrome are found dead in the lot. Dead cattle have been found near the feed bunks, watering devices, and within the group of cattle in the pen, it was the usual history that pen riders observed the cattle as normal, only to return an hour or so later and find a dead steer. Signs or evidence of struggling have been completely absent. The most consistent necropsy lesion observed was that of hemorrhage and edema in the tissues located near the thoracic inlet and extending forward along the trachea. Bloat was often included as a necropsy lesion. Distention of blood vessels anterior to the diaphragm was often noted. In some reports gas formation was present in the affected tissues.

Tissues have been collected at necropsy by a number of veterinarians and specimens were submitted to the diagnostic laboratory in Kansas State University, to diagnostic laboratories in other states, and to a number of commercial laboratories. Results from the various laboratories have been confusing and varied, with the majority of findings involving Clostridium organisms. The species reported were Chauvoe i, septicum, sordellii, novyi, and perfringens. One isolate of Cl. Carma was also reported.

During a field investigation members of the Diagnostic Laboratory necropsied two feed lot steers, weighing over 1,000 pounds, that had died suddenly. Necropsies were completed within two hours of the time of death. At the time of necropsy specimens were removed from the peritracheal lesions and were inoculated on agar plates and placed into portable, disposable anaerobic gas packs. Tissues were also inoculated into meat mash tubes and representative tissues were placed under refrigeration by dry ice. Specimens were fixed in formalin for histopathologic examinations. The laboratory results of bacteriologic studies on these tissues did not reveal Clostridium organisms. Histopathologic studies suggested congested vessels in the peritracheal tissues and areas of hemorrhage. Major organs did not reveal changes. Careful dissection and examination of the heart muscle was negative for pathologic lesions.

Careful studies at one lot on feed consumption per day per head, as related to occurrence of death losses, failed to correlate the occurrence of losses with high consumption or low consumption of feed.

During the several months that this condition existed, cattle in some pens were...
vaccinated with Clostridium bacterins (C.S.) as was the usual procedure. In other pens repeated vaccinations were evaluated, using multivalent antigens to attempt to control the death loss to the syndrome. Attempts were made to evaluate the use of two injections of multivalent Clostridium bacterins. In some lots it was reported that a decrease in the number of deaths was observed, while in other lots the effect was questionable.

During the past several months the occurrence of the syndrome had decreased in all areas that had previously experienced exceptional losses. In a number of large feedlots the bivalent bacterin is the only one used and death losses to the sudden death syndrome have diminished as in other lots using multivalent bacterins.

DISCUSSION

It is not intended that this descriptive, diagnostic phrase of sudden death syndrome become a catch-all, such as the shipping fever complex. It is a term used by many feedlot veterinarians when referring to losses in long term feedlot cattle that are found dead without clinical signs of illness and with similar necropsy lesions of peritracheal hemorrhage and edema.

In 1967 Niilo2 investigated losses in four feedlots over a period of eight months. Sixty-seven animals were necropsied and examined, and diagnoses were made on all but 11 animals. He suggested that some of these animals may have died due to metabolic disorders associated with feeding practices. Niilo also suggested that many of the so-called sudden death losses probably had demonstrated clinical signs but these signs evidently were not observed prior to death.

In 1967 Niilo2 investigated losses in four feedlots over a period of eight months. In experimental infections of *Cl. novyi* he observed noticeable clinical signs which were progressive and lasted for one to three days. Sudden deaths did not occur in animal inoculated with *Cl. novyi* organisms.

Mills1 described the pathogenesis of bloat. As intraruminal pressure increases, organs such as the liver and spleen become ischemic. Blood is redistributed into peripheral circulation from the abdominal reservoirs such as the liver and spleen. If we consider the 1,000 pound beef animal on full feed of concentrate ration, periodic subclinical bloat no doubt does occur. Also, the liver and spleen are known to harbor Clostridial organisms. Should the redistribution of blood from the liver occur, and carry numbers of Clostridial organisms to the peripheral circulation, then these organisms could seed the areas such as the thoracic inlet area and foci of infections could occur.

Turner4 reported that in his feedlot practice the two injections of multivalent Clostridium bacterins have controlled the losses due to sudden death syndrome. Other veterinarians have not been so successful in controlling losses, using vaccination procedures.

If this syndrome is truly a Clostridial infection, we might further consider the fat animal consuming 25 pounds a day of 90% concentrate ration. Does such a concentrate ration produce physiological environmental conditions within the tissues of the animal, that growth cycles of Clostridial organisms are so enhanced that in some animals the Clostridia overcome immunity and peracute fatal infections occur.

Tissues examined in our laboratory have revealed negative findings in some
instances and Clostridium species such as chauvoei, novyi, and sordellii in others. It is understood that many tissues submitted are actually unfit specimens, as some necropsies are performed several hours after death occurred.

The laboratory diagnostician is responsible to report his findings. His responsibilities may extend beyond the laboratory on occasions when the isolation of a specific organism may not be compatible with a disease syndrome.

To alter vaccination programs in large feedlot operations is sometimes necessary. To suggest revaccination of large numbers of cattle after they have become acclimated and on full feed is quite another consideration. The cost of a biologic is small compared to the cost of handling large numbers of animals during the feeding period.

In October of 1971 a questionnaire submitted to a number of feedlot veterinarians in Kansas revealed the following information. The occurrence of the sudden death syndrome has decreased during the past year but is still a problem. The use of multivalent Clostridial bacterins did not control the losses in a majority of the feedlots. A large percent of the veterinarians reported the primary cause of the conditions was more physiological then infectious.

REFERENCES

CASE REPORT
ISOLATION OF M. PARATUBERCULOSIS FROM A COW AT ROUTINE SLAUGHTER

W. D. Richards and R. L. Muham

*Mycobacterium paratuberculosis* was isolated from granulomatous lesions from a ten-year-old cow consigned to a packer for routine slaughter. Histopathologic findings were compatible for Johne’s disease even though caseation and calcification observed in lymph nodes was contrary to the classic histopathologic description of Johne’s disease. Herrold’s Egg Yolk Agar supplemented with mycobactin was the only one of eight laboratory mediums which supported the growth. The use of this medium with each routine mycobacteriology case could provide an effective surveillance method for Johne’s disease in cattle with granulomatous lesions regardless of the reason for slaughter.

The diagnosis of Johne’s disease is complicated by lack of specificity of skin test reagents and by the similarity of clinical signs to other disease conditions. The etiologic agent, *Mycobacterium paratuberculosis* proliferates in the intestine and is frequently shed in the feces of the infected animal. Saprophytic acid-fast bacilli exist as a part of the normal flora of feces from healthy and infected animals and cannot be differentiated from pathogenic acid-fast bacilli on the basis of cellular morphology. For this reason, the microscopic demonstration of acid-fast bacilli in stained smears of feces is not generally considered confirmative for the diagnosis of Johne’s disease.

Recent developments in culture methods1 have made possible the successful isolation and identification of *M. paratuberculosis* from feces or tissue specimens. *M. paratuberculosis* is dependent upon mycobactin,2 an extract of *M. phlei* for multiplication *in vitro*. This is a report of an isolation at the Diagnostic Services Laboratory of *M. paratuberculosis* from tissues collected from a cow in which Johne’s disease was not suspected. Routine use of medium containing mycobactin could provide an effective means of surveillance for Johne’s disease.

CASE REPORT

A ten-year-old bred cow was purchased by a livestock dealer and was consigned to a packing company where it was slaughtered on April 28, 1970. Tissue specimens

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From the Diagnostic Bacteriology Section (Richards) and the Diagnostic Pathology & Toxicology Section (Muham). Animal Disease Diagnostic Laboratory, Animal and Plant Health Service, USDA, National Animal Disease Laboratory, Ames, Iowa 50010.

518
were submitted to the Diagnostic Services Laboratory for histopathologic and mycobacteriologic examination.

**GROSS PATHOLOGIC CHANGES**

The meat inspector observed a caseous lesion in a posterior mediastinal lymph node and described the mesenteric lymph nodes as "swollen and hard". The cow's condition was described as "thin" and "suspected of being infected with Coccidioidomycosis."

**HISTOPATHOLOGIC FEATURES**

Portions of the mediastinal and mesenteric lymph nodes were received in 10% formalin. These tissues were processed for histopathologic examination. Sections were stained by the hematoxylin and eosin, new fuchsin, Auramine O, Brown and Brenn and Goodpature procedures.

Microscopic examination of the mediastinal lesion revealed a large mass of necrotic debris, surrounded by a thin connective tissue capsule, which was in turn surrounded by lymph node tissue. The necrotic material appeared to be caseous and some mineralization had occurred. Numerous aggregations of epithelioid cells were seen in the lymph node tissue. These cells contained large numbers of short bacilli which were Gram-positive with the Goodpasture and B & B stains. They were also observed in the new fuchsin and Auramine O preparations which indicated that they were acidfast. A small number of these organisms were seen in the necrotic material. The mesenteric tissues were an almost solid mass of epithelioid cells, (Figure 1) with little normal lymph node tissue remaining. These cells also contained large numbers of bacilli which were Gram-positive and acidfast (Figure 2).

**MYCOBACTERIOLOGIC FEATURES**

The specimens submitted in chloramine T were processed and cultured according to the procedures described in the manual "Laboratory Methods in Veterinary Mycobacteriology". The laboratory mediums routinely employed in general mycobacteriology cases were inoculated with derivatives of a composite of mediastinal and mesenteric lymph nodes. The mediums were, Lowenstein-Jensen, two slants; Stonebrink medium, two slants; Middlebrook 7H10 agar, one slant; Herrold's egg yolk agar, one slant containing mycobactin and two slants without mycobactin.

Primary colonies of *M. paratuberculosis* appeared after 24 days incubation at 37 C. Colonies appeared only on the single slant of Herrold's Egg Yolk Agar with mycobactin (Figure 3). All medium slants were incubated for eight weeks according to routine procedure but no additional colonies appeared.

* Case Number 70-05553-1816
The cellular and colonial morphology of the isolate was typical of *M. paratuberculosis*. The mycobactin dependency of *M. paratuberculosis* was confirmed by subculture of the organism on Herrold’s medium with mycobactin while Herrold’s medium without mycobactin failed to support growth of a subculture.

DISCUSSION

Diagnostic Services personnel began in 1967 to develop their capability to culture *M. paratuberculosis* from intestinal tissues, mesenteric lymph nodes and animal feces. Since that time, isolations have been made from cattle, sheep and goats located in widely separated geographic areas of the United States. The interest of practicing and regulatory veterinarians in the laboratory aspects of Johne’s disease has increased sharply since a cultural method became available for isolating and identifying the etiologic agent.

Frequently, bovine mesenteric lymph nodes are histopathologically “compatible for mycobacteriosis” but no isolation is made on mediums commonly used to culture mycobacteria other than *M. paratuberculosis*. This discrepancy may be attributed to the presence of *M. paratuberculosis* in the tissue examined. It is conceivable that *M. paratuberculosis* organisms could be present in mesenteric lymph nodes of cattle with Johne’s disease because of the anatomical relationship of these nodes to the intestinal tissues which are commonly infected. Histopathologic examination of the infected mesenteric lymph nodes should reveal the acid-fast bacilli and tissue changes compatible with mycobacteriosis. The *M. paratuberculosis* organisms would not be culturable on any medium lacking mycobactin. The discrepancy between the histopathologic and cultural examinations could be explained by this theory.

Jubb and Kennedy stated that caseation and calcification in lymph nodes may occur in sheep infected with *M. paratuberculosis* but not in cattle. Smith and Jones stated that the absence of necrosis and calcification is of importance in the diagnosis of Johne’s disease in cattle.

The caseous, mineralized mediastinal lesion described in this report may have been due to some other cause. However, in view of the fact that some acid-fast organisms were found in the necrotic debris, it seems reasonable that they were directly involved. Most certainly the epithelioid response in lymph nodes from both body cavities was due to the acid-fast bacilli.

The case reported here is the first at this laboratory in which *M. paratuberculosis* was isolated from tissues collected from a cow consigned, under routine conditions, to a slaughter plant. Neither the cattle dealer nor the meat inspector suspected Johne’s disease in the cow. It was not possible to identify the herd from which the cow originated.

It is the opinion of the authors that careful histopathologic examination and the use of culture mediums containing mycobactin could provide an effective surveillance method for detecting Johne’s disease in cattle with granulomatous lesions regardless of the reason for slaughter.
Figure 1.— Microphotograph showing mesenteric lymph node tissue consisting almost entirely of epithelioid cells. H & H; X 250.

Figure 2.— Microphotograph showing acid-fast bacilli in cytoplasm of epithelioid cells. Single fluorescing organisms are white dots or rods. The masses of fluorescing organisms appear as white, foci. Auramine O stain; X 250.
Figure 3.— Photograph showing the abundance of *M. paratuberculosis* colonies on Herrold's Egg Yolk Agar medium containing mycobactin. The amorphous mass (arrow) is culture growth left by the transfer loop during subculturing; X 8.

REFERENCES

ASSESSING AND DEFINING A STATE'S VETERINARY DIAGNOSTIC NEEDS

Sidney R. Nusbaum, John F. Quinn, Vaughn Seaton, Kenneth Weide

Assessment and definition of one's position within a state obviously requires a thorough looking inward and outward. These are not easy things to do. First, there never seems to be time. If one makes time he must be convinced of the necessity of the project or he may well assume that it is daydreaming and feel guilty. There is a very real problem in attempting to be truly objective in appraising the situation within one's own laboratory. You are used to it; you have helped to create it, and a critical examination is rare. Finally, the assembling of the whole picture concerning the "outside world" is a long, difficult job. And yet it is partly because of the complexity of the job that one must consciously practice a program of continuing examination and appraisal. It is all too easy to come to believe that one is doing the best possible job inside the laboratory and an ultimate job of servicing the needs of a state. This may rapidly lead to false complacency, yielding poor service, and being outmoded.

The assessment process is not a simple matter. It is very easy to fall into the trap of opinion—if one asks the state veterinarian's office what is needed he will get a definite opinion, and an equally definite opinion from the various college personnel, and a plethora of ideas from practitioners. Likewise, the use of surveys and statistics must be handled with great care and discrimination. To do a thorough job there must be a compilation of the total knowledge followed by a thorough job of distillation. The purpose of the assessment will make a difference in what manner it is presented, and how broad the project should be.

Diagnostic laboratories are rapidly becoming beneficiary and victim of an expanding body of knowledge and increasingly sophisticated methods. Beneficiary, because new precision of disease definition is possible which was only suggested a few years ago. Victim, because the new methods require investment in manpower, space and equipment which strain the resources of the most lavishly endowed institution. In view of the investment that must be made it is essential that laboratory administration insofar as possible plan and anticipate for the future so that the most efficient use can be made of resources. A laboratory should be prepared to expand or contract particular services as their importance grows or lags. To effect this flexibility the laboratory must have full information about the needs of the population it serves. This information is essential for internal planning for both the large and small laboratory.

In addition to utilizing survey information for internal planning it is becoming increasingly obvious that this knowledge is necessary for external relations, particularly in maintaining essential economic support for the laboratory. Rarely do our actions speak sufficiently loud that budget administrators will year after year disburse funds without questioning the value of the expenditure. Particularly in these times of intense competition for financial support, the laboratory should be able to
demonstrate truthfully and forcefully that it is performing a valuable service which justifies the faith of the sponsor. If this justification cannot be demonstrated then administrators will favor units which can validate their worth. The validation of one's services may be unattractive or even repugnant to some scientists, but they had best be prepared to demonstrate their value or there may not be an opportunity to demonstrate their skills. Accurate, logical demonstrations of laboratory services, not merely listing of tests and numbers, but how these tests apply to satisfying a public need is the best validation that can be made.

In addition to utilizing survey information for internal planning and for the maintenance of support of budgets, survey information is also essential in securing initial support for the laboratory which hopes to be newly funded or greatly enlarged. The following is a case history of the accumulation and presentation of the facts in New York State. As with any medical case history it should serve as an example only and not necessarily as a model. This example is presented rather than a methods and technique discussion because we feel it demonstrates more completely several matters. As an example it may be useful for others but only to be applied within the context of their own situation.

Throughout the 1960's New York provided minimal support for a general diagnostic laboratory. To remedy the situation a proposal was submitted in November 1969 for a complete service diagnostic laboratory. In addition to capital funds the proposal suggested an annual expenditure of about $400,000. A broad appeal was made for support to animal industry, public health, recreation, ecology, and pet groups; the Legislature responded favorably by granting funds far in excess of that which had been given in previous years, but not sufficient to meet the needs of the proposal. The Legislature also took a far-reaching step in directing that a committee of outside authorities investigate the needs of the state for diagnostic facilities, in effect to validate or negate the proposal that had been made. Drs. Quinn, Seaton and Weide were selected for that investigational committee. The remainder of this paper will constitute a report of the methods employed by the committee and a review of the findings.

Prior to the committee assembling in New York each member was provided with a folder of basic information. This material had been collected during preparation of the Proposal and in large part had been previously distributed to legislators and interested laymen. Thus, when the members of the committee arrived they had some familiarity with the conditions within the state. First meetings were with Department of Agriculture and Markets and Bureau of Budget personnel and with representatives of the Veterinary College. These meetings helped define the problem and assignment.

At these meetings there was a realization that much information was needed which was not available and also that formal meetings with officials would not be sufficient to do a complete job. On-site visits would be necessary as would utilization of a broad group of resources both within and outside the state. A second visit consisted of a four-day, fourteen-hundred mile trip across the state. Preparations were made for this trip by scheduling stops with key persons and asking them to arrange for others of their choice to be at the site. In this way the groups were varied, and a degree of spontaneity was arrived at which would not have been possible if the planner had arranged all details. Interviews and observations were con-
VETERINARY DIAGNOSTIC LABORATORY FACILITIES – STATE OF NEW YORK

Analysis and Recommendations

CONTENTS

I. SUMMARY AND CONCLUSIONS ......................................................... 1
   A. Value of Livestock Industry in New York State ......................... 2
   B. Losses from Animal Diseases ............................................... 2
   C. Relationships of Animal Disease Problems .............................. 3
   D. Deficiencies ........................................................................... 4
   E. Savings .................................................................................. 4
   F. Recommendations of the Committee ......................................... 5

II. INTRODUCTION ............................................................................ 7

III. DEFINITION OF THE PROBLEM .................................................... 12
    A. Animal Disease Potential ...................................................... 12
    B. Value of Animals in New York State ...................................... 13
    C. Estimated Losses in New York State from Animal Diseases ....... 17
    D. Public Health Inter-relationships ......................................... 21
    E. Ecology and Conservation Inter-relationships .......................... 25
    F. Preparedness and Laboratory Complexity ............................... 26

IV. NEED FOR CENTRAL VETERINARY DIAGNOSTIC FACILITIES ....... 30
    A. Current Facilities and Services Available in New York State .... 30
    B. Income from Animals in New York State ............................... 31
    C. Population Dependence on Agriculture ................................... 32
    D. Need for “Insurance” and Knowledge .................................... 33
    E. Fallacy of Relying on other Laboratories for Assistance .......... 35
    F. Potential Volume of Work ...................................................... 36
    G. Examples of Individual Diagnostic Accomplishments .............. 37
    H. Advantages of a Veterinary Diagnostic Laboratory ................. 39

V. WHAT CAN A LABORATORY EXPECT TO ACCOMPLISH? ................ 42

VI. PROPOSED CENTRAL DIAGNOSTIC FACILITY .............................. 45
    A. Administration ...................................................................... 45
    B. Location ............................................................................... 45
    C. Physical Plant ...................................................................... 46
    D. Operating Budget .................................................................. 47
    E. Regional Laboratories ............................................................. 47
    F. Continuing Education ........................................................... 48
    G. Cost Comparisons .................................................................. 49

VII. ACKNOWLEDGEMENTS ............................................................... 50
ducted at poultry, beef, dairy and horse farms, poultry and mastitis laboratories, two colleges, and a major race track. Committee members had the opportunity to meet with animal industry representatives at all levels, veterinary practitioners, state and federal regulatory officials. Under these circumstances data and statistics took on new meaning. Later the committee met to digest, organize, and write the report. Time limitations will permit only the briefest outline of the material contained in the report. The report was fifty pages long. Its composition favored its being read. Printing was double-spaced, a “Summary and Conclusion” section was placed at the front for the convenience of the reader who preferred to scan rather than study, and a detailed table of contents made locating information a simple matter.

(See preceding page for illustration of contents)

Despite its place at the beginning of the report, the “Summary and Conclusions” section will be discussed last.

The introduction included general information including the importance of animal food supplies to the general economy and well-being of the nation and the state. The fact that efficiency in animal production was essential and that disease was inefficient led into the point that disease control, utilizing a diagnostic laboratory, maximizes food potential. The failure to maximize this food potential threatens every household budget. The part that the laboratory plays in supporting the practitioner in difficult cases and in helping monitor and thus measure disease incidence was pointed out. In addition, there was information concerning developing problems of disease husbandry, chemical residues, and population concentration problems. In this section the contrast was drawn between the support which was given public health laboratories as contrasted with the failure to support veterinary diagnostic laboratories in the state.

III. Definition of the Problem

A. Animal Disease Potential

New York’s location makes it particularly prone to introduction of foreign disease. Over 6,000 ships traverse the St. Lawrence Seaway yearly. Many violate international law by disposal of animal products. The international airports of New York receive almost 60,000 flights a year from foreign countries and this figure does not include Canada or Mexico-originated flights. The potential for introduction of foreign disease is dramatically shown.

B. Value of Animals in New York State

Most New Yorkers think of their state as a manufacturing and industrial state and are not aware of the large investment in domestic animals, and yet we possess almost one-half billion dollars worth of cattle, swine, sheep, and poultry, an unmeasured but large population of horses, and over 410 laboratories are licensed to use research animals.
C. Estimated Losses in New York State and Animal Diseases

It is difficult to do much more than estimate losses from animal disease, but the committee used several parameters including a higher than national death rate of New York’s calves, piglets, and lambs. Documentations of a 400 thousand dollar direct loss from Dutch Duck Plague in 1967 was given along with the estimate that indirect costs probably would double that figure. An outbreak of influenza causing the closing of a race track in New York served as an illustration of how much the state, horsemen, and associated businesses lost under these conditions.

New York was shown to have some unique problems because of concentration factors of human and animal populations, and thus the public health inter-relationships are particularly important. The committee listed some of the diseases transmitted from animal to man and pointed out that there was a minimum of information about this subject because of the absence of adequate veterinary diagnostic laboratories. At the same time they contrasted this with the number of tests conducted by the State Health Laboratory on humans for animal-transmitted diseases, and pointed out that full knowledge would have done much to combat infection.

D. Ecology and Conservation Inter-relationships

The importance of animal disease in ecology and conservation was demonstrated in several ways. Besides the problem of disease from domesticated animals to wild animals and vice versa the very real dollar value of wild life was illustrated by the fact that New York claims an outdoor recreation industry of more than five billion dollars a year. Much of this revolves around animal life. One-half billion dollars is spent on hunting and fishing in New York each year. The fact that the state has the dubious distinction of having one of the highest levels of wild life rabies in the country illustrated the problem of human-domestic wild animal disease.

F. Preparedness and Laboratory Complexity

The committee pointed out that a laboratory must be well rounded, that a single capacity is not sufficient. It gave instances of the new and developing methods which illustrated the need for depth. In discussing preparedness in a laboratory complexity the committee pointed out “that a properly funded and equipped laboratory stands in readiness to cope with any and all diseases of domestic animals”, that a specialized laboratory had very definite limitations. They then editorialized to state “there is no government function easier to justify than a well funded, well staffed, state diagnostic laboratory, for its function has direct effect on losses from animal diseases and assures the consumer of quality animal products.” Illus-
trations were then given to demonstrate the variety of specialized skills and equipment that are necessary.

IV. Need for Central Veterinary Diagnostic Facilities

A. Current Facilities and services available in New York State.

Evaluating the present facilities the committee concluded that the state was dramatically unprepared to render adequate diagnostic services. This conclusion was balanced against the following data:

B. Income from Animals in New York State

In 1968 eight hundred thirty-four million dollars was derived from animal products without including income from horses. The total value of the horse industry cannot be measured in New York State, but it is known that 170 million dollars in parimutuels were directed to the state in 1969. The committee estimated that the total income from all animal sources might exceed five billion dollars.

C. Population Dependence on Agriculture

The due of farmers was demonstrated by the fact that fresh, wholesome meat and milk supplies were made available to the people, that over 1.3 million jobs are indirectly dependent on agriculture, and if the state lost any of its producing ability a serious consequence would be suffered in increased costs to consumers.

D. Need or "Insurance" and Knowledge

The committee felt it ironic that New York State, a comparatively prosperous state, could not provide an insurance coverage for 1) the diagnosis and surveillance of animal disease 2) the protection of a sizeable portion of food supply, 3) protection against diseases of animals transmissible to man, and 4) methods to monitor residues in animal products.

This section also dealt with the deficiencies in support of disease regulatory personnel and the fact that the lack of laboratory support discouraged young practitioners from entering general practice.

E. Fallacy of Relying on other Laboratories for Assistance

The committee was aware of the argument that materials could be sent to other laboratories and they illustrate the delays and difficulties that are associated with such a procedure.

F. Potential Volume of Work

An estimate of the work to be accomplished by a complete laboratory was provided by the state veterinarian’s office which contrasted sharply with what is now being done.

G. Examples of Individual Diagnostic Accomplishments

To prove the point of what can be accomplished there were examples given of the control of such diseases as vibriosis, tuberculosis, and brucel-
NUSBAUM, QUINN, SEATON, WEIDE

Letosis in New York, and other diseases in other states. The conclusion was “such laboratories do not cost, they pay, and pay handsome dividends”.

H. Advantages of a Veterinary Diagnostic Laboratory

Finally, this section was summarized by the several areas where advantage will be gained if a diagnostic laboratory is available.

V. What can a Laboratory Expect to Accomplish

In some respects the section on what a veterinary diagnostic laboratory can expect to accomplish was a restatement of other material and covered alleviation of animal disease problems, public health protection, and the ability to provide information and definition of animal disease problems. Particular emphasis was placed upon the time factor in that personnel and facilities would be immediately available to abort crises. The report pointed out that while contributing great good to limited fields the extensive mastitis and poultry laboratory system in New York was at best inefficient in serving all segments of animal industry, for they suffer from dual administration and concentrate an excess effort on two species. The committee believed that at least five to ten million dollars would be saved each year in New York with an adequately funded diagnostic laboratory.

VI. Proposed Central Diagnostic Facility

Specific recommendations of the committee as regards actions to be taken will not be discussed here. The proposed program did provide for a complete facility with recommendations for administration. In large part it was in agreement with the College’s proposal of 1969 but did differ in size and some other areas.

SUMMARY AND CONCLUSIONS

The “summary and conclusions” was placed at the beginning of the report and provided the reader with little time to invest the opportunity to gain a complete view of the ideas and principles with a minimum of effort.

The value of livestock industry in New York State was shown in relation to the entire state’s activity.

Losses from animal diseases were pointed up in regard to the consumer and to the financial situation of the state.

Relationships of animal disease problems were fitted into the entire state scene. The present deficiencies were covered and the savings that could be effected by a proper laboratory service were pointed up.

Finally, the recommendations of how the situation might be improved were stated.

DISCUSSION

This paper was meant to be an example of what a survey definition assignment
ASSESSING AND DEFINING A STATE'S VETERINARY DIAGNOSTIC NEEDS

may entail. First, a total compilation of the situation as viewed by all concerned. Few states have provided the opportunity for their laboratories to provide a total service, therefore greatest emphasis must be given to those areas which are "most significant". The designation of "most significant" requires complete knowledge plus some Solomon-like discretion and wisdom.

Note that in this study it was deemed worthwhile to go far afield from traditional agriculture and public health information. But when 1.8 million people in a state purchase hunting and fishing licenses they have a vested interest in wildlife health which should be responded to. Similarly, agricultural statistics for New York fail to reflect the number or value of horses, yet when parimutuel taxes represent the seventh largest single source of income legislators and taxpayers should be concerned and informed.

References were made to the pet industry and its economic importance, public health significance and psychological values, but I believe the committee felt it unwise to emphasize these factors because the study was sponsored by agricultural interests and there may have been the suggestion that public funds should not be lavished too freely in what is regarded by some as a luxury industry.

Each state has its individuality in people and in customs, in government, and attitudes. The report carried an extra degree of validity because the investigators could quote direct conversations and observations from their trip to the places referred. (Similarly, I profited from the impressions that were gained on these trips, for they re-enforced the necessity to revisit old, well-known areas and look for new ones before drawing conclusions; just because we are not aware of a problem or a need does not mean it does not exist).

Finally, a report without an audience is of little value. This report was created in a manner to keep an audience. It was largely couched in laymens' terminology; facts were frequently presented several times, but in different ways to take advantage of the knowledge that a stranger in a field is usually not able to comprehend with a single exposure to facts.

The position of the "Summary and Conclusion" in the report not only made it easy for the busy legislator to draw conclusions but also whetted the reader's interest.

The paper generally covers the areas suggested at the beginning of this paper, -flexibility and planning, justification of support, and in this case, justification for new facilities. Similar reports might be more specific and deal with single areas or provide more complete technical detailing.

Possibly the greatest value of the use of this report as an example of surveying is the demonstration of the many areas which should be explored, and the variety of sources of information available.

The purpose of this survey was achieved in that it did accurately evaluate the
needs of the state; it was read by legislators and the Governor, and they did take action to implement the recommendations.
NEED FOR PERSONNEL SAFETY AND HEALTH
PROGRAMS IN VETERINARY DIAGNOSTIC LABORATORIES

by
Vaughn A. Seaton

This subject of health and safety in veterinary diagnostic laboratories is not new certainly, however I don’t believe many of our laboratories have specific and formal health and safety programs in effect. It shall be my purpose to briefly discuss it, perhaps to stimulate some latent interest, and to propose that the AAVLD initiate a study and explore the needs for health and safety plans for veterinary diagnostic laboratories. We may find that we have obligations and responsibilities legally which have been largely ignored in addition to these pertaining to health alone. It may be advisable to propose a recommended safety and health program for reference and use in our respective veterinary medical laboratories.

In discussing this subject on this program, I do not wish to imply that a panic situation exists in our laboratories or that calamitous events are upon us in so far as health and safety hazards to our personnel. I simply wish to emphasize that a judicious look should be taken at the problem.

A logical starting point for our discussion would be to assess the severity of the known threat of accidents and laboratory affiliated illnesses among our laboratory personnel. To my knowledge this information has not been compiled and is not available for veterinary diagnostic laboratories. Accurate figures may be nearly impossible to obtain.

In lieu of any solid information on accident and illness incidence in veterinary diagnostic laboratories, let us consider for a moment the more obvious and likely types of problems, then likely sources of the problems and finally a few thoughts on some preventive measures.

The first things we think of are the soonoses such as bacterial, viral, fungal, and other types of infectious agents. We are working with some of these diseases constantly, others occasionally and some rarely. This fact I believe not only immediately presents the obvious problems but also presents the most important, effective and easiest preventive medicine solution. That is simply to employ common sense aseptic technique measures in the handling of all cases and materials. Using aseptic technique throughout the laboratory in all our operations and activities and doing them routinely and almost unthinkingly whether a hazard is expected or not is obviously our best safeguard.

I shall not dwell on the rudiments of aseptic technique nor on the zoonoses per se. You are as aware of both the techniques and diseases as I am. The more important aspect is how we come in contact with infectious agents, usually accidentally. Contaminated laboratory clothing, aprons, gowns, gloves and instruments are the most common source. This occurs by direct contact. Splashing of water, disinfectants and body fluids are also common methods of contaminating clothing, instruments and laboratory equipment such as centrifuges, microscopes and worktable surfaces.
NEED FOR PERSONNEL SAFETY AND HEALTH
PROGRAMS IN VETERINARY DIAGNOSTIC LABORATORIES

G. Briggs Phillips in a publication titled "Casual Factors in Microbiological Laboratories Accidents and Infections" published in 1965, studied four microbiological institutes including those laboratories at Fort Detrick, Maryland. The purpose of his investigations was "to identify the casual factors related to accidents and occupational infections occurring in microbiological laboratories." This publication might well serve as a guide for further studies in so far as cause of laboratory accidents and infections. This study was referred to me by Doctor James Sullivan, Safety Officer of the National Animal Disease Laboratory at Ames, Iowa. Phillips states in his study that "less than 20 per cent of the infections were caused by recognized and recorded accidents. As many as 80 per cent of the infections were caused by unsafe acts that occurred without realization or recognition." He also states that "more than three quarters of the injuries were caused by unsafe acts. Unsafe conditions caused 10 per cent of the accidents." Keep in mind that his study concerned strictly large microbiological laboratories. His study did lead to a set of safety rules for infectious disease laboratories. These rules cover many areas and while they are basically a combination of common sense and aseptic technique, they are well worth reviewing and could equally as well apply to veterinary diagnostic laboratories in general as to microbiological laboratories in particular.

While we recognize the potential problem among our professional and technical personnel, it is obvious that an even greater risk can occur among the untrained technical and clerical assistants should their duties bring them into contact with contaminated worktables, instruments, cultures and laboratory waste materials. For instance "mailed in" specimens which may be unpacked and distributed to certain sections of the laboratory by persons untrained in asepsis have a very real potential for direct contact and contamination.

In addition to direct contact and contamination, we should give a little thought to aerosols produced primarily by specimen preparative including waring blenders and centrifuges especially in cases of tube or bottle breakage. We are especially vulnerable to aerosol born infection through the eyes and nose and mouth.

In our present day diagnostic laboratories with a greater emphasis on chemistry and toxicology than ever before, we have a significant threat of toxicosis. This occurs not only from the toxic suspect material submitted to us for analysis but equally as great or greater is the high percentage of very toxic chemicals and solvents which we use during the analysis. We have a veritable dynamite situation in our midst not only from the toxic standpoint but from the explosion standpoint. External storage of highly volatile solvents necessary for pesticide extractions and other common toxicological procedures is a must. Storage of these materials within the laboratory building is extremely hazardous. Many of our laboratories do not have explosion proof refrigerators, freezers, drying ovens, and incubators. Yet we put highly volatile substances in these pieces of equipment for incubation, processing or storage. The explosion proof aspect of which I speak is a closed electrical control system rather than a "strong box" approach.

I would like to emphasize ventilation problems throughout the laboratory but especially so in chemistry laboratories. Some of these compounds are extremely hazardous upon inhalation and some of the instrumentation in these areas create problems including radiation emmission. Negative air pressures, fume hoods...
and instrument exhaust vents to the outside are imperative. Inhalation problems are a clear and present danger.

Now that we've cited some obvious problems of infective agents and toxic materials by contact, aerosol and inhalation, we should mention some of the other safety factors such as cleaning perchloric acid fume hoods, microbiological hoods, having proper lighting, electrical circuit overloads, safe steps and stairs and hallways. We must not forget that the public uses these facilities as well as our laboratory personnel. I might add that one of our very real threats to personnel health is the inadvertant ingestive of infective agents and toxic agents by means of our time honored coffee break. Eating and drinking in laboratories is an especially dangerous activity. Table surfaces are easily contaminated, coffee cups, the coffee urn, the sinks we wash them in and even in some instances toxic or infective agents can be carried by flies, roaches, ants or other unscheduled visitors.

I think we have only touched on some of the obvious problems and I have made no attempt to completely document the risks.

In draft is a publication entitled "Laboratory Safety at the Center for Disease Control" published by the United States Department of Health, Education, and Welfare, Public Health Service which is an extensive effort and is designed to present "a broad program of preventive medicine designed to protect health of employees who may encounter biological or chemical hazard in the laboratory or field."

In this publication such broad topics as 1) Restricted areas in the laboratory for certain employees only, 2) Protective clothing, 3) Preventive medical services, 4) Reporting accidents and illnesses, 5) Designation of known biological hazards and areas, 6) Signs indicating hazard areas, 7) Control of air flow, 8) Visitors in the laboratory, 9) Dangerous chemicals, 10) Immunization records are extensively discussed. This publication also can serve as an important resource for application to health programs in Veterinary Diagnostic Laboratories.

Now I'd like to mention a couple health programs employed in two laboratories that I am familiar with. I don't offer these programs as answers particularly, but rather as points of departure.

In one diagnostic laboratory a health program for all animal caretakers has been initiated as follows for a new employee:
1.) Physical examination, 2.) Tuberculosis skin test, 3.) Tetanus inoculations — Two, and 4.) Rabies vaccination.

The National Animal Disease Laboratory has a health program under the direction of Doctor James Sullivan and the nursing staff which goes something like this:

1.) At time of initial employment a serum sample is obtained and routinely checked for psittacosis, brucellosis, and leptospirosis. This is true except for purely administrative personnel. Titers may be checked again for those three agents at irregular intervals as deemed advisable. All new employees receive a Mantoux followed by the tine test later as indicated.

2.) If an employee will be working with specific disease agents for which a vaccine is available, they are inoculated before they are permitted to work with that agent. The vaccines used for persons in specific areas of research of diagnostic work at NADL include the following; rabies, anthrax, Eastern, Western, and Venezuelan encephalomyelitis, certain str-
NEED FOR PERSONNEL SAFETY AND HEALTH PROGRAMS IN VETERINARY DIAGNOSTIC LABORATORIES

Aims of influenza virus, mumps, tetanus and diptheria.

3.) A Mantoux test is administered every six months and chest plate annually for tuberculosis research or diagnostic workers.

4.) Each six months a chest plate is made of those persons working with mycotic agents.

5.) Persons working with fixed tuberculin tissues are x-rayed and Mantoux tested annually.

6.) When a person ceases employment at NADL, a serum sample is again obtained and specific titers determined at that time. The remainder of the serum is frozen and retained in serum bank should there be a need for checking further titers at a later date. This is a protection legally on the one hand and may be of diagnostic significance on the other hand.

Doctor Sullivan has indicated to me that medical surveillance programs have been shown to be very valuable. In incidences where a potential problem has manifested itself, a surveillance program not only aids in diagnosis and recognition but also in control.

I would say in summation, if immunizations for the various diseases and pathogens available to us can be justified anywhere, it seems the justification is as great or greater in veterinary diagnostic laboratories where people are working directly with the agents. Especially when we are dealing with "the unknowns" constantly as compared to a microbiology research laboratory where they are dealing with "known" agents.

So then the question is what type of health programs should be encouraged? The answer to that it seems to me is a combination of medical and legal factors. The greatest danger is the possibility of medically untrained personnel making claims against the parent organization, the laboratory and specific individuals for not informing them of the danger involved in their work, for not educating them to protect themselves and it could be argued that gross negligence is involved if the laboratory does not at least have a health program for its employees - regardless of what specific items are included in it.

In such instances even if the health program were not too effective medically it might prove more effective legally.
The major goal of most safety programs is the prevention of work-connected injuries and their attending costs in terms of human suffering, man hours lost, damage to programs or property or other factors which ultimately increase the overall cost of the product or service being provided.

To achieve these objectives in a biological laboratory, consideration must be given to the specific biological hazards of the work under way.

In general, there are three major areas of consideration which a well-rounded biological safety program must attempt to control. These include:

1. Personnel Protection—the protection of laboratory personnel against infection with pathogenic agents worked with.
2. Program Protection—the prevention of unplanned contamination or infection of laboratory media or animals used in experiments or diagnostic assay procedures.
3. Biological Agent Containment—the control and containment of all biological agents in a manner which will minimize the possibility of laboratory associated disease outbreaks within the immediate laboratory community.

High security biosafety program planning and operations were first pioneered at Fort Detrick and other facilities within the U.S. Army Biological Laboratories during the 1940's.

Although one might assume that the degree of occupation exposure of the need for containment were unparalleled, the last two decades have seen the creation of a number of similar facilities including the USDA's Plum Island Animal Laboratory for the study of exotic diseases of animals, NASA's Lunar Receiving Laboratory for the assay and characterization of Lunar samples, and the National Institutes of Health's Virus Oncology Laboratory.

The obvious need for such facilities, based on our knowledge of certain disease entities or the lack of knowledge but potential hazard relating to the escape of biological agents from others, are not universal criteria which necessitates only the construction of expensive high security laboratories in the future. As a consequence, laboratory design and construction in the 70's will continue to center around the need for "moderate security facilities."

Fortunately, the emphasis on the design and operation of high security laboratory during the past three decades have not centered exclusively on costly mechanical systems or equipment, but has been paralleled by a search for information on the number, costs, and causes of laboratory-acquired infections.

This search has been stimulated in part by the ever-increasing list of laboratory "faux pas" resulting from the inapparent contamination of laboratory media or animal cross infection, as well as the replacement of the philosophy that a scientist's serological profile is in some way a measure of his worth by more realistic moral, economic and legal concepts.
One of the most comprehensive studies involving the frequency and severity of laboratory-acquired infections entitled “Causal Factors in Microbiological Accidents and Infections” was written by G. Briggs Phillips and published by the U. S. Army Biological Laboratories, Ft. Detrick, Maryland, in 1965.

The author states that even though he had found over 600 articles on laboratory-acquired infection, he and other authorities in the field believe that these reports represent only a fraction of such accidents actually occurring.

Phillips’ study provides a good deal of basic information on biological accidents which do much to help one gain on insight into the overall dimensions of this problem. His work shows that in general, laboratory-acquired infections are a good deal more expensive than the typical industrial injury in terms of man hours lost and associated medical expense.

His study also suggests that the ratio of disabling laboratory infections to non-biological disabling injuries in the same laboratory appears to be more closely related to the scope and efficiency of the medical surveillance program than it is to the purported relationship of biological and nonbiological accidents.

A third, but highly significant conclusion found in comparing industrial and biological accidents, is that in contrast to the ease with which the underlying “cause” of the great majority of industrial accidents could be determined, the specific cause of biological accidents was extremely illusive and could be determined in less than 30% of the cases.

In the majority of cases where the cause of laboratory infections could be determined with any degree of assurance, obvious flaws in the techniques or equipment employed were usually apparent. A partial list of incriminated causes for such accidents would include breakage of vessels containing infectious material, pipetting accidents, syringe malfunctions, autoinoculation with hypodermic needles, or post-mortem equipment, etc.

Many laboratories have adopted a set of basic guidelines or common sense rules of conduct which will do much to minimize, but will not entirely prevent, accidents of this type. An excellent example of the type of general safety rules for work in biological laboratories can be found in the text “Microbial Contamination Control Facilities” which was published by the Van Nostrand Reinhold Publishing Company of New York in 1969.

In general, all such guidelines tend to identify laboratory areas or operations which are hazardous and to limit the degree of exposure by controlling the flow of traffic and materials into and from such areas, by restricting eating, drinking and smoking to specified areas, by prohibiting mouth pipetting, and by mandatory use of rubber gloves and other inexpensive safety aids for certain laboratory activities.

Since 1950, numerous studies have been conducted in an effort to identify factors which contribute to laboratory infections which are not associated with an overt accident. These studies clearly showed that a large number of laboratory activities generated biological aerosols of varying concentrations ranging from the low count aerosols created by unplugging test tubes or streaking plates to the high count aerosols generated during blending or high pressure animal room cleaning procedures.

Once the significance of aerosols as a cause of laboratory infections was firmly established, a number of steps were taken to help control exposures of this type.
Personnel safety devices ranging from modified surgeon's masks (through gas or particle arrestance mask) to ventilated hoods or suits become available and were used in keeping with overall program priorities. (Needless to say, their acceptance was sometimes difficult to obtain because of restrictions placed on the user.)

Safety techniques were devised for the use of existing equipment in many of the smaller, less well funded laboratories, and specific pieces of maximum containment equipment such as safety centrifuge tubes and the safety blender began to appear in many laboratories.

Containment equipment, including ventilated safety cabinets and small animal isolators, become commercially available. Equipment of this type not only protected laboratory personnel but provided an effective means of controlling biological pollutants originating in the laboratory.

Limitations relating to the introduction or removal of material, available work space and freedom of movement convinced many scientists that the bio-safety engineer had failed "to build a better mouse trap" and stimulated a continuing search for less restrictive containment equipment.

The laminar air flow or clean room system, originally developed to provide a controlled environment for the manufacture of electronic components required by the aerospace industry in the early 60's, was quickly recognized as a valuable biomedical tool in areas where air purity was important.

Laminar air flow systems which provided a continuous stream of filtered, particle free air were quickly developed for use in surgical suits and burn centers of our hospitals as well as the tissue culture production and packaging units of many laboratories.

As the horizontal flow of air used in most of the early laminar flow units was obviously an impractical system for containing aerosols created during the course of many laboratory procedures, a number of basic modifications were required before such units could be used for work with pathogenic agents.

Biosafety specialists interested in this problem soon found that a significant degree of containment could be achieved by utilizing a vertical stream of air consisting primarily of recirculated, filtered air mixed with approximately 10% makeup air from the room.

Vertical laminar flow biological hoods created in this manner provide a high degree of control over airborne contamination of either room or hood origin.

Although laminar flow hoods afford a substantial degree of user protection, they like many other products on the market today, are frequently over-sold and the prospective purchaser or user should have some appreciation of their limitations.

The degree of protection afforded by laminar flow hoods, although substantial, falls short of the absolute containment capabilities of the older cabinets of the Class III type.

The laminar flow system reaches maximum efficiency only in the absence of items which interfere with the flow of air. The careless placement of equipment or overload work surface, can create eddy currents which may escape from an improperly balanced hood.

Undetected flaws in the filter unit can permit the recirculation of contaminants or pathogens within the unit and can be prevented only by the periodic use of appropriate monitoring procedures.
In certain high priority situations, primary containment equipment designed to control biological hazards at their point of origin, are supplemented by secondary barrier systems.

Secondary barrier systems, a term commonly applied to high security laboratories where a maximum effort is made to prevent the escape of the agents worked with into the environment, affords the best in biological pollution abatement and control systems.

Facilities of this type generally differ from conventional laboratories in that they have:

1. Well-planned room arrangement to facilitate movement of personnel and materials.
2. Change room and shower systems.
3. Both steam and gas sterilizers.
4. Incinerators for the disposal of carcasses and other potentially contaminated flammable wastes.
5. Air lock or ultraviolet chambers to separate areas having varying potentials for contamination.
6. Elaborate air handling and sewerage treatment systems.

As the intended use and operation of most of these systems are familiar to all of us, additional comments are required only with respect to the design criteria for air handling, sewage treatment and biological waste incinerator systems.

Air handling systems in biological laboratories usually employ 100% replacement air instead of utilizing the conventional recirculation system employed in most public or private buildings.

In addition, differential air pressure zones and air filtration systems are included to control the directional flow or air within the facility and minimize the release of biological agents into the atmosphere.

At the NADL, dust filters are located at the exhaust port of all animal rooms and biological filters are located in the exhaust air plenum from all laboratory or animal isolation units.

High efficiency biological filters employed at our laboratory are capable of removing 90% of all particles in the 1 μ size range and reducing an aerosol of T3 coliphage having a mean diameter of 3 μ by 99%.

When priorities are higher, increased filtration efficiency can be obtained with absolute filter media (99.97%/0.3w) or air incineration.

Sterilization of liquid sewage in a biological laboratory is usually accomplished by heat treatment employing a temperature of 274°F and a retention time of 30 minutes.

In most high security laboratories, sterilization is accomplished by either a batch system or a continuous flow heat exchanger system.

The batch system usually employed in smaller laboratories utilizes steel tanks for both the collection and subsequent treatment of raw sewage; while sewage in the first tank is being cooked and cooled, raw sewage is being collected in a second tank. This system is optimal when a day’s accumulation can be processed in a single operation.

Larger laboratories find the continuous flow-heat exchanger system more advantageous in terms of and hours and thermal energy required.
In this system, heat exchangers are used to preheat raw sewage with sterile, high temperature sewage. The temperature of the preheated sewage is raised to the desired level by steam injectors: Treatment time is controlled by the flow rate through a series of retention tubes.

Continuous flow-heat exchanger systems are designed to handle from 50,000 to 150,000 gallons per day.

Incinerators for the disposition of animal carcasses and other types of contaminated flammable wastes must not only provide the desired bulk reduction in keeping with existing antipollution standards, they must do so in a manner which will prevent the release of pathogens into the atmosphere.

Pathological incinerators or crematories are multichamber units which include a primary or ignition where the bulk of the wastes are disposed of and a secondary or combustion chamber where gaseous products of incomplete combustion in the primary chamber, including biological aerosols, are destroyed.

To provide assurance that all biological agents are destroyed during the incineration process, incinerators should not be charged until the primary chamber has been preheated to a minimum temperature of 1,000°F.

An additional safety factor is achieved by restricting gas velocity in the secondary chamber to 9' per second at 1,400°F.

Fortunately, the time temperature exposure required to assure thermal destruction of biological agents is above the thermal stability levels for the bulk of the odor-producing products of incomplete combustion (1200-1400°F) as well as that of most insecticides, pesticides and other agricultural poisons (1800°F).

Although any comprehensive bio-safety program demands an understanding of biological hazards as well as the equipment and procedures for their control, overall success is more closely related to the human element than to the degree of mechanical specialization available.

The best programs are found in organization where top administrative support is both available and apparent.

One of the best and most practical methods of demonstrating administrative backing and gaining the support of key members of the scientific staff involves the development of written safety regulations which are specifically applicable to the institution involved.

By careful selection of personnel responsible for the development of a working draft as well as those responsible for reviewing, modifying and approving the finished document, one not only gains a great deal of valuable input information, but profits by establishing the concept that regulations governing employee conduct are largely formulated by the employees themselves.

Safety regulations, prepared in this manner, provide all employees with a list of minimal restrictions governing conduct in a biological laboratory setting.

This procedure also provides the laboratory director or chief a channel which can be used to realign or modify the regulations in keeping with changes in programs assignments or priorities or with changes in the equipment available for agent containment and control.
SUMMARY

Experience has shown that the majority of laboratory acquired infections cannot be traced to a single overt act or occurrence, but are usually the result of aerosols generated by any one of a number of commonly employed laboratory techniques or procedures.

Equipment and facilities for the control and containment of biological aerosols at their point of origin as well as the overall laboratory level are reviewed and discussed.

The desirability and benefits resulting from the development of written safety regulations, based on specific laboratory programs, are enumerated.
DIAGNOSTIC LABORATORIES AND EXTENSION VETERINARY MEDICINE

By

Homer K. Caley

It is an extreme pleasure for me to have the privilege of speaking to this knowledgeable and distinguished group. It is my hope that I will give you a little insight on the relationship of the Extension Service and the Diagnostic Laboratory at Kansas State University.

My association with the Diagnostic Laboratories and Laboratory personnel extends almost 20 years and as a private practitioner I must confess that I had my frustrations in dealing with you. It seems as if every tissue or blood sample would get placed on a bus dock and deteriorate to the point of being impossible to use. If my memory serves me right, the kindest letter I ever received from the Diagnostic Lab was one that carefully explained that the sample was not suitable for diagnosis.

Shortly after I moved to Kansas State University as the Extension Veterinarian I started holding meetings with lay groups on cattle, sheep, dairy, and swine. Farmers and ranchers will talk to extension people and as a result of this I received several complaints every week against the Diagnostic Lab. At the time it was quite difficult for me to remain calm, cool, and collected while some livestock producer berated my colleagues and friends in the Diagnostic Lab, but as I look back I'm sure this is when I decided that the Extension road and that of the lab are similar and that we must both work together to get the job done.

We, the Extension Service, and the Diagnostic Lab are a major part of the public relations for the college of Veterinary Medicine as well as for each other. It is my belief that the Extension Service represents the entire university regardless of the state involved and that the next to Extension, the Diagnostic personnel are the second most important contact; and while I list Diagnostic personnel second it is because of extensions county and district office contacts that have access to more people. This would be based on numbers alone.

Every facet of the university is on the P.R. staff, but it just happens that Extension and the Diagnostic Lab have more in common than most groups.

In my travels across Kansas I took the name and addresses of all the producers that had what I considered legitimate complaints. Some of these were registered in a voice furious manner at a large public meeting. I informed them that I would run down the problem.

Not once in 6 years of tracing down complaints has the Diagnostic Laboratory been guilty of not notifying the veterinarian, but many times the referring veterinarian had not bothered to relay the information to the producer and while these would be some opposition to this it would eliminate confusion if the producer received a copy of the letter that goes to the referring veterinarian.
At the present time when I receive a complaint I immediately contact our lab
director and he notifies both parties of the results and date the letter was sent. I
frequently write to the producer. Veterinarians involved know this will be done and
from this busy start complaints have now almost stopped. It is my opinion that
our number 1 problem has been nearly eliminated.

There is no formal administrative arrangement between the Diagnostic Lab
and the Extension Veterinary Medical section, therefore we have a maximum of free-
don on deciding what must be done. Decisions on a give and take basis can usually
be arrived at by discussing the problem over the phone and when I can be of assis-
tance to the lab I try to work it in with what I am doing and when I need help the
diagnostic lab personnel comes through. There is no particular concern about who
receives the credit for the important thing is to get it done.

The following topics are those on which I believe we work together best:
Communication – The extension service has the communications in force and it is
relatively simple to get information to our Kansas veterinarians either by our pres-
ent publications or by using a special release. We also have a direct line through the
county agricultural agents office to the producer and in addition, we have access to
radio stations, newspapers, and T. V. broadcasts. It is very common to hear some
favorable information from the diagnostic lab mentioned either as an information
release from the director or a general release that will show up on a T. V. broadcast,
newspaper article, or in “Notes from the Extension Veterinarian.”

The end result is that cooperation between extension and the diagnostic lab
does make it possible to alert veterinarians and producers to the actual disease sit-
uation in the area.

I also try to mention our regulatory personnel in a favorable light when ever
possible and in some instances I have publicly defended their actions against their
critics by using our radio and T. V. facilities. An example of this is V.E.E.
Follow-Ups – Fequently the Diagnostic Lab receives specimens from the various
areas of the state that they never hear from again. They do like to know how the
problem worked out. We can and do follow up on some of these problems and
give a first hand report back to the lab. Nearly any area of the state that uses the
Diagnostic Laboratory is never far from the Extension Service.
Collect specimens – At times our laboratory personnel cannot find time to go to
a location in the state that needs help. Quite often this is in an area where some
member of the Extension Veterinary Medical section is going to be. We will do a
necropsy or collect material as instructed to do by Dr. Anthony and check it in to
him when we return. This saves man power and travel expense for the lab and keeps
us involved in what is going on in the state.

In some cases it is easily determined that a trip by the Diagnostic Lab would
have been wasted and quite often the problem can be solved at the time of our visit
without submitting material. By screening some of the trouble calls for the Diag-
nostic Lab they can concentrate on more important disease problems and we can
be concerned more about management for disease prevention. If in the process of
checking problems we need additional specialists we call on the Extension staff to
provide them. For example Dr. Hyde, range specialist, answered a trouble call from a Beloit, Kansas veterinarian on poisonous plants and they solved the problem. Capability of our Diagnostic Lab — Our laboratory cannot possibly handle all the material that could be sent in to them. At the present time we have a clamor for help with nitrate and prussic acid testing and while our laboratory runs many such tests, we recommend commercial laboratories whenever possible. In our educational meeting throughout the state we try to explain what the lab can do and also its limitation. This prevents laboratory personnel from having to write letters explaining why they cannot run one test or the other.

Involvement— Quite frequently the Extension Veterinarian gets involved by the county agricultural agent between an irate client and the local veterinarian. There is usually charges and counter charges hurled by both sides and at times even communications have completely stopped. Help is needed and the agent called in. A sincere effort is made by the lab and Extension Service to get the interested parties together and back to talking. We have been quite successful at this and it usually requires help from the County agent, Diagnostic Lab, and Extension Veterinarian. Once the communications are started I'm ready to leave, but Dr. Anthony will hang around and eventually give them a diagnosis.

Meetings— An essential part of Extension Veterinary Medicine is meetings and frequently this requires help. I use some local veterinarians for programs but quite often I request and receive help from the director of the Diagnostic Lab. This is not used more than 4 or 5 times a year, but it helps us out. Meetings are planned that will increase livestock producers contact throughout the state and eventually result in improved public relations with this group. It will also increase the contact for the Extension service at the same time. Two meetings of this type known as “Cowboy College” will be held in Dodge City and Garden City, Kansas in November. We will have about 80 of the top feedlot cowboys in the state in these sessions. During the years I have tried to work with clinicians and other staff members. Some of them have worked out well but it has not been worthwhile to try to work with the clinic staff. It is usually easier to do it yourself than it is to get an administrative decision on participation.

Field Trials— For the past four years I have been conducting field trials on various drugs, vaccines, antibiotics, and management practices associated with bovine respiratory problems. During this time Dr. Anthony has been very cooperative with lab work as well as advice and council. Dr. Merwin Frey, Iowa State University, has also been most helpful.

In summary there really isn't anything unique about our Diagnostic Laboratory and the Extension Veterinary Medical section. We function when there is a job to do without concern about where the credit goes. Our association is for our mutual benefit and while this frequently results in more work for both of us, I believe the satisfaction of a job well done is worth the price we have to pay.
INTER RELATIONSHIP OF THE DIAGNOSTIC LABORATORIES AND REGULATORY MEDICINE

by

Dr. Glenn B. Rea

An animal disease regulatory program without competent diagnostic assistance is like a "One arm paper hanger with the seven year itch"—it just cannot and will not get the job done.

In a way I feel kind of foolish talking on this subject, especially to a group of diagnosticians. I would feel just as foolish talking to a group of regulatory people because both groups ought to know the value of diagnostic services to a regulatory function. Perhaps, however, it is good to remind each other of some things that we take for granted.

First of all, what are the broad goals of a livestock disease (and pest) regulatory program?

1. To prevent, control and where practical and economically feasible, eradicate diseases of livestock and in our state as in many others this includes all animals in captivity and not just meat, milk, fiber and pet or pleasure animals.

2. To prevent and control and, as above, when feasible, eradicate those diseases which are transmissible from animals to man (Public Health).

3. In many states assure a disease free, healthy, wholesome meat supply to our public, (consumer protection).

As far as the diagnostic laboratory is concerned, these three broad goals of a regulatory program might as well be considered as one.

Therefore, what are the most important features of a good diagnostic facility?

In my book accuracy must be first. Sloppy technique—The use of antigens and other products that are not properly titrated or further checked; mediocre equipment and instruments not calibrated, i.e., P. H. meters, Spectrometers, etc., failure to check personnel or procedures and lack of uniformity of evaluation are some good examples of sloppy or inaccurate technique.

To be really effective, speed is probably the second most important. The owner in most cases has taken what steps he can, if he is a good husbandman, to satisfy the needs of his stock from a disease standpoint. When he gets beyond his depth, he calls the practicing veterinarian. Between these two they probably take care of 95% or maybe 98% of the disease problems on the farm or ranch.

When the veterinarian is doubtful of his diagnosis, or when he needs assistance, he goes to the diagnostic laboratory. From the time the owner called the practitioner until he hears results from the laboratory, he is in a state of anxiety, he may be losing animals, production, or both. Though accuracy should never be compromised, if a procedure gives a rather clear indication of outcome before all steps are completed, a phone call to the veterinarian or sometimes the owner (if you accept direct accessions) will allow him to at least start treatment and/or preventative measures based on some legitimate findings.

Good judgment must be used both by the practitioner and the owner, in considering whether or not treatment should be started before final confirmation. In some cases the situation may be an—either—or—circumstance, that is, it might be
only the differentiation between two diseases, both of which may respond to the same treatment. Many situations would not be hampered even if the treatment for a suspected disease had to be stopped and another one instigated.

Still other situations might be better served by waiting until a final conclusion has been reached. In any case a diagnostician will seldom err if he contacts the party or parties involved as soon as he has useful information. If the procedure is going to take a long time, just a call to say you are still working is important. If there are no facts on which to start treatment, at least the owner knows you are working on his problem and have his interest at heart as against his waiting in silence not knowing whether or not he is being served and/or whether you are simply taking time to improve your golf game.

“Old Alex Bell’s Baby” is not very costly to use and it can dispell a lot of false notions and will greatly enhance the diagnostician’s rapport with the people he serves.

It is quite easy for a laboratory worker to feel independent because usually you cannot alter the speed of your procedure and it is easy to feel that communication is unnecessary before you have a definite answer, but this is not so.

The third important service is disease surveillance. The constant effort a diagnostic laboratory gives to an industry as a whole provides one of the best and most complete tools available against the establishment of a zoonosis, or even more important, the establishment of an exotic disease.

The fourth important service that a diagnostic facility can render is a review of the history and epidemiology of the disease circumstance with the practitioner, state epidemiologist, and/or the regulatory representative. Here again some situations are too common place to lend themselves to such a critique; but consider a possible situation which occurred as a result of an exotic disease exercise in our state over a year ago — 1969. This is a good example of both surveillance and epidemiology.

An alert practitioner and an accurate and complete diagnostic procedure would have saved the establishment of an exotic disease if it had in fact been real instead of an exercise. Not only that, but by discussing the procedures used and the involvement of many agencies not ordinarily oriented to disease control, a great portion of our state officialdom is now aware of the importance of constant vigilence.

Previous arrangements had been made with a rancher, a practitioner, the chief of our diagnostic laboratory, and the extension veterinarian. No one else other than the state veterinarian who organized the procedure had any knowledge that this exercise was about to take place, what it was, or who was to be involved.

The following situation was presented by the practitioner in writing (having been previously prepared by the state veterinarian) at noon on Father’s Day, June 15, 1969. It set forth the events that presumably had happened up to that time, posed a problem, and left all further action up to the area regulatory official. Names and places are not fictional and neighboring ranchers and ancillary state officials and activities were actually contacted.

**EMERGENCY DISEASE EXERCISE 1969**

**“RIFT VALLEY FEVER”**

Events Prior to this date and hour : — 12:00 Noon June 15, 1969.
During the forenoon of Tuesday, June 3rd, at about 8:30 A.M., Dr. Jack Moye of Junction City was called to the Powell Ranch at Crown Point on Highway 36 by Mr. Eldon Powell, to examine sick and dying sheep and cattle. The Powells run a pure-bred herd of Angus cattle and a considerable number of sheep.

Dr. Moye’s tentative diagnosis was Bluetongue, but something that he could not quite explain caused him to question this decision and he returned to the ranch later in the day with Dr. Gene Shortlidge, assistant state veterinarian and area disease control supervisor. Dr. Shortlidge agreed with Dr. Moye’s original diagnosis but also noticed that the deaths seemed to be higher in ratio per number of animals involved and more rapid than commonly seen in Bluetongue. Both veterinarians decided that further investigation was necessary and so called Dr. Dean Smith at the diagnostic laboratory at O.S.U. The following is Dr. Smith’s report—

"Dr. Guy E. Reynolds and I went to the premises with Dr. Moye, where we met Dr. Shortlidge on the afternoon of June 4, 1969. We observed sick and dying lambs, a few ewes appeared to be sick, and there were four sick cows; two healthy appearing cows had aborted. Two of the sick cows had a Leukopenia, salivated excessively, and appeared weak. At least one of the cows had some stomatitis. Several necrotic areas were noticed around the mouth and udder and there were shallow erosions on the lips. Three calves had died. There were several sick and dying lambs; they appeared to be sick only about a day before death. Ten lambs had died altogether.

One sick and two dead lambs were necropsied; also, one calf was necropsied. One of the lambs was severely decomposed although it had been dead only six hours. There were tiny grey foci of necrosis about 2–3 mm. in diameter throughout the liver of the sacrificed lamb. Liver lesions in the other animals showed severe necrosis. The livers were enlarged and soft. There were petechiae and ecchymoses of the serosal and mucosal surfaces of the heart and the gastrointestinal tract. There was a catarrhal foul smelling stomatitis.

Material from infected animals was inoculated into mice on June 5, 1969. These mice were dead on June 6, 1969, and Dr. Henkel (disease control supervisor) was called and told we might possible be dealing with an exotic disease. He consulted with the state veterinarian and the federal veterinarian in charge, and it was decided to await more positive diagnosis before taking any further action.

Histological sections were read on June 10, 1969, and these showed changes very suggestive of Rift Valley Fever. These lesions consisted of miliary Necrotic areas sometimes becoming confluent in the liver and there was also congestion and hemorrhage of all tissues studied.

Again, Dr. Henkel was called. A quiet investigation stimulated by earlier suspicions, showed no unusual disease syndrome in the immediate area surrounding the Powell ranch. It was decided to send material to the Federal Laboratory and await positive diagnosis before taking further action.

Material was sent to the Federal Laboratory on June 9, 1969 and we received a call the morning of June 15, 1969, confirming our tentative diagnosis of Rift Valley Fever.

Mr. Powell and one of his workers appeared to have had the “flu” the past week. Dr. Reynolds had what appeared to be the “flu” on June 6, 1969.
DR. GLENN B. REA

Very Truly,
Dean H. Smith, D.V.M.
Veterinarian in Charge
Veterinary Diagnostic Lab.

Since investigation was set in motion by you, Dr. Shortlidge, and the ranch and the involved veterinarian located in your area, Dr. Smith, reported directly to you upon receiving word from the Federal Laboratory. He has not and will not make any other notification or public statement. You take it from here. — The Powells are awaiting an immediate visit from you within the next few hours, in fact. Certain other officials are also waiting immediate word from you concerning this situation.

A quarantine has not yet been placed on the ranch. Noting the very cooperative attitude on the part of the Powells, Dr. Rea solicited their agreement not to move any animals from the premises until a definite diagnosis could be made.

**THIS IS AN EMERGENCY—WHAT ARE YOU GOING TO DO ABOUT IT?**

Upon receipt of the statement of the situation, Dr. Shortlidge immediately called his superior, Dr. Henkel, who is the head of our Disease Control Section, who immediately called me (the state veterinarian). I called the director of agriculture, who then notified the governor. At the same time, and at my suggestion, the governor was told that we might need the services of the National Guard, state highway engineer, Fish and Game Commission, and Public Health Services. I called our federal veterinarian in charge and he informed his people in Washington.

(The following was pieced together by Dr. Shortlidge when questioning the Powells.)

On May 25th, a Mr. Chidester Smythe, a Canadian Missionary to Kenya, Africa, arrived at the Powell Ranch on a visit from Lethbridge, Canada. He had arrived from Lethbridge only three days prior (May 22) from Nairobi. The evening of the 26th, Mr. Smythe complained of aching joints and muscles, accompanied by nausea and dizziness. He vomited twice.

The morning of the 30th, Mr. Smythe felt much improved and returned to Lethbridge by plane. Subsequent information received in a letter by the Powells, indicated Mr. Smythe had a relapse shortly after arriving home. He is better now but has had some problems with his vision.

The Powells employ several people on their ranch and have numerous visitors. Several of these people have complained of the symptoms of the "flu", very similar to those experienced by Mr. Smythe. At least one child and one woman both had vision impairment. Mr. Eldon Powell had the "flu" the past week, but is well now.

In the course of conversations with Mr. Willard Powell, Eldon Powell's father, he was heard to remark on several occasions that the mosquito and culicoides infestation in and around the ranch was the worst the week of May 25th that he could remember in all his years at Crown Point.
Further details would only bore you but, to make a long story short, everyone cooperated beyond our wildest dream. Quarantines were prepared, fiscal people arranged for indemnities, excavation equipment was made available for trenching and burying and helicopters sent to spray surrounding areas. In fact the exercise was so real that the people in charge of the helicopters actually had one started, the rotors were really turning, and they wanted to know just which area they were supposed to spray.

That was the closest thing to a breakdown in communication or a backlash that we had. We had many people ask repeatedly, “You are sure this is just an exercise?” The wildlife people made arrangements to survey and trap deer and just about everyone who could possible be involved in an actual exotic outbreak had a part to play. This included legal staff and the press.

A command post was actually set up and veterinarians traveled to the surrounding ranches and talked to the owners.

This situation was, after all, only an exercise, but it served the purpose of bringing home to all involved the necessity of complete team work across the board. It was exotic all right, but it definitely illustrated the interrelationship of diagnostic facilities and a disease regulatory program. It does more than that, of course, but was used today not only to tell an interesting story but to show the importance of diagnostic procedures. You can let your imagination run wild in contemplating what might have happened had the original tentative diagnosis as Bluetongue been left with no follow-up.

Regulatory disease control work is generally confined to small routine programs and the usual laboratory work connected with them. However, it is most important that all personnel continuously have their radar locked on to surveillance of livestock, constantly looking for exotic diseases.

Likewise the laboratory must continuously have the capability and procedural knowledge to set in motion at almost a moment’s notice any one of its facilities to assist in the diagnosis of a suspected disease. Our disease control staffs must be closely integrated forces of both state and federal personnel working both in the field and in the central offices. Many times an interstate health certificate, a request for a permit, or a telephone inquiry may be the key information that sets the whole force, including the laboratory, in motion.
SOME DIAGNOSTIC AND HEALTH PROBLEMS
AT THE SAN DIEGO ZOO

by

Lynn A. Griner, D.V.M., Ph. D

I might preface this report by saying that we at the Zoo have more problems in diagnosis than we have solutions. It is these problems that make our work both fascinating and frustrating. It would not be practical to list all of the problems encountered, since new ones arise each day. I have therefore selected a few general topics related to health problems at the San Diego Zoo.

Animal behavior, both normal and abnormal, is frequently involved in the creation of health problems. Perhaps the greatest deficit in our work with some 1700 species of animals, is our incomplete knowledge of the normal behavior pattern of many of these species. There are many questions that we should have the answer to, such as .. Is an animal primarily solitary except at breeding time, or is it a troop or herd animal? Is there a hierarchical social structure within the group? What is the role of the dominant individual? What are the space requirements? What are the feeding habits is the animal a grazer or browser? Is the animal herbivorous, carnivorous or omnivorous? What is the reproduction or breeding behavior? Is the species monogamous? Answers to these and many other questions would add to our ability to more adequately manage and maintain animals in a better state of health. Partial answers to some of these questions can be obtained by close, frequent observation of the animal in the captive environment, but this takes considerable time.

Partial answers to questions on feeding habits and the type of feed that should be offered in a ration, can be obtained by study of the alimentary tract at post mortem. We of course are aware of the ruminant digestive system of the Artiodactyla, and the cecal colic system of Equidae. However, there are many variations in the anatomy of the alimentary tracts in other animals. As an example, all primates do not have a simple stomach type of digestive system. The Langur or leaf-eating monkeys have a semi-compound stomach, that is, their stomach has several compartments somewhat resembling a rumen. This anatomic feature indicates that the animal’s diet should consist primarily of leaves, bark, buds and nuts, and that rapidly fermenting carbohydrates should be restricted. We once received a shipment of very rare primates .. in answer to our inquiry as to the food habits of the animal, we were informed that bananas, papaya, melons and other tropical fruits were raised in the country of origin. Such fruits were offered the animals and they died from acute gastric dilatation and acidosis. At necropsy we found a semi-compound stomach which had lost all muscle tone due to over-distention.

For many years, zoos, herpetological parks and laboratories, have generally been unsuccessful in keeping Tuataras, (Sphenodon punctatus), a strange, "living fossil" lizard from New Zealand. As a result, export of the animal has properly been severely curtailed. Failure in keeping these reptiles now appears to be related to a lack of knowledge regarding the animals’ feeding habits and metabolic needs. It appears that most tuataras had been kept too warm and were over-fed. This reptile has one of the lowest metabolic rates known, (Sharell)³. Body temperatures of 76 tuataras
on Stephens Island averaged 11°C (52°F). The highest temperature recorded was 13.3°C (56°F). "No other reptiles, from the tropics to cool mountain regions, were found with a temperature lower than 14.4°C (58°F)." In 1923, R.D.D. Milligan carried out experiments which showed that the metabolic rate of tuataras was very low. He measured the output of CO₂ when breathing and found that the active rate was much less than in other vertebrates. He watched one tuatara at a temperature of 9°C for one hour, during which time no breathing was observed. If we obtain new tuataras we must provide an environmental temperature with a range of 8°C to 15°C, and in addition we must not over feed them.

On the opposite end of the metabolic scale would be the hummingbirds. According to Greenwalt, hummingbirds have the highest energy output per unit weight of any living warm-blooded animal. Its energy output per unit of body weight, while hovering, is about ten times that of a man running nine miles an hour. "A man's actual daily energy output is about 3500 calories. The daily output of a hummingbird leading its ordinary life - eating, flying, perching, sleeping - if calculated for a 170-pound man, is equivalent to about 155,000 calories". Hummingbirds actually use sugar as their principal food, and will consume sugar at a rate of about half their body weight per day. To make sure that our hummingbirds have adequate energy intake, we provide sugar water, fresh fruits and live fruit flies, and have been quite successful in keeping these birds. It does appear that their life span is comparatively short.

The problem of malnutrition can perhaps be decreased as we gain additional knowledge of metabolic needs and feeding behavior. Malnutrition however does continue to occur as a result of behavior factors, the more important of which is individual dominance and territoriality. This behavior problem is greatest in the large flight cages for birds. At one time, malnutrition was the single greatest mortality factor in the rain forest, in spite of the fact that adequate feed was available. Following several days of close observation, it was obvious that certain birds had established territories around the few feeding stations and would drive intruding birds from the feed. We have succeeded, to a considerable extent, in correcting this situation by increasing the number of feeding stations and distributing the feed at variable space levels. In addition, fresh fruits are impaled on short branches of the trees, thus increasing food availability.

Animal behavior in the form of territorial dominance may create other health problems, such as stress and trauma. In birds, the more frequent traumatic injuries will go un-noticed unless skin of the calvarium is reflected, revealing penetrating puncture wounds through the skull and hemorrhage in the brain. These lesions are made by the sharp beaks of the aggressors.

Traumatic injuries resulting in death, is the most frequent of the mortality factors at the San Diego Zoo. In 1969, 20% of the 973 animal deaths were due to trauma. In 1970, 22.5% of the avian and 20% of the mammalian deaths were due to traumatic injuries, commonly inflicted by cage mates or by collision with fences while trying to escape from an aggressor. Traumatic injuries and deaths appear to be the product of animal behavior and restricted environments.

Behavioral patterns involving traumatic injuries, include social hierarchy and territorial defense, both of which can be considered as aspects of reproduction behavior and are thus sex-oriented. Traumatic injuries may at times be reduced by increasing the size of the exhibits, and/or restriction of the number of animals, especially males,
in the enclosure.

The latter is frequently a necessity in exhibits of mixed species of artiodactyla. For maximum reproduction of mixed groups of antelope, it is often advantageous to have only one male at a time present in the group. Males of the other species can be rotated to coincide with estrous of the female. Occasionally a female may be the dominant animal. In such cases temporary or permanent removal of the female may lead to stabilization of the group.

Health problems however are not restricted to animal behavior patterns. Infectious and physiological diseases, while of less frequency, do occur. These disease processes at times present problems in clinical and post mortem diagnosis. A few of these diseases will be discussed briefly.

Recently we have had a number of cases of acute, croupous pneumonia in our snakes. The pneumonia has been of sudden onset, with early death, and has been characterized grossly by a thick layer of translucent mucus covering the lumen of the lung. The mucus covering apparently interferes with gas exchange in the lung, resulting in asphyxiation. Histopathologic studies of the lung reveal minimal damage to the mucosal epithelium or other cells of the lung. There is an increase of granulocytes in the lung tissue and in the mucus. The number of granulocytes in the lung however is not believed to be as great as one would expect to see in a bacterial infection. Smears of the mucus demonstrated many gram negative and gram positive bacteria of variable morphologies. A pseudomonas has been isolated. Epidemiologic investigations reveal that the disease has occurred primarily in rattlesnakes and one cobra. The majority of these reptiles have been housed in cages in the corridor or in adjacent exhibits of the Reptile House. It was further learned that pesticide strips (containing organic phosphorous insecticide) had been hung in the corridors to control mites on the reptiles. The use of these pesticide strips appears to coincide with the occurrence of the pneumonia. We are currently faced with the question — is the pneumonia caused by the pesticide or is it of bacterial origin? We believe the former is the more likely, but this remains a problem of further study.

Avian malaria has been recognized as a summer disease problem in the Zoo's penguin collection. It has occurred only after periods of hot weather. The clinical history is that the birds go off feed for a day and are found dead the next day. Clinical diagnosis of the disease has generally been unsuccessful. Rarely have we been able to find the parasites in the peripheral circulation, nor have the parasites been demonstrated in the blood of newly arriving penguins. Post mortem and histopathologic diagnosis has not been a problem. The gross pathology is characterized by pulmonary, pleural, and air sacedema, hydropericardium, swollen and congested liver and spleen. Histologically, exerythrocytic schizonts can be found in the cytoplasm of capillary endothelial cells, as well as some reticulo-endothelial cells.

Alveolar periodontitis, lumpy jaw, or soft tissue necrosis and abscessation, is a frequent disease problem in marsupials, to which we have given the name, rightly or wrongly, of actinomycosis. Gram smears of exudate demonstrate numerous bacteria of variable morphology and tinctorial properties. Filamentous gram negative bacilli are nearly always present. We have had poor success in isolating and identifying the etiological organism. Treatment of these cases also leaves much to be desired. This is perhaps due to the fact that the disease is often advanced before it is reported to the Health Department.
Another disease problem of marsupials has been the comparatively high incidence of arterial calcification. In 1590 mammals necropsies, 21 cases of arterial calcification were observed. Sixteen of these cases occurred in mammals of the order Marsupalia, and 13 of these were in wallabies. At this time we have no explanation for the higher incidence of arterial calcification in wallabies. Could the wallaby be an animal model for arterial calcification?

Parasitism is always a problem for zoos since most new animals from the wild will be parasitized to some degree. One problem is how do you determine the significance of a parasite. For example at what time do trichomonads or Balantidium constitute a health problem? Identification of parasites is usually difficult to obtain.

For many months we had been losing exotic caprine animals from a verminous pneumonia. Lung worms were first noted after the animals were moved to newly constructed exhibits on a hillside and wet bottom area that had previously been used to hold a variety of ungulates for short periods of time. Before the sheep and goats were placed in the new exhibits, the area was permitted to dry out and most vegetation was destroyed. After introduction of the animals the area was irrigated to reestablish a cover of vegetation. The nematode had tentatively been identified as Mueleria sp. This much of an identification indicates that it has an indirect life cycle, with a snail serving as intermediate host. We were unsuccessful in finding an anthelminic to remove the nematodes from the definitive hosts, so we have tried to eradicate the snails from the area. First we discontinued irrigation of the exhibits and then the entire area was sprayed with "Polytrap", a sticky plastic material which coats the vegetation. When snails eat the sprayed vegetation, their mouth parts apparently stick together and they die from starvation. It is too early to evaluate but at the moment the results are encouraging.

An interesting parasite problem is found in the Cape Hyrax (Procavia capensis). In our series of 44 necropsies on hyrax, 17 have had stomach ulcers. Small nematodes, between 1/4 to 1/8 inch in length, are found in the stomach or attached to the mucosa. Fecal examinations of the hyrax have been negative for ova or larvae. Thus far we have not seen an ova. Larvae were found in the feces of one hyrax that had developed diarrhea just prior to death. Nematodes were abundant in the stomach and an ulcer was present. The problem is how do we examine the animals for this specific parasite? How can we evaluate the effectiveness of an anthelminic? We have started testing some of the animals by means of gastric lavage, with good success, and it now appears that this may be our most accurate method of diagnosing parasitism of the stomach.

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SERUM AND PLASMA NEURAMINIC ACID LEVELS IN CLINICALLY HEALTHY CATS AND IN CATS EXHIBITING CERTAIN DISEASES

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The concept that certain substances increase in the blood during a disease is not new. For example: in tissue destructive diseases such as White Muscle Disease, encephalomyelitis and hepatitis the SGOT levels are elevated; in a metabolic disease such as Diabetes Mellitus or when glucocortecoids are administered the patient's blood glucose level is elevated; and in nephridiates the blood BUN and Creatinine levels can be increased. Similarly, increased quantities of a compound identified as Neuraminic Acid have been found in the blood of man (10), horse (6), dog (1) and cattle (3) during an infection.

Neuraminic Acid has several synonyms: N-acetyl Neuraminic Acid and Sialic Acid. The compound and its significance has been studied in European and Asian laboratories more extensively than in the U.S.A. ones. The compounds have been found in the mucoproteins of saliva, in serum mucoproteins, in cell membranes, in antibodies, enzymes, hormones, fibrinogen (7) and milk (7,12). The structural formula of N-acetyl Neuraminic Acid has been portrayed as (7):

The biochemists show it to be attached to glactosamine by an ether linkage and the glactosamine to be attached to the cell membrane by aspartic or succinic acid residues. The compound is anionic and aids in maintaining water, Na and K ions in the cell membrane and aids in forming the microeive keeping macro-molecules from entering viable cells. It is antigenic (2,11) and is removed from the various moieties in which it is found by the enzyme neuraminidase, which is produced by numerous microorganisms including Myxoviruses (5), Streptococcus species (4), Vibrio cholera and some strains of Clostridium perfringens (8).

Since a search of the Veterinary literature contained no report of the Neuraminic Acid levels in the blood of the Feline, this work was undertaken.

MATERIAL AND METHODS

Blood samples were drawn from a series of mature and from seven cats 10-12 weeks old. The blood was obtained either from the jugular or the cephalic veins or,
in moribund cases, from the heart after light ether anaesthesia. Three to 5 ml. of blood were obtained from each animal and the blood was permitted to clot. In the phase of this study concerned in part with the effect of liver damage on the Neuraminic Acid levels, the blood sample was treated with heparin in order to obtain the plasma.

The cats composed five groups: Group 1 consisted of the so called “normal” cats which had been submitted to the Veterinary Clinic for spay or castration; Group 2 consisted of cats having miscellaneous conditions associated with their admission to the clinic; Group 3 consisted of half-grown kittens which had been exposed to the virus of Feline Panleukopenia; Group 4 consisted of cats exposed to Feline Rhinotracheitis virus under controlled conditions; and Group 5 consisted of two series of cats whose plasma became available after an evaluation of their cholinesterase levels, a subject of another study. This group was comprised of 22 clinically normal or pretreatment cats, 20 of which were then treated with Carbon Tetrachloride. Their plasma was used for Neuraminic Acid determinations within one week of liver damage.

Neuraminic Acid levels were determined in the serum and plasma by a method adapted for the Coleman, Jr., colorimeter (1,9). The standard curves were prepared using synthetic N-acetyl neuraminic acid (Sigma.) Each determination was performed in duplicate and a standard check sample, run along with each group of specimens. The values of Neuraminic Acid were recorded in millimoles (mM) per 100 ml. of serum (or plasma).

RESULTS

Table 1 contains data accumulated in establishing the expected serum range for Neuraminic Acid in cats which were mature and clinically healthy. The range was found to be 269 plus or minus 42 millimoles/100 ml. of serum.

Table 2 contains a list of localized conditions and the serum Neuraminic Acid levels found in them.

Table 3 contains the Neuraminic Acid levels of sera from 10-12 weeks old cats exhibiting signs of Panleukopenia after exposure to that virus. The average level was 384 mM/100 ml.

Table 4 contains the Neuraminic Acid levels in cats having miscellaneous suppurative lesions. The values ranged from 345 to 510 mM/100 ml. of serum.

Table 5 contains the values found in sera of cats with the signs and lesions of Feline Rhinotracheitis. The range was from 375 to 800 mM/100 ml.

Table 6 contains the Neuraminic Acid levels in the plasma of cats which were clinically normal. The value was found to be 240 plus or minus 29 millimoles per 100 ml. of plasma.

Table 7 contains the plasma levels of Neuraminic Acid after the cats had been treated with Carbon Tetrachloride to damage their livers. The average value obtained was 229 mM/100 ml. of plasma.

DISCUSSION

Reports of work performed with dogs in which pyrexia had been produced by preventing body heat loss indicate that Neuraminic Acid levels are not increased;
SERUM AND PLASMA NEURAMINIC ACID LEVELS

however, the reports continue by stating fevers caused by infections are accompanied with elevated levels of Neuraminic Acid (1). This investigation with cats revealed that pregnancy, localized conditions (Table 2), and toxic liver damage (Table 7) are associated with "normal" levels. Also diseases outside the cat's body, so to speak, like Otis Externa and encapsulated abscesses (Table 2) are associated with the usual levels of the compound.

The increased values of two standard deviations or more above the average which were found in the infectious processes of this study (Tables 2, 3, 4, and 5) indicate that the etiological agents elaborate a neuraminidase and that some etiological agents elaborate it in larger quantities than others. The levels of Neuraminic Acid in the Feline Panleukopenia cases were lower than those found in the Feline Rhino-tracheitis cases, for example. The explanation of the elevated value found in the cat with the Carcinoma (Table 4) which had undergone radiotherapy becomes difficult when neuraminidase production is its only basis, however. Apparently, another mechanism wherein a non-specific enzyme like thrombin in the clotting mechanism, which releases Neuraminic Acid in addition to fibrinopeptides A and B from fibrinogen, is involved. Other workers (6) have reported that extensive tissue destruction is associated with increased Neuraminic Acid in the serum without explaining also the probably mechanism of its releases (6). Nevertheless, the data of this study indicate that Neuraminic Acid determinations in the Feline can be a diagnostic aid in differentiating infectious diseases from non-infectious or sharply circumscribed ones in the animals body which are not associated with active tissue destruction.

SUMMARY

Neuraminic Acid levels were determined in 25 sera and 22 plasmas of clinically normal cats. The serum value was 269 millimoles per 100 ml. with a standard deviation of 42 and the plasma value was 240 millimoles per 100 ml. with a standard deviation of 29. Feline patients in this study with infectious diseases and others with non-localized tissue pathology associated with autolysis contained higher levels of Neuraminic Acid in their blood than did clinically normal ones.

REFERENCES

4. J. of Bacteriology 100:354-357 (1968)
Table 1

Neuraminic Acid Levels in "Normal" Adult Cats

<table>
<thead>
<tr>
<th>Neuraminic Acid Levels (mM/100 ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>280</td>
</tr>
<tr>
<td>310</td>
</tr>
<tr>
<td>270</td>
</tr>
<tr>
<td>280</td>
</tr>
<tr>
<td>280</td>
</tr>
</tbody>
</table>

Average value= 269± 42 millimoles/100 ml. serum

Table 2

Seven Cats Having Physiological or Disease Conditions Associated with Slight or No Increase in Serum Neuraminic Acid

<table>
<thead>
<tr>
<th>I.D. of cat</th>
<th>Condition</th>
<th>mM/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3418</td>
<td>Pregnant</td>
<td>200</td>
</tr>
<tr>
<td>5034</td>
<td>Otitis Externa</td>
<td>270</td>
</tr>
<tr>
<td>4428</td>
<td>Neoplasm, Mandible</td>
<td>253</td>
</tr>
<tr>
<td>2610</td>
<td>Neoplasm, dorsum right front foot</td>
<td>255</td>
</tr>
<tr>
<td>4832</td>
<td>Encapsulated Abscess (to be drained)</td>
<td>300</td>
</tr>
<tr>
<td>5657</td>
<td>Diarrhea for 2 weeks</td>
<td>320</td>
</tr>
<tr>
<td>5960</td>
<td>Urinary Calculi</td>
<td>330</td>
</tr>
</tbody>
</table>

* The first two figures refer to the page of the data book on which this cat's complete I.D. and Blood findings are found.
Table 3

Seven Cats Exposed to Panleukopenia Virus and Showing a Moderate Increase of Serum Neuraminic Acid

<table>
<thead>
<tr>
<th>I.D. of cat</th>
<th>Signs and WBC count</th>
<th>mM/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>5135</td>
<td>Depressed; WBC count, 1750</td>
<td>296</td>
</tr>
<tr>
<td>5236</td>
<td>Depressed; WBC count, 2400</td>
<td>366</td>
</tr>
<tr>
<td>5640</td>
<td>Typical signs of Panleukopenia; WBC count, 150</td>
<td>455</td>
</tr>
<tr>
<td>5741</td>
<td>Typical signs of Panleukopenia; WBC count, 2750</td>
<td>335</td>
</tr>
<tr>
<td>5842</td>
<td>Typical signs of Panleukopenia; WBC count 800</td>
<td>310</td>
</tr>
<tr>
<td>5943</td>
<td>Typical signs of Panleukopenia; WBC count 150</td>
<td>410</td>
</tr>
<tr>
<td>6044</td>
<td>Typical signs of Panleukopenia; WBC count, 250</td>
<td>520</td>
</tr>
</tbody>
</table>

Average = 384

Table 4

Five Cats Exhibiting Moderate to High Serum Neuraminic Acid Levels

<table>
<thead>
<tr>
<th>I.D. of cat</th>
<th>Disease or Kind of Suppurative Lesion</th>
<th>mM/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>4125</td>
<td>Undifferentiated Carcinoma and 3180 r of radiotherapy</td>
<td>345</td>
</tr>
<tr>
<td>5337</td>
<td>Encephalitis</td>
<td>400</td>
</tr>
<tr>
<td>5455</td>
<td>Myiasis, foot lesion</td>
<td>430</td>
</tr>
<tr>
<td>5539</td>
<td>Multiple Skin Abscesses, some draining</td>
<td>490</td>
</tr>
<tr>
<td>5556</td>
<td>Pyrothorax (2 cheat grass awns found at necropsy)</td>
<td>510</td>
</tr>
</tbody>
</table>
Table 5

Serum Neuraminic Acid Levels in Cats Exhibiting Signs and Lesions of Feline Rhinotracheitis

<table>
<thead>
<tr>
<th>I.D. of Cat</th>
<th>mM/100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>46305</td>
<td>375</td>
</tr>
<tr>
<td>47315</td>
<td>382</td>
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<tr>
<td>45309</td>
<td>650</td>
</tr>
<tr>
<td>5758</td>
<td>460</td>
</tr>
<tr>
<td>4017</td>
<td>440</td>
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<tr>
<td>4016</td>
<td>460</td>
</tr>
<tr>
<td>3963</td>
<td>800</td>
</tr>
<tr>
<td>4120</td>
<td>660</td>
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Table 6

Plasma Neuraminic Acid Levels of Apparently Normal Cats, mM/100 ml.

<p>| | | | |</p>
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<thead>
<tr>
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<td>250</td>
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<tr>
<td>190</td>
<td>250</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>190</td>
<td>220</td>
<td>270</td>
<td></td>
</tr>
</tbody>
</table>

Average = 240 millimoles/100 plasma
S.D. = 29
Table 7

Plasma Neuraminic Acid Levels in 20 Cats Whose Livers Had Been Damaged by Carbon Tetrachloride, mM/100 ml.

<p>| | | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>280</td>
<td>300</td>
<td>210</td>
<td>180</td>
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<tr>
<td>210</td>
<td>270</td>
<td>250</td>
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<tr>
<td>230</td>
<td>220</td>
<td>190</td>
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<td>220</td>
<td>210</td>
<td>190</td>
<td>250</td>
</tr>
<tr>
<td>270</td>
<td>290</td>
<td>190</td>
<td>220</td>
</tr>
</tbody>
</table>

Average = 229 mM/100 ml.
USE OF TRACHEAL ORGAN CULTURES FOR VIRUS PROPAGATION AND BIOASSAY OF MYCOTOXINS

by
William M. Colwell, DVM, PhD
Raleigh, N. C.

Organ culture may be defined as the in vitro cultivation of embryonic rudiments or fragments of organized living tissue. The technique was apparently first practiced in 1907 by Harrison, a physician, who explanted a piece of nerve tissue, and observed its development in vitro.

Let us compare and contrast organ culture and tissue culture or cell culture. In order to prepare for cell culture we begin with a piece of tissue such as a calf kidney, a chick embryo, or an existing cell monolayer in the case of established cell lines, and allow the tissue to be digested by the enzymatic action of trypsin until it is broken down into its component cells. We then suspend the cells in an appropriate culture medium and allow them to incubate. The cells attach to the glass or plastic surface, and grow to form a monolayer. Useful as the cell monolayer is to us in the research or diagnostic laboratory, we must admit that it is an artificial tissue. The cells rapidly become de-differentiated and lose many of their specialized functions. An experienced histologist is often hard-pressed to identify the original source of cultured cells. Living cells may likewise be propagated in suspension or "spinner cultures". This is also an artificial system.

In contrast to monolayer or suspension cultures, the cells of organ cultures retain their normal relationships with surrounding cells, remain differentiated, and many cellular functions continue in an apparently normal manner. The contractions of cardiac muscle, ciliary movement, mucus secretion and phagocytosis have been observed to continue for extended periods of time in living tissue explants maintained as organ cultures. Organ cultures may be considered as a system intermediate between tissue culture monolayer and the intact living animal. Almost every tissue of the body has been grown in organ culture.

Embryologists have utilized organ cultures to work out the developmental sequence of organs and tissues. Physiologists have used cultured tissue fragments to study the effects of vitamins, hormones and carcinogens on the cells and tissues of an isolated system. Antibody production has been demonstrated in organ cultures. The envasiveness of tumors has been studied by co-cultivation of malignant and normal tissue fragments. A large number of viruses and mycoplasma have been propagated in the cells of organized tissues maintained in culture. Tissue explants have also been used to study the effects of toxins on isolated cells or tissues.

A renewed interest in organ culture techniques seems to have begun with the work of Tyrell and Bynoe (12) in England and McIntosh et al. (8) in the United States who utilized tracheal organ cultures in the isolation of previously undescribed viruses from patients with common colds. Some of their isolates have produced a cold-like syndrome in human volunteers. One such virus which was characterized
by McIntosh et al. resembles the avian infectious bronchitis virus.

Several laboratories in the United States and England became interested in the use of organ culture techniques in veterinary medicine. Colwell and Lukert (4), successfully propagated six serotypes of infectious bronchitis virus and Newcastle disease virus in the ciliated cells of tracheal explants prepared from young chickens, and maintained in organ culture. Tracheas were aseptically removed from the chicks, and cut into thin rings. The rings were placed in 35mm plastic culture dishes containing 0.7 ml of a standard tissue culture medium and incubated at 37°C in an atmosphere of 5% CO₂ in air. Ciliary activity was easily observed at 100x or 200x magnification by use of an inverted microscope. Cytopathology was observed within 24 hours after tracheal rings were inoculated with virus. The ciliated cells lining the tracheal rings became infected, rounded up, and were sloughed off into the lumen of the explant. Within 1-4 days all ciliary movement has ceased in inoculated rings, but continued in a normal manner in uninoculated control rings. Specific immunofluorescence was demonstrated in cells of smears prepared from infected organ cultures.

Johnson, Newman and Wills (5) and Johnson and Newman (6) have also utilized chick tracheal organ cultures for infectious bronchitis virus propagation, titration and as an indicator system for serum neutralization. Butler (1) has propagated fowl pox and infectious laryngotracheitis as well as infectious bronchitis and Newcastle disease viruses in tracheal organ cultures. Cherry and Taylor-Robinson (3) have used tracheal explants to study several avian viruses and mycoplasma.

Shoyer and Easterday (11) have utilized tracheal tissues from fetal calves as a model system in which to propagate infectious bovine rhinotracheitis virus. Similar culture systems were used by Campbell et al. (2) and Kita et al. to grow bovine parainfluenza 3 virus.

Perhaps the most recent adaptation for organ culture was reported by Nair et al. These workers found that the ciliated cells of chick tracheal explants are very sensitive to aflatoxins and a number of other mycotoxins. As little as 0.2 µg/ml of aflatoxin B₁ caused complete cessation of ciliary movement in the cultures within 48 hours. Nine other mycotoxins were found to be toxic in a range of 0.13 to 150. µg/ml. (10).

Organ cultures are not extensively used in diagnostic laboratories at the present time. They do appear to offer potentially useful model systems for some laboratory purposes, however. Some of the possible applications for organ culture techniques include:

1. Virus isolations and propagation.
2. Mycoplasma isolation and propagation.
3. As an indicator system for serum neutralizations involving viruses or mycoplasma.
4. As a bioassay system for mycotoxins.

SUMMARY

Explants of living tissue were first grown in vitro more than 60 years ago. Most early applications were in embryology and physiology studies. During the past decade, however, organ cultures have been adapted for use in virus propagation, sero-
logical studies and toxicology. Tracheal explants from various species have been most frequently used. Organ culture techniques are not difficult, and are potentially useful in diagnostic work, as well as in the research laboratory.

REFERENCES

APPLICATIONS OF RECOMMENDED FLUORESCENT ANTIBODY TECHNIQUES FOR VIRAL DISEASES IN VETERINARY DIAGNOSTIC LABORATORIES

by
E.A. Carbrey, VMD, MS, and W.C. Stewart, DVM, MS

INTRODUCTION

The word “recommended” employed in the title of this paper is an obvious euphemism for the words approved, standard and official. The ability of a diagnostician to employ fluorescent antibody (FA) methods in the laboratory requires the attributes of discernment and discrimination. Yet FA techniques are sufficiently difficult that failure to observe certain basic rules can lead to trouble. The worker who believes he is being creative when he omits the positive and negative controls is perhaps the worst offender but often honestly believes he is saving time and, therefore, money.

It is the intent of this paper to first consider some basic attributes of viral FA techniques and second to describe briefly some methods with certain viruses that are worthy of recommendation to diagnosticians. General rather than precise directions will be offered with an emphasis on the considered application of the scientific method.

There are basic technical problems involved in developing a reliable FA technique for use as a routine laboratory procedure. The in-depth precision demanded in the selection, preparation and standardization of items such as virus strain, hyperimmunization schedule, antiserum, conjugate preparation and cell culture propagation, is inevitably controlled by economic factors. Excellence is a worthy goal in the preparation of diagnostic reagents but sometimes hard decisions must be made as to reasonable limits. Some of these areas may be considered as they apply to veterinary diagnostic FA techniques.

VIRUS STRAIN

In the preparation of hyperimmune serum for conjugation a suitable virus source must be obtained. The first problem is the selection of the appropriate strain. The Western Hemisphere Committee on Animal Virus Characterization has provided a list of recommended reference virus strains. These strains should be considered first for use in the preparation of antiserums or as the positive virus controls in FA
techniques. In some cases it may be an advantage to use a more virulent strain of the virus for the later stages of hyperimmunization. Consideration must also be given as to whether the conjugate will be multivalent and react with all strains of a particular virus including those that do not cross react serologically in other techniques. The FA method provides a less specific response with most viruses, and strains with antigenic differences will often cross fluoresce as in the case of bluetongue and African swine fever. However, with vesicular stomatitis it is necessary to prepare separate conjugates for the New Jersey and Indiana types because a conjugate prepared against one strain will not stain cells infected with the heterologous strain.

Some effort should be made to purify the selected virus strain prior to animal inoculation. Cytopathogenic viruses can be cloned using the agar overlay method with subinoculation from isolated plaques. Viruses that are not cytopathogenic such as hog cholera (HC) can be less adequately purified by the limiting dilution technique in which transfers are made from the highest infective dilutions of the virus. However, with vesicular stomatitis it is necessary to prepare separate conjugates for the New Jersey and Indiana types because a conjugate prepared against one strain will not stain cells infected with the heterologous strain.

If cost is not a factor the next step might be propagation of the virus in gnotobiotic animals or cell culture derived from them. However, in the absence of reliable methods of preventing intrauterine virus infection this step is not a reasonable one. Contaminating orphan viruses in the selected virus strain may not interfere with its use in the application of the FA method. However, steps taken to produce antibody with it will probably result in increased titers against the contaminants as well.

SERUM SOURCE

The animal species selected for antiserum production should be capable of replicating the virus. There is an economic advantage to using the largest species available. The natural host animal gives the best antibody response and is most desirable provided an attenuated strain is available for the initial exposure. Small laboratory animals are of little value in producing adequate amounts of antiserum. The rabbit is not recommended for preparing antiserums for viral conjugates. Conjugates prepared from rabbit antiserums in our laboratory have consistently produced high non-specific background fluorescence that could not be removed by absorption with tissue powders. Animals should be obtained from the best possible source, preferably from a production unit capable of supplying animals of consistent quality. For example, animals obtained by hysterectomy, deprived of colostrum, and raised in isolation are excellent though expensive. Repeat orders from such a source will usually be of the same quality.

Prior to virus exposure a large volume of serum should be obtained from the animals. This preinoculation serum is used to prepare a normal conjugate for a reference reagent and should contain all of the extraneous antibodies that will be present in the antiserum conjugate. Inasmuch as it is not possible to obtain enough
preinoculation serum to prepare adequate supplies of normal serum conjugate for routine use, it is extremely helpful to be able to obtain serum from other animals of the same background, i.e., hysterectomy obtained, colostrum deprived.

HYPERIMMUNISATION SCHEDULE

The objective of the immunization procedure is to introduce as much pure viral antigen and as little other foreign protein into the production animal as possible. Antivirus conjugates must stain viral antigen in a matrix of cell proteins. The ideal initial exposure is made with a parenteral dose of an attenuated strain of the virus followed in 14-21 days with a small dose of the most virulent strain available. A period of 60-70 days is then recommended before administering a large inoculum for hyperimmunization. The hypering dose of virus may be obtained from the same species in the form of viremic blood or may be a cell culture harvest obtained from homologous cells. The dose should be cooled to 4°C and infused intravenously. Due to the large volume of inoculum required this is the most desirable route although the intraperitoneal route may also be used. The hypering dose should overwhelm the antibody defenses in the circulatory system thus enabling the excess virus to reach susceptible cells. For example, in preparing anti-HC serum as much as 5 ml. of virus blood per lb. body weight is infused. Fourteen to twenty-one days later the animals are bled out for maximum antibody titer.

If the large hypering dose of virus is obtained by propagation in a heterologous system then it may be necessary to produce a negative control serum by infusing large amounts of noninfectious heterologous blood or cell culture harvests into healthy animals. A conjugate prepared from such a sensitized animal might provide a better negative control of normal background fluorescence for comparison with the antiviral conjugate.

CONJUGATE PREPARATION

Preparation of conjugates for the direct staining of virus infected cells in culture or tissue section depends on starting with a potent, high titer antiserum so that heavy adsorption of the conjugate with tissue powders can be performed. Some of specific staining properties are removed in this process. Consequently, most convalescent serums do not contain sufficient antibody to make adequate conjugates. Although there are faster methods the following technique is recommended as having provided consistently good conjugates.3

The gamma globulin is precipitated from the serum with saturated ammonium sulfate solution, one-third volume for pig serum and one-half volume for cow, horse and sheep serums. The precipitate is dissolved in distilled H2O and reprecipitated twice more with saturated ammonium sulfate. After dialysis against saline (0.85% NaCl) the protein concentration is adjusted to 1% and the pH to 9.0) with a carbonate-bicarbonate buffer solution. Fluorescein isothiocyanate (FITC) is dissolved in a small amount of the buffer solution and added to the globulin solution to produce a final concentration of 1 mgm. per 20 mgm. of protein. The solution is stirred overnight at 4°C. to permit chemical binding of the FITC and gamma globulin. It is

advisable to try several commercial sources of FITC to find the brand that works best in a specific system. The excess FITC is removed on a G-25, coarse Sephadex column and the conjugate is absorbed with rabbit liver powder, 1-2 gm. per 10 ml. of conjugate. The liver powder is made into a paste by adding 2.5 ml. of saline per gm. of powder before adding the conjugate. The liver powder is removed by high speed centrifugation and the conjugate is dialyzed against phosphate buffered saline, pH7.2, for 48 hours as a final purification step.

To determine the optimum dilution of the conjugate to be used, twofold dilutions are prepared and infected cell cultures and tissue sections are stained. The dilution at which specific staining of viral antigen is just beginning to fade is determined. The optimum or stock dilution of the conjugate selected should be twice this concentration. The diluted conjugate has the advantages of reducing the background fluorescence and reducing the concentration of conjugated antibodies against other agents that may have been present in the serum.

A necessary complement to the above is a conjugate for negative control purposes prepared in the same way from the preinoculation serum of the hyperimmune donor animal. An alternative source for the normal serum conjugate is from animals obtained from the same production unit or farm. The normal or negative control conjugate is diluted to a concentration that gives the same intensity of background fluorescence as the antiviral conjugate on a negative cell monolayer or section. The normal serum conjugate thus provides a zero level of staining for comparison with the hyperimmune conjugate. This is of particular value when specific diffuse viral fluorescence is found throughout the section.

CELL CULTURE IMMUNOFLUORESCENCE

The most important attribute of any cell culture used for immunofluorescence is susceptibility to infection by the virus and the ability to produce intracellular viral antigen. The cells are most susceptible when in a state of active metabolism as is found in a cell culture as it approaches 70-80% confluency. After the cell cultures become confluent and are placed on maintenance medium the number of infected cells, the concentration of viral antigens, and therefore the intensity of fluorescence are usually less than that found with an actively growing cell culture. Care should be taken to provide high nutrition levels for the cells and eliminate toxicity problems related to soiled glassware and coverslips, water impurities, incompatible serums, etc. The cell line should be passaged regularly with as short a time interval between passages as possible.

Primary cell cultures are not as desirable as established cell lines in which certain passage levels have been found to provide cultures that are consistently susceptible to the virus of interest. The cell sheet of the primary cell culture stained with conjugate has a higher background of nonspecific fluorescence as compared with a cell line culture or even a secondary cell culture. Different batches of primary cells vary considerably in susceptibility to virus infection.

Established cell lines that can be stored frozen in aliquots at a low passage level in liquid nitrogen and used through passage levels of known susceptibility are a more reliable source of susceptible cell cultures. However, minor variations in cell susceptibility will occur throughout the different passage levels and positive virus
controls must be used on all batches of cells to provide assurance of virus infectivity. Of course, uninoculated cell cultures should be stained with the viral conjugate for a negative control.

An experiment was performed on three different sources of PK-15 cell cultures; one provided by a central tissue culture supply and the other two produced in our laboratory using two different batches of cell culture medium of similar quality. Leighton tube coverslip cell cultures were prepared from the 3 cell lines 3 times a week and inoculated with 2 strains of HC virus. Each coverslip culture received a standard inoculum of each virus strain. One of the virus strains, Strain A, had been adapted to cell culture by many passages on the PK-15 cell line and the other virus strain, Ames, was a highly virulent virus strain of blood origin. The total plaques on 40 randomly selected microscopic fields were counted on each coverslip.

The variation in plaque counts among the 3 cell lines and between the 2 viruses throughout the 4 passages was considerable (Table 1). The data were analyzed independently for each virus and the mean counts were found to vary significantly (P<0.05) from cell line to cell line for each of the two viruses. Significant passage to passage variability (P<0.10) was found only with the Ames strain.

The interaction between cell line and virus strain was highly significant (P<0.01). The mean plaque counts of virus strain by cell line were compared graphically (Fig. 1). Strain A virus had a lower mean count than the Ames strain on cell line I while on cell line III the Ames strain had the lower plaque count. However, the 2 virus strains had almost the same mean plaque counts on cell line II. This variation in cell culture susceptibility to different virus strains may account for the low plaque counts obtained with some field strains of HC virus.

From the above data it is obvious that cell lines may vary in their susceptibility although the cells look healthy and are growing well. Diploid cell lines lose susceptibility and grow poorly as high passage levels are reached. Each passage grows out more slowly, the cells look rough, and eventually the line is lost. As this critical passage level, usually between the 60th and 70th, is reached the cultures should be discarded and a new start made with a low passage culture from frozen cells. If a laboratory wishes to perform cell culture immunofluorescence using cell lines it is recommended that a deep freeze facility for storage of aliquots of low passage cells be obtained.

The use of controls with cell cultures must be emphasized. However, plaques of infected cells can be followed advantageously as they increase in size and number following incubation in replicate cultures. Unexposed and infected cell monolayers must be stained with both normal and hyperimmune conjugates particularly when most of the cells in the culture are infected and the entire cell sheet has a diffuse fluorescence. The presence of diffuse fluorescence in both the uninfected and infected cell sheets stained with immune conjugate may be due to infection of the cell cultures with a contaminant virus that cross fluoresces with the virus against which the conjugate was prepared. This contaminant virus is usually introduced into the cell line by fetal serum supplements but may also be the result of aerosol contamination.

On the other hand, cell sheets in poor condition that have dead or dying cells may have a high degree of nonspecific fluorescence when stained with normal and hyperimmune conjugates.
TISSUE SECTION IMMUNOFLUORESCENCE

An important aspect of this technique is the skill, care, and precision employed in the preparation and staining of the tissue sections. Tissue sections may be prepared improperly and the interpretation of such sections inevitably leads to incorrect findings.

The condition of the tissue has considerable bearing on the quality of the sections since nonspecific staining is a much greater problem than with cell cultures. The use of controls is imperative and should include a section cut from a block of tissue with virus infected cells that will have characteristic bright fluorescence when stained with the immune conjugate. A section of the specimen tissue stained with normal serum conjugate will provide the observer with a zero level of background fluorescence to compare with that of the specimen stained with the hyperimmune conjugate. Germinal centers of lymphoid tissues and aggregations of leucocytes and macrophages of uninfected tissues should stain with equal intensity with both normal and hyperimmune conjugates. An alternative method of providing controls is to stain replicate sections with the hyperimmune conjugate mixed with normal and hyperimmune serums. Of course, the hyperimmune serum-conjugate mixture should inhibit the specific immunofluorescence while the normal serum-conjugate mixture does not. In actual practice some difficulties are found with the use of this method. The immune conjugate already diluted to the optimum point receives another doubling dilution with the addition of an equal amount of normal serum and decreased fluorescence will be observed in the positive section. In addition, the transfer of FITC from the immune globulins to normal serum proteins by chemical recombination in the normal serum-immune conjugate mixture may account for the partial reductions of fluorescence observed in virus infected tissues stained with this mixture.

BOVINE VIRUSES

Fluorescent antibody conjugates prepared against infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD), and parainfluenza (PI-3) are available and recommended for use in bovine cell cultures.

The IBR conjugate may be used on tissue sections from aborted fetuses. Coverslip cell cultures are inoculated with tissue suspensions and examined daily for cytopathic effects. From the appearance of the cytopathic changes it may be possible to select the appropriate conjugate for staining the coverslip culture. Cytopathic changes due to IBR virus may be detected in 24 hours while changes due to BVD and PI-3 may take longer. If cell destruction has not been observed by 48 hrs. 2 coverslip cultures should be stained. If negative, additional inoculated coverslips should be processed at 72 and 96 hours. In this manner, a virus may be isolated and identified with the specific antiviral conjugate in 24-48 hours in the case of IBR or PI-3 viruses and 72-96 hours with BVD virus. Noncytopathogenic strains of BVD virus can be detected by immunofluorescence with this technique.

Tissue sections may be cut from aborted fetuses and stained with IBR con-

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569

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*Diagnostic Services, Animal Health Division, National Animal Disease Laboratory, P. O. Box 70, Ames, Iowa 50010.*
jugate. The concentration of the viral antigen and intensity of the specific staining usually permits a confirmation of the virus infection in spite of the usual poor quality of fetal tissues. The staining of control sections with the normal, bovine serum conjugate is a necessary part of this procedure. Equivocal findings should be confirmed by isolation of the virus in cell culture.

The lesions of vesicular stomatitis (VS) resemble those of foot and mouth disease and a rapid laboratory confirmation is necessary to differentiate the two viruses. Bovine cell cultures infected with VS may be stained with antiviral conjugates after 18-24 hours of incubation. It is necessary to use two conjugates, one each for the New Jersey and Indiana types since the two strains do not crossfluoresce.

SWINE VIRUSES

The use of anti-HC conjugate for the detection of HC antigen in cell cultures and tissue sections has been widely accepted and has provided a standard for comparison with FA techniques employed with other viruses. There is an urgent need, however, for the routine use of the positive and negative controls. Work is currently underway to prepare and make available a normal swine serum conjugate for negative controls as described earlier in this paper.

An excellent conjugate may be recommended for use in staining cell cultures infected with pseudorabies virus. The technique is performed in a similar manner to that with HC virus using PK-15 cell cultures. The viral antigen is initially seen as bright clumps of densely staining perinuclear material and later, as the cell degenerates, is found throughout the cytoplasm.

A system for isolation and identification of transmissible gastroenteritis (TGE) virus using a swine testicle cell line or primary swine kidney cells and antiviral conjugate has been used in our laboratory. However, some cases with suitable histories fail to yield isolations of TGE virus. Further studies are currently underway to develop a cell culture adapted strain of the virus and obtain information on specimens using tissue section, cell culture, neutralization test, and pig inoculation.

EQUINE VIRUSES

A conjugate for equine rhinopneumonitis virus may be used to stain infected equine cell cultures. The limitations of this technique are the lack of equine tissues for cell culture propagation and the poor transfer qualities of equine cell lines. Most of the equine cell lines established in our laboratory cannot be maintained beyond 25-30 passages. However, there is an equine dermal cell line that can be taken to higher levels. Equine rhinopneumonitis virus may also be isolated on bovine or rabbit kidney cell cultures and stained with the conjugate.

When processing specimens from cases of respiratory disease in horse, bovine cell cultures should be inoculated also and stained with PI-3 conjugate. This virus has been found to be a cause of respiratory illness in the horse.

*The American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852*
BOVINE VIRUSES

The only conjugate to be recommended is one for contagious ecthyma (CE) virus. Sheep kidney cell cultures may be infected with CE virus and stained with the conjugate in 48-72 hours. As with other pox viruses it is difficult to maintain the CE virus by passage in cell cultures. However, the fluorescence of the viral antigen following initial infection of the cell culture is excellent.

SUMMARY

The application of a potent antiviral conjugate is recommended to the diagnostic virologist provided he uses negative and positive controls. If a cell culture method is used the cells must be susceptible to the virus. This may require facilities for proper storage of frozen cell lines and a high level of competency in tissue culture propagation. If a tissue from the infected animal is sectioned then there must be skill and attention to detail in this area. Control sections from all tissues should be stained with normal conjugate or serum-conjugate mixtures.

Reference virus strains should be used whenever possible and a bank of reference antiserums should be developed and maintained. Future work should be directed towards the production of new and better conjugates and the development of more precise systems for their utilization.

Table 1. Comparison of the Susceptibility of Three Sources of PK-15 Cell Cultures Using Two Strains of Hog Cholera Virus

<table>
<thead>
<tr>
<th>Passage</th>
<th>Line I St. A</th>
<th>*</th>
<th>Line I St. A</th>
<th>Line II St. A</th>
<th>*</th>
<th>Line III St. A</th>
<th>Ames</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>159</td>
<td>265</td>
<td>103</td>
<td>345</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>171</td>
<td>146</td>
<td>149</td>
<td>784</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>204</td>
<td>22</td>
<td>55</td>
<td>602</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>244</td>
<td>26</td>
<td>172</td>
<td>137</td>
<td>195</td>
<td></td>
</tr>
</tbody>
</table>

* Total number of virus plaques found in 40 fields.
Fig. 1. Comparison of mean plaque counts of Strain A (cell culture adapted) and Ames (virulent, blood source) hog cholera virus on three sources of PK-15 cell cultures through four passages.

REFERENCES


INVESTIGATIONS OF EPIZOOTIC BOVINE ABORTION IN COLORADO AND SOUTH DAKOTA

by
D. E. Reed, R. E. Pierson, C. A. Kirkbride and J. P. McAdaragh

Epizootic bovine abortion (EBA) was first reported as a separate disease entity in 1956. The disease was characterized clinically by subcutaneous edema, ascites and hemorrhages in fetal tissues and a pronounced fetal hepatopathy. After several workers reported unsuccessful attempts to isolate an etiologic agent, Giroud in France and Storz et al. in California reported the isolation of a bacterium of the genus *Chlamydia* from cases of EBA. The EBA syndrome was later reproduced experimentally by inoculation of the chlamydial agent into pregnant cattle. Serologic studies provided further evidence of the role of chlamydia in bovine abortion.

In field cases of abortion, a presumptive diagnosis of EBA can be made when the above mentioned fetal gross lesions are noted in conjunction with histopathologic changes of generalized reticuloendothelial hyperplasia and vasculitis in the brain. A confirmed diagnosis requires isolation of chlamydia from fetal or placental tissues. Although less satisfactory than isolation of the organism, demonstration of a rise in chlamydial antibody level after abortion can be used to aid in diagnosing EBA.

MATERIALS AND METHODS

Over the past four years, a large outbreak of EBA in a single herd in Colorado and four smaller outbreaks in South Dakota were investigated. Specimens obtained from these cases and examinations performed are summarized (Table I). With the exception of the Colorado herd which was investigated through the Department of Clinical Sciences, Colorado State University, specimens were obtained as routine diagnostic accessions to the South Dakota Animal Disease Research and Diagnostic Laboratory.

Laboratory procedures used in investigating these outbreaks were as follows:
1. Necropsy: fetuses and placentas were examined for gross pathologic changes. Tissues were collected in sterile plastic bags for microbiology procedures and in 10% neutral formalin for histopathology procedures.
2. Histopathology: tissues preserved in formalin were cut at 10 microns and stained by standard hematoxylin and eosin (H&E) methods.

From the Animal Disease Research and Diagnostic Laboratory, (Reed, Kirkbride and McAdaragh) South Dakota State University, Brookings, South Dakota 57006 and the Department of Clinical Sciences, (Pierson) Colorado State University, Fort Collins, Colorado 80521.

Approved for publication by the Director, Agricultural Experiment Station, South Dakota State University as Journal Series No 574.
3. Bacteriology and Mycology: stomach contents of fetuses were cultured on Blood Agar and Saboraud’s medium.

4. Mycoplasma: mycoplasma were detected by culturing fetal and placental tissues on Frey’s M94 medium. Samples were inoculated into broth tubes and agar plates and incubated at 37°C in a 10% CO₂ atmosphere.

5. Cell Culture: fetal and placental tissues were examined for the presence of viral agents by culture through two passages on bovine kidney or adrenal cell cultures.

6. Complement Fixation (CF) test: serum samples obtained from dams of aborted fetuses were tested for the presence of group specific chlamydial CF antibodies. When available, paired serum samples (first taken at the time of abortion and second taken two to four weeks later) were tested to determine if a rise in chlamydial antibody could be detected.

7. IBR FA: The fluorescent antibody test on frozen fetal tissue sections was to detect infectious bovine rhinotracheitis virus.

8. Chick Embryo: Six to eight-day developing chick embryos were inoculated with fetal and placental tissue extracts in efforts to detect the chlamydial agent of EBA. Procedures used were according to Storz and McKercher. When inoculated chick embryos died 3 to 4 days post inoculation, yolk sac impression smears were prepared and stained to detect chlamydial elementary bodies.

RESULTS

The results obtained are summarized (Table 1.) Positive results obtained under the headings of gross and microscopic lesions indicated the presence of lesions similar to those reported in cases of confirmed EBA. The negative results in bacteriologic and mycologic examinations indicated only that significant pathogens were not recovered.

In some cases E. coli, Streptococcus sp. or Proteus sp. were recovered but not considered to be significant. In one instance a mycoplasma organism was isolated. The mycoplasma was first isolated in developing chick embryos and later transferred to mycoplasma medium where its identity as mycoplasma was confirmed. No viral agents were isolated or detected by the IBR FA procedure. Inoculation of chick embryos failed to detect the presence of chlamydial agents.

The results of CF tests performed on serum samples submitted from four of the herds are given (Tables III and IV).

DISCUSSION

A confirmed diagnosis of EBA requires isolation of the chlamydial agent of EBA from fetal or placental tissues. By this criterion, none of the 5 cases investigated were confirmed as EBA. Serologic studies on the S.D. 3 outbreak, however, revealed a highly significant increase in chlamydial antibody level 12 days after abortion. Studies by Storz and McKercher indicated that a rise in antibody level 12 days to 4 weeks after abortion could be considered as strong evidence that chlamydia were involved in the abortion. The possibility does exist, however, that a different chlamydial infection such as sporadic bovine encephalomyelitis (SBE) might be responsible for the positive serologic response seen in the S.D. 3 outbreak.

In two other instances (S.D. 2 and S.D. 4), four-fold increases in CF antibody
levels were seen after abortion. While it is generally conceded that a four-fold rise in titer is significant, the low magnitude of response and the small number of animals demonstrating a response tend to cast considerable doubt on its significance in these cases. The essentially negative serologic findings in the Colo. 2 outbreak tend to rule out chlamydia in the etiology of this particular abortion outbreak.

The significance of the mycoplasma agent isolated from the S.D. 1 fetus is not known. Although it is possible that it played a role in this case of abortion, there have been no reports where an abortion syndrome has been experimentally reproduced with bovine mycoplasmal agents.

To summarize the findings from 5 suspected EBA outbreaks, good evidence for chlamydial involvement was found in only one instance. Investigations of three outbreaks yielded results indicating the probable absence of chlamydial involvement. These findings suggest that there may be etiologic agents in addition to chlamydia responsible for the clinical syndrome of EBA.

TABLE I

Suspected EBA Outbreaks: Specimens obtained, Number of Abortions and Herd Size.

<table>
<thead>
<tr>
<th>Case Designation</th>
<th>Specimens</th>
<th>Number of Abortions</th>
<th>Herd Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colo. 1</td>
<td>4 fetuses, serum 2 placentas</td>
<td>32</td>
<td>51</td>
</tr>
<tr>
<td>S.D. 1</td>
<td>1 fetus</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>S.D. 2</td>
<td>3 fetuses, serum</td>
<td>5</td>
<td>--</td>
</tr>
<tr>
<td>S.D. 3</td>
<td>1 fetus, serum</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>S.D. 4</td>
<td>3 fetuses, serum 1 placenta</td>
<td>5</td>
<td>60</td>
</tr>
</tbody>
</table>
TABLE II

Results of Laboratory Examinations on Suspected EBA Outbreaks.

<table>
<thead>
<tr>
<th>Case Designation</th>
<th>Colo. 1</th>
<th>S.D. 1</th>
<th>S.D. 2</th>
<th>S.D. 3</th>
<th>S.D. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross Lesions</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>decomposed</td>
<td>Pos</td>
</tr>
<tr>
<td>Microscopic Lesions</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>decomposed</td>
<td>Pos</td>
</tr>
<tr>
<td>Bact. &amp; Mycology</td>
<td>NT</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>NT</td>
<td>Pos</td>
<td>NT</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Cell Culture</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>CF Test</td>
<td>Neg</td>
<td>NT</td>
<td>equivocal</td>
<td>Pos</td>
<td>equivocal</td>
</tr>
<tr>
<td>IBR FA</td>
<td>NT</td>
<td>NT</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Chick Embryo</td>
<td>Neg</td>
<td>Mycoplasma</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

Pos = Positive results obtained  
Neg = Negative results obtained  
NT = Not tested

TABLE III

Results of Chlamydia CF Tests: Unpaired Sera

<table>
<thead>
<tr>
<th>CF Titer</th>
<th>Number of Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLORADO 1</td>
<td></td>
</tr>
<tr>
<td>1:2</td>
<td>1</td>
</tr>
<tr>
<td>1:4</td>
<td>1</td>
</tr>
<tr>
<td>1:8</td>
<td>1</td>
</tr>
<tr>
<td>&lt;1:8</td>
<td>15</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>CF Titer</th>
<th>Number of Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOUTH DAKOTA 4</td>
<td></td>
</tr>
<tr>
<td>&lt;1:2</td>
<td>37</td>
</tr>
<tr>
<td>1:2</td>
<td>4</td>
</tr>
<tr>
<td>1:4</td>
<td>4</td>
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<tr>
<td>1:8</td>
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</tr>
<tr>
<td>1:16</td>
<td>4</td>
</tr>
<tr>
<td>1:32</td>
<td>2</td>
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</tbody>
</table>
TABLE IV

Results of Chlamydia CF Tests: Paired Sera

COLORADO 1

<table>
<thead>
<tr>
<th>Cow Number</th>
<th>1st sample</th>
<th>2nd sample</th>
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<tbody>
<tr>
<td>1</td>
<td>1:4</td>
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</tr>
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<td>2</td>
<td>1:4</td>
<td>1:4</td>
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</table>

SOUTH DAKOTA 2

<table>
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<th>Cow Number</th>
<th>1st sample</th>
<th>2nd sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:4</td>
<td>1:4</td>
</tr>
<tr>
<td>2</td>
<td>1:8</td>
<td>1:16</td>
</tr>
</tbody>
</table>

SOUTH DAKOTA 3

<table>
<thead>
<tr>
<th>Cow Number</th>
<th>1st sample</th>
<th>2nd sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:20</td>
<td>1:40</td>
</tr>
<tr>
<td>2</td>
<td>1:320</td>
<td>1:40</td>
</tr>
</tbody>
</table>

SOUTH DAKOTA 4

<table>
<thead>
<tr>
<th>Cow Number</th>
<th>1st sample</th>
<th>2nd sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:16</td>
<td>1:32</td>
</tr>
<tr>
<td>2</td>
<td>1:2</td>
<td>1:32</td>
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<tr>
<td>3</td>
<td>1:2</td>
<td>1:2</td>
</tr>
<tr>
<td>4</td>
<td>1:32</td>
<td>1:32</td>
</tr>
</tbody>
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REFERENCES


3. Frey, M. Veterinary Medical Research Institute, Ames, Iowa. Personal Communication.


DIAGNOSIS OF MYCOTIC ABORTION IN CATTLE

by


Mycotic infection of the placenta of a cow was first reported by Theobald Smith in 1920. Dr. Smith described the lesions of the uterus, placenta and fetus associated with a fungus of the genus *Mucor*. The gravid bovine uterus was obtained intact from an abattoir.

Since the disease was first described, numerous reports of bovine abortion associated with mycotic placentitis have appeared. Fungi of the genera *Aspergillus*, *Mucor*, *Rhizopus* and *Absidia* are most commonly involved, although several reports exist of occasional cases of bovine mycotic placentitis associated with fungi of other genera.

Some of the earliest work wherein the disease was artificially reproduced was reported by Gilman and Birch in 1925. They produced uterine infection in two of five pregnant cows by injecting intravenously *Mucor* sp. isolated from an aborted bovine fetus. Through this and a more recent report, it has been established that a fungemia of pregnant cows with *Mucor* sp. or *Aspergillus* sp. frequently results in colonization of the caruncles and placenta with consequent placentitis and abortion. The original portal of entry of the fungus has not been completely agreed upon. Coitus and inhalation have both been postulated as means of infection. Hillman and McEntee reviewed their findings and those of others and concluded that fungal spores most likely enter the bloodstream through ulcers in the forestomachs, especially the omasum. Considering the probability that cows are frequently exposed to large numbers of causative fungal spores and that ordinarily only individual cows within a herd are affected, some triggering mechanism is thought to be required to precipitate infection.

The incidence of bovine mycotic abortion in various surveys has been found to range from less than 1.0% to 12.5%. In New York, Hillman found the portion of mycotic abortion diagnosed each year from 1940 to 1966 varied from 0.0% to 16.4% with an average of 5.6%. Mycotic placentitis caused 11.5% of all the abortions investigated in the New York laboratory during this time and comprised the largest group of abortions of known etiology. In considering the incidence as reported by various workers, the differences in criteria used in establishing the diagnoses must be kept in mind.

Ordinarily, mycotic placentitis occurs sporadically and frequently only single individuals within a herd are affected. However, Turner reports that 10 (29%) abortions out of 34 investigated from a single herd were associated with mycotic infection. Most of the fungi involved were Phycomycetes but several different species and genera were identified. The variation in etiologic agents in these cases again indicates that a predisposing factor is involved in production of the disease rather than a common source of infection.

MATERIALS AND METHODS

July 1, 1968 a survey as to the causes of bovine abortion and stillbirth was
started at the South Dakota Animal Disease Research and Diagnostic Laboratory. By completion of the survey, July 1, 1971, 1556 bovine abortions or stillbirths had been investigated. The specimens included 485 placentas. Cases in which serologic examination only was done are not included in this total. Specimens examined were those submitted by veterinary practitioners in South Dakota and surrounding states. The type and number of specimens submitted varied with what was available to the practitioner. In some cases, only portions of a placenta from a cow known to have aborted were submitted. In other cases, an aborted fetus, placenta, blood sample from the dam, as well as feed and water samples were submitted from a single case. In the average case, the number and type of specimens submitted was usually between these extremes.

In addition to examination for mycotic placentitis, the following procedures were carried out depending on the specimens submitted:

1. Necropsy examination of the fetus and placenta.
2. Histopathologic examination of the placenta, lung, liver, lymph nodes, muscle, myocardium, spleen, kidney and brain. Other tissues with gross lesions were also examined microscopically.
3. Bacteriologic culture of the abomasal content. When no abomasal content was submitted the available tissues were cultured.
4. Culture on bovine embryonic kidney (BEK) cell culture of a pool of lung, liver, kidney and spleen from the fetus. Placenta was also cultured for viruses on BEK but was handled separate from other tissues.
5. Immunofluorescent examination for IBR was performed on cryostat sections of kidney and placenta.
6. Abomasal content, fetal tissue pools and placenta were cultured for mycoplasma.

METHODS USED IN EXAMINING ABORTION SPECIMENS FOR MYCOTIC PLACENTITIS

When an entire fetus was examined in the laboratory or when the tied-off abomasum of an aborted fetus was submitted, the wall of the stomach was punctured with a sterile needle and the contents drawn into a sterile disposable syringe. The contents were cultured on Sabouraud’s* and Mycobiotic8 agars at 25°C and 37°C. Colonies were picked and transferred to potato dextrose agar* slants for identification. Teased mounts of these cultures were used to identify organisms; when necessary, slide cultures were prepared.

Sections of paraffin mounts of fetal tissues and placenta were stained with hematoxylin and eosin by standard methods. All placental tissues and lung and skin suspected of being infected with fungi were stained by Gomori’s methenamine-silver nitrate technic10. The tissue sections were then examined microscopically.

A diagnosis of mycotic abortion was made only when mycotic elements could be demonstrated in the tissues of the fetus or placenta and associated with lesions of inflammation.

*Difco, Detroit, Michigan
RESULTS

Results of the examination of 1556 bovine abortions and stillbirths over a three year period are summarized (Table 1). Mycotic placentitis was diagnosed in 60 cases and made up the second largest group of abortions of known etiology. It accounted for 3.85% of all the cases examined and 11.36% of the diagnoses made. Of the 485 placentas examined, 60 (12.4%) had lesions of mycotic infection.

The diagnoses of mycotic abortion are summarized for each year of the survey (Table 2). This infection accounted for 2.9% of all abortions investigated in 1969, 3.38% of the abortions in 1970 and 4.48% of the abortions in 1971.

The monthly incidence of mycotic placentitis over a three year period is illustrated (Fig. 1). The peak incidence appears to be during the early spring months of February and March. The incidence is lowest during the summer and early fall.

<table>
<thead>
<tr>
<th>Total No of Diagnoses</th>
<th>% of Total Cases</th>
<th>% of Total Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBR</td>
<td>278</td>
<td>17.87</td>
</tr>
<tr>
<td>Mycotic Abortion</td>
<td>60</td>
<td>3.85</td>
</tr>
<tr>
<td>Vibriosis</td>
<td>49</td>
<td>3.15</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>22</td>
<td>1.42</td>
</tr>
<tr>
<td>EBA</td>
<td>13</td>
<td>.84</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>8</td>
<td>.52</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>6</td>
<td>.38</td>
</tr>
<tr>
<td>BVD</td>
<td>3</td>
<td>.19</td>
</tr>
<tr>
<td>Other</td>
<td>89</td>
<td>5.71</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>528</strong></td>
<td><strong>33.93</strong></td>
</tr>
</tbody>
</table>

Table 1. Summary of the results of the examination of 1556 bovine abortions and stillbirths over a three year period.
Table 2. Summary of the diagnosis of bovine mycotic placentitis over a three year period at South Dakota State University.

* From July 1 of the previous year through June of the year given.
GENERAL FINDINGS OF NECROPSY AND HISTOPATHOLOGIC EXAMINATION

The primary lesions of mycotic abortion occur in the placenta. Chances of diagnosing the disease are greatly reduced when the placental tissue is not available for examination.

Macroscopic examination of a placenta infected with fungi reveals distinct lesions. Part or all of the maternal caruncle often adheres to the placental cotyledon giving it a greatly thickened appearance especially around the outer circumference. This area of the cotyledon may be rolled inward toward the center of the structure producing a cupped appearance. On some occasions most of the maternal caruncles rupture at the peduncle and are expelled attached to the cotyledons. It appears that this occurs more often when mucoraceous fungi are involved than when *Aspergillus* spp. causes the infection.

Intercotyledonary areas of the placenta are thick and leathery. The amount of placental necrosis observable grossly varies from severe to practically none. The majority of infected placentas are tan to brown with a distinctly necrotic appearance. Occasionally the cotyledons are dark red while the thickened intercotyledonal areas are opaque and white. Extensive adventitious placentation is common.

Gross lesions of the placenta in cases of mycotic placentitis are quite distinctive and the experienced diagnostician can make a tentative diagnosis of this disease on finding these lesions. It is well to note, however, that certain bacterial infections of the placenta, such as brucellosis and vibriosis, produce lesions which are quite similar.

The most outstanding microscopic lesions of mycotic placentitis occur in the tissues around the periphery of the cotyledons. Large aggregations of polymorphonuclear cells occur in and beneath the chorionic villi. Varying amounts of necrotic tissue debris fill the spaces between the villi. This debris represents the maternal tissue which has adhered to the fetal placenta. Hyphae are present in greatest numbers in this necrotic material. Where large amounts of maternal tissue remain adhered to the placenta, hyphae may be seen in it especially in the blood vessels. Thrombosis, perivascular inflammation and necrosis accompany the mycotic elements.

A complete description of the placental and uterine lesions of this disease was given by Cordes, et al. and Hillman and McEntee.

In approximately 25% of mycotic placentitis cases the fungus invades the fetus. Circumscribed lesions of the skin from less than one centimeter to ten centimeters in diameter occur most frequently on the head and neck. The skin affected by these lesions is thick, wrinkled and dry. On macroscopic examination, the areas resemble lesions of ringworm. The presence of well defined skin lesions provides ample evidence for a diagnosis of mycotic placentitis.

Microscopically, hyperkeratosis and inflammation are apparent in these lesions. Hyphae are most frequently seen on the surface of the epidermis and in the hair follicles and are made more easily visible with one of the special fungus stains.

Aside from skin lesions, swelling and congestion of peripheral lymph nodes are the only other gross lesions frequently seen in the bovine fetus aborted due to mycotic infection. The fetal tissues are usually fresh and show no signs of the fetus having been retained in utero after its death. The lungs are often partially expanded,
indicating that the fetus was alive during birth. Most of them live for a very short
time and are usually dead when found.

Other rather common features of fetal victims of mycotic placentitis are emaciation and dehydration of the carcass. The skin appears slightly wrinkled as if the body had shrunk within it leaving the skin too large.

The emaciation and dehydration of the fetus can be rationally attributed to the interference with its nutrition by the severe placentitis. Other conditions which interfere with exchange of nutrition from the dam to the fetus may produce similar results. In the authors' experience, fetuses aborted due to vibriosis often have a similar appearance.

Histopathologic examination of lung tissue from a fetus aborted due to mycotic infection may reveal bronchopneumonia. In these cases there are collections of polymorphonuclear cells, exudate and cell debris in the bronchioles. On rare occasions hyphae may be demonstrated in the bronchioles.

GENERAL RESULTS OF CULTURE OF THE ABOMASAL CONTENTS OF ABORTED BOVINE FETUSES

Culturing the abomasal content of aborted bovine fetuses for fungi produces some interesting findings but does not provide conclusive evidence for establishing a diagnosis of mycotic abortion. Of 1142 fetal stomachs cultured, 811 produced no growth. Approximately 42% of the other 331 contained mixed cultures and the remainder contained pure culture of yeast or mold. The number of colonies produced from a drop of abomasal fluid varied from none to many. Numerous species of molds and yeasts were isolated including: *Mucor* sp., *Aspergillus fumigatus*, *A. terreus*, *A. glaucus*, *A. niger*, *Penicillium* sp., *Monosporium* sp., *Absidia* sp., *Geotrichum* sp., *Streptomyces* sp., *Cephalosporium* sp., *Alternaria* sp., *Cladosporium* sp., *Rhodotorula* sp., *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. parasitopsis*, *Aureobasidium* sp. and *Phoma* sp.

The source of these organisms is unknown. The means of collecting the specimens precluded any great chance of contaminating them. Fungi have not been reported in the stomach content of normal unborn fetuses. Many of the fetuses examined appeared to have been dead several hours before being expelled and it is difficult to imagine how the organisms gain entrance to the digestive tract of these animals.

The Phycomycetes are not cultured as frequently from abomasal contents, or fetal and placental tissue as is *Aspergillus* sp. The former organisms appear less able to maintain their viability in the tissues and may be demonstrated by histopathologic means when they cannot be cultured.

A tentative differentiation can be made between *Aspergillus* sp. and the mucoraceous fungi on the basis of morphology as seen in tissue sections. The hyphae of *Aspergillus* spp. tend to be of even diameter with parallel sides and to contain numerous septae. The hyphae of the mucoraceous fungi are usually of uneven diameter and contain few septae.

Other examination procedures may be carried out to provide supplementary information for making a diagnosis. Smears of stomach contents may be stained by various methods and examined for mycotic elements. Demonstrating the presence
of fungi in this manner does not provide any more conclusive evidence as to their significance than does demonstrating them by culture.

Smears from the placental cotyledon may be digested with 10% KOH and examined microscopically for hyphae. Demonstrating mycotic elements by this means in smears of placental tissue with gross lesions characteristic of mycotic placentitis provides enough evidence for a tentative diagnosis. On this basis a rather firm diagnosis may be issued on the same day the specimen is examined.

Because fungi are quite ubiquitous and the placenta is, under ordinary conditions, exposed to great contamination from the environment, the significance of any fungi recovered by culturing placental tissue must remain doubtful. On the other hand, recovery of a fungus in large quantities and in nearly pure culture from placental tissue in which the presence of mycotic elements and inflammation is demonstrable provides reasonable evidence that the organism is the etiologic agent.

SUMMARY

Mycotic placentitis has been shown to be a major cause of bovine abortion in many areas of the world. In South Dakota it was found to be second only to IBR as a cause of abortion in cattle. In examining 1556 cases, mycotic placentitis was diagnosed in 60 (3.85%). During a three-year period the yearly incidence ranged from 2.9% to 4.45% of all the bovine abortion cases investigated. The highest incidence was during the months of February and March.

A firm diagnosis of mycotic placentitis can be made by: 1. finding characteristic lesions of dermatomycosis on the fetus and demonstrating by microscopic examination of these lesions, mycotic elements associated with signs of inflammation. 2. finding characteristic gross and/or microscopic lesions of placentitis and demonstrating mycotic elements associated with these lesions, 3. finding fetal bronchopneumonia associated with mycotic elements.

The abomasal contents of 1142 fetuses aborted or stillborn from various causes were cultured for fungi. Of these, 331 (28.9%) contained molds or yeasts. The fact that only 60 (12.4%) of 485 placentas examined had lesions of mycotic infection indicates that demonstrating the presence of fungi in the stomach contents does not provide adequate information upon which to base a diagnosis of mycotic placentitis.

The incidence of mycotic placentitis in 1556 cases was about 4%. The incidence of mycotic placentitis found by examining 485 placentas included in the above cases was 12.4%. This emphasizes the importance of examining this tissue when seeking the cause of bovine abortion. It also suggests that many of the cases wherein no placenta was available and no diagnosis was reached may have been due to mycotic infection. The true incidence of mycotic placentitis could be as much as three times the 4% indicated.

REFERENCES

AN APPRAISAL OF FETAL SEROLOGY FOR THE DIAGNOSIS OF BOVINE ABORTION AND CONGENITAL DEFECTS


SUMMARY

Testing fetal bovine serums for antiviral antibodies is of value in determining the etiology of congenital anomalies and abortion. Presence of specific antibodies indicates prenatal infection, but not a positive cause-effect relationship. Current knowledge does not permit a negative serologic test to be used to eliminate a virus from etiologic consideration. Caution is required in the interpretation of these tests because it must be positively ascertained by observation, history or necropsy that the calf or fetus had not ingested colostrum. This assurance is provided when the calf is lifeless or unable to stand at birth or when the sampling veterinarian is in attendance at birth because of dystocia or caesarian section or by observing absence of milk in the stomach at necropsy.

Of 77 term calves with history of colostrum deprivation, 4 had BVD-MD antibodies and none had IBR antibodies.

Of 11 aborted fetuses, 2 had BVD-MD antibodies and 3 of 12 fetuses had traces of IBR antibody.

A syndrome of congenital cataracts and cerebellar deficiency demonstrated at necropsy can be convincingly ascribed to prenatal BVD-MD infection if BVD-MD antibody is present in neonatal calf serum collected before nursing. The diagnostic value of low levels of IBR neutralizing substance in aborted fetuses requires further study.

Serological testing of bovine neonates has potential for surveys devoted to determining the etiology of various bovine congenital disorders as well as usefulness in the diagnosis of individual cases.

A finding with potential diagnostic utility has emerged from experiments 3,4, 7,8,9,10 and field observations2,3, relating bovine virus diarrhea-mucosal disease (BVD-MD) infection with abortion and congenital cerebellar hypoplasia and cataracts. Before ingestion of colostrum, some calves from susceptible dams infected by BVD-MD during pregnancy were found to possess serum antibody. This was in-

From the Department of Large Animal Medicine, Obstetrics and Surgery and the Department of Veterinary Microbiology, New York State Veterinary College, Cornell University, Ithaca, New York. This study partially supported by NIH Grants 5-S01-RR 05462-09 and R01 HD05757-01.

588
terpreted as evidence of a fetal immune response. A summarization of 3 different studies\textsuperscript{3,4,7,10} indicated BVD-MD serum antibody was present in 20 of 26 colos-trum-deprived calves from susceptible cows inoculated with BVD-MD virus during pregnancy. These data, (summarized in Table I) indicate 15 or 20 normal calves and 4 of 4 calves with cerebellar or ocular defects were immune at birth.

A study comparing the serologic status of calves born from 3 dams immune at the time of BVD-MD inoculation with calves from 4 dams susceptible when in-oculated suggested fetuses will develop BVD-MD only if the dam is susceptible (seronegative by neutralization test) and the fetus is immunocompetent at the time of exposure (see Table II).

In one BVD-MD herd epizootic\textsuperscript{2} in which 29 pregnant cattle subsequently produced 19 normal calves, 8 aborted fetuses and 2 calves with congenital cerebellar hypoplasis, presuckel serums were available from 10 normal calves, 2 aborted fetuses and 2 calves with cerebellar disorders. Results of BVD-MD neutralization tests (Table III) indicated both aborted fetuses, both calves with cerebellar anomalies and half of the normal calves were BVD-MD immune at birth.

To probe the diagnostic value of fetal serology and to gain a preliminary esti-mate of antibody prevalence in bovine neonates, serums from aborted and stillborn fetuses and calves from normal births, dystocia and caesarian sections were tested for BVD-MD and infectious bovine rhinotracheitis (IBR) antibody.

**MATERIALS AND METHODS**

SERUMS were collected by ambulatory clinicians attending dystocia and by surgeons performing caesarians. Dairymen participating in herd health programs were asked to call the investigators whenever a calf was born and to withhold colos-trum until the specimen was collected. Blood and pleural fluid were aspirated from aborted fetuses presented for diagnosis. Whenever possible, dam’s serum was collected simultaneously.

SERUM NEUTRALIZATION (SN) TESTS to detect BVD-MD and IBR anti-body in serums of neonates and their dams were conducted with secondary embryonic spleen cells in microtiter plates using equal amounts of undiluted serum and virus suspension containing approximately 100 T.C.I.D.\textsuperscript{50} of test virus. Serums from dead calves and aborted fetuses were diluted 1:5 for retesting when initial results were obscured by hemolysis. Fetal serums found possessing neutralizing activ-ity were retested simultaneously with dams serum using 2-fold serial dilutions with 2 replicates per dilution and 50% end-points approximated by inspection.

**RESULTS**

TERM CALVES:

As summarized in Table IV, BVD-MD neutralizing antibody was detected in 2 of 35 serums from calves from normal births, and 2 of 15 serums from calves born following dystocia and in none of 27 serums from calves delivered by caesarian sec-ction. Three of the 4 dams producing the positive calves had antibodies in serums collected on the day of parturition. No specimen was available from the fourth. Of 65 cows producing BVD-MD negative calves, 49 (75\%) were BVD-MD positive at
parturition. IBR antibody was not detected in any of these serums, but 42 (61%) of 69 of the dams were IBR positive.

ABORTED FETUSES:

As summarized in Table V, BVD-MD antibody was detected in serum of 2 of 11 aborted fetuses. Traces of IBR antibody (at a titer 1:2) were detected in the serum of 3 of 12 aborted fetuses and in pleural fluid obtained at necropsy from one fetus with positive serum. All these fetuses were markedly decomposed and no microbiologic procedures.

DISCUSSION

Unlike the human infant which transplacentally acquires the immune status of its mother, the bovine neonate lacks maternally bestowed antibodies until acquisition by nursing. The detection of antibody in aborted fetuses or presuckle calves could suggest any of the following:

1) transplacental antibody transfer contrary to existing dogma; 2) test error; 3) unobserved colostrum ingestion; 4) active fetal immune response to infection acquired in utero. Our experiences, as well as those of Bognar1, Kendrick4 and Kniazeff, et al.5, with BVD-MD support the interpretation that this antibody indicates prenatal infection. This interpretation was chosen by McKercher in attributing congenital bovine hydranencephaly to prenatal bluetongue infection6.

In diagnosing prenatal infection by serology on calves born alive and able to nurse, it is critical to determine that the specimen was collected prior to nursing. If the calf is first seen standing, it is useful to carefully examine the teats of the cow for evidence of nursing and if possible ascertain at necropsy that the calf had no milk in its stomach. Ascertainment of colostrum deprivation is no problem with dystocias, abortions, stillbirths and ataxic calves which are unable to rise.

The stage of gestation at the time of maternal infection seems to be one determinant of fetal capacity to respond immunologically. In BVD-MD studies2,3,4, 7,10, it appeared that calves born of dams exposed at less than 75 days of pregnancy rarely developed antibodies, while a significant portion of calves from dams exposed in the last 180 days of pregnancy developed antibodies. This finding may partially explain data suggesting most BVD-MD abortions occur in cattle infected early in gestation. Firm conclusions on the nature of the fetal response to BVD-MD require further study.

Since it has been convincingly demonstrated that BVD-MD infection of pregnant cattle can produce congenital cataracts and cerebellar hypoplasia, BVD-MD etiology should be considered when this syndrome is observed. The finding of BVD-MD antibody in presuckle serums of these patients completes the triad needed for a definitive diagnoses. The finding of BVD-MD antibody in 4 of 77 term calves and 2 of 11 aborted fetuses suggests that prenatal BVD-MD infection is not uncommon under natural conditions and examination of this attribute should be exploited in diagnostic procedures. In the absence of clinical, pathological and virological evidence, it is presumptive to state that merely finding antibodies constitutes definitive proof that BVD-MD virus produced the abortion or anomaly. Likewise, a negative serologic test by itself is insufficient evidence to rule out the virus as the
etiologic agent because exposure may have occurred early in pregnancy before the fetus was competent immunologically or the fetus may have been aborted before antibody production occurred. Thus this procedure should be used as a adjunct to existing diagnostic techniques.

Aside from cerebellar and ocular defects, other anomalies (alopecia, brachygnathism, and multiple ovine anomalies) have been reported associated with maternal BVD-MD infection. Neutralization tests on presuckle serums from a series of anomalous neonates offer a simple method of determining the extent of the association between the anomaly and BVD-MD.

The finding of IBR antibody in serums of 3 of 12 aborted fetuses suggests potential use in diagnosis. The IBR antibody levels were very low and therefore further studies are needed before the diagnostic value of such findings can be appreciated.

Despite these interpretive difficulties, serologic testing of fetuses and neonates offers a hitherto unexploited addition to the diagnostic armamentum.

ACKNOWLEDGEMENTS

The authors thank Drs. Tex Taylor, Kenneth Twisselmann and Edward Wiebe for serum collections. Dr. Talmadge Brown for necropsies, Dr. Ronald Schults for consultations and Mrs. Eleanor Tompkins for technical assistance.

TABLE I

Summary* of BVD-MD Neutralization Tests on Serums of Colostrum-Deprived Newborn Calves from Susceptible Dams Inoculated with BVD-MD Virus During Pregnancy:

<table>
<thead>
<tr>
<th>Number Tested</th>
<th>Number Positive</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Calves</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Ataxic Calves</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>(with cataracts and cerebellar lesions)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Calf with Alopecia</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Brachygnathous Calf</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>26</td>
<td>20</td>
</tr>
</tbody>
</table>

TABLE II

The Effect of Serologic Status of Pregnant Dams When Inoculated with BVD-MD Virus on the Immune Response of the Fetus*

<table>
<thead>
<tr>
<th>Pregnant Dams</th>
<th>Colostrum Deprived Neonates**</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVD-MD Seronegative</td>
<td>3/4+</td>
</tr>
<tr>
<td>BVD-MD Seropositive</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* Scott, et al.7.

** Numerator is number of calves positive to BVD-MD neutralization test; denominator is number of calves tested.

+ The 3 positive calves were from seronegative cows inoculated at 116, 146 and 150 days of pregnancy. The negative calf was from a seronegative cow inoculated at 79 days of gestation.
TABLE IV

**BVD-MD Antibody in Colostrum-Deprived Term Calves**

Ithaca, New York 1971

<table>
<thead>
<tr>
<th></th>
<th>Normal Births</th>
<th>Dystocia Births</th>
<th>Caesarian Births</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BVD-MD</strong></td>
<td>2/35</td>
<td>2/15</td>
<td>0/27</td>
<td>4/77</td>
</tr>
<tr>
<td><strong>SN Results</strong></td>
<td>5.7%</td>
<td>13.3%</td>
<td>0%</td>
<td>5.7%</td>
</tr>
</tbody>
</table>

*Numerator is number of serums positive to BVD-MD neutralization test; denominator is number of calves tested.

TABLE V

**SeroLogic Status of Twelve Aborted Fetuses**

<table>
<thead>
<tr>
<th>Neutralization Test Results</th>
<th>BVD-MD*</th>
<th>IBR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal Blood</td>
<td>2/11</td>
<td>3/12</td>
</tr>
<tr>
<td>Pleural Fluid</td>
<td>0/2</td>
<td>1/2</td>
</tr>
</tbody>
</table>

*Numerator is number of tests positive by neutralization test; denominator is the number tested.*
REFERENCES


It is unfortunate that anomalies have been looked upon as "side show freaks" in the past. It is now apparent that many of these conditions are not only economically important, but also professionally stimulating. The veterinary profession can no longer neglect this area. Finger et al. reported a frequency of 0.25% congenital defects, while Herschler et al. indicated that 36 calves in every 1000 were abnormal. These figures indicate the importance of anomalies. (4,8) Through routine necropsy procedures we have found some rather interesting and important while others are simply interesting and unique.

*Diprosopus* is an example of the so called freak type of monstrosity. This type of condition creates considerable public interest but is not economically important.

Conversely internal *hydrocephalus* is an important disease, especially in calves. Past research indicates that this condition is due to single autosomal recessive gene. (1,6) We find considerable difference in severity of this condition and have the opinion that there is more than one type of this disease. For example, we have observed various degrees of *hydrocephalus* which may or may not be associated with cerebellar hypoplasia or muscle dystrophy. The later was observed by Urman and Grace in 1964. (17) In addition, microphthalmia and variation of length of the maxillae or mandible may be observed with hydrocephalus. We have observed one case of internal hydrocephalus in pigs. *Cerebellar hypoplasia* has recently been attributed to intrauterine virus diarrhea-mucosal disease infection but hydrocephalus was not apparent. (11,18).

*Cerebellar agenesis* has occasionally been observed but the cause is not known. (3, 15) *Microencephaly* has been described but the etiology is unknown. (15) Reportedly there is concomitant small cranium. The case we observed was contained in a normal size bony compartment.

*Hypomyelinosogenesis or hereditary neuraxial edema* in Hereford calves as described by Cordy and associates in 1969 was characterized by extensor spasms. (2) They suggest that this is related to an autosomal recessive. We have observed a similar condition in calves that were born apparently normal but exhibited signs affected on the second or third day after birth. Gross appearance of the brain was normal or the gyri flattened.
Arthrogryposis is a congenital contracture of the joints. It has been observed in most breeds of cattle but is most often observed in Charolais and Herefords in the United States. Cleft palate and hypoplasia of the patella are often associated with arthrogryposis. Hydromyelia and syringomyelia of the cervical spinal cord may also be associated with arthrogryposis. This is considered to be a genetic recessive condition. Leipold et al. has reported this in calves. (14) Rarely it occurs in other species such as swine.

Osteopetrosis (metaphyseal dysplasia) has recently been recognized in Angus cattle. It is characterized by slightly premature birth (251-272 days) small size, brachygnathia inferior with impacted teeth, open fontanelle, small or absent foramina of the skull and lack of bone marrow. Microscopically there is a lack of spongiosa. This may be confused as an abortion, but examination will aid in identification and the cause of the condition is considered to be due to a recessive gene. (13) Osteopetrosis occurred in 6 calves from 27 first calf heifers bred to their father.

The cause of encephalocele in calves is not known, but is probably a genetic recessive in swine. Cyclops may be associated with hydrocephalus. White heifer disease is associated with persistent hymen. Defects may occur anterior to the hymen but in any case the ovaries are active and there is accumulation of secretory products in the uterus. (7) Little is known about the etiology, although some have proposed the possibility of sex chromosome related trait.

Production of a small calf such as the one brought to us which appeared fully mature but weighed 60 pounds, may or may not be inherited. We have seen this primarily in one herd.

Polydactyly and adactyly are observed in several different species.

Renal hypoplasia is rarely observed. (this may be dysplasia.) We have observed this in a newborn calf. The individual kidney weights were 19 and 20 grams. Normally they should weigh in the neighborhood of 60 grams.

Septal defects of the heart commonly occur in cattle. They may vary in size and location and may be associated with other malarrangements. (5) For example, we have seen the pulmonary artery and aorta exit from the heart in a common trunk to bifurcate later. Congenital alopecia may or may not be lethal. The calves that are born alive, die from exposure because of the lack of hair. There are reduced numbers of teeth associated with this condition.

Crooked head or asymmetrical cranium has been observed only in Herefords at our Laboratory. These calves may live and mature. As they mature there appears to be a certain amount of compensation. The genetic relationship is not known. Crooked calves have been described by Shupe and Coworkers. (16) They have incriminated lupine plant toxins. We have observed this in lambs but do not know the etiology. Field investigations failed to reveal the plants which have been incriminated in the past.

Syndactylism of Holstein — Friesian cattle is a recessive trait with incomplete penetrance. The right front foot is first to be affected followed by the left front, right rear and left rear in that order. (10,12) Affected cows are quite subject to stress and die rather young.

Coxa Plana in pigs was observed by us recently. The cause is not known but the incidence indicates the possibility of a single recessive trait. (9).

A few of the numerous anomalies of livestock have been discussed. There are
NEONATAL ANOMALIES IN DIAGNOSTIC LABORATORIES 597

many others, many of which there is little information available. This is partially due to our lack of interest in the past as diagnostic pathologists. A central library for accumulation of data and specimens would be advantageous, not only to the veterinarian, but also to the livestock owner. The economics of anomalies in food animals warrants more studies in this area.

REFERENCES


LABORATORY TECHNIQUES FOR DEMONSTRATING NEBRASKA CALF DIARRHEA VIRUS*

by

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Two methods have been employed to detect the Reo-like neonatal calf diarrhea virus (NCDV). The easiest method is the fluorescent antibody (FA) technique on fecal smears. The other is direct examination of feces by electron microscopy.

First we shall cover the FA procedure. Conjugate was prepared as previously described. For the FA method to be successful, calves sampled have to be selected and at least 8 to 10 samples examined per herd. Large numbers of FA positive cells are present in the feces for only a short time. During the first 4-6 hours after the onset of diarrhea, the infected cells over the outer two-thirds of the small intestinal villi are lost into the lumen. To detect these cells, specimens should be collected from animals in the early stage of illness and, if possible, from the same animal at hourly intervals for 3 or 4 times. The best samples are generally yellow and liquid. Specimens should be collected directly from the calf. The easiest way to stimulate defecation is to insert a thermometer or gloved finger into the rectum. Wide mouth baby food jars are good collecting containers. After collection, the samples should be immediately refrigerated. If more than a couple of hours will elapse between collection and examination, the specimens should be tubed and quick frozen.

Before making the smears, the samples are gently inverted to mix and then a cotton-tipped applicator is quickly immersed into the feces, so as not to get a large amount of feces on the swab, and rolled or smeared on the slide. By using only one applicator per slide, the smears are not too thick. The slides are air dried in front of a fan, fixed in cold acetone for 10 minutes, rinsed with phosphate buffered Saline (PBS), flooded with conjugate and placed in a humidity chamber at room temperature for 30 minutes. The slides are then rinsed, flooded with PBS for 3 washes for 10 minutes each, dried under a fan, and mounted in buffered glycerine just before they are read.

For a slide to be positive there must be numerous cells with bright fluorescence. An occasional greenish cell is not considered positive.

If all the samples are negative by FA and there is a particular interest in the herd, the specimens are examined by electron microscopy. Examination of feces for the Reo-like virus by electron microscopy is feasible, for at least during the acute stage of illness viral titer in the feces is about 10^8/ml. Fernelius et al, have repor-

* Presented at the meeting of the American Association of Veterinary Laboratory Diagnosticians at Oklahoma City, Oklahoma on October 26, 1971.
ted that they prefer direct EM examination of feces to the FA method for detection of the Reo-like virus. They followed several experimentally infected calves and found that while both FA positive cells and viral particles were present in the feces during the diarrheic stage, a large number of viral particles were still present in the feces several days after diarrhea had subsided while no FA positive cells were found. They also reported that these Reo-like virus particles reacted specifically with antiserum against NCDV in the immuno-electron microscopy test.

The procedures used for electron-microscopic examination of feces are as follows:

1. Fluid diarrheic feces are subjected to centrifugation in a clinical microcentrifuge at 10,000 g for 3 minutes;
2. A droplet of supernatant diarrheic fluid is placed on a parlodion coated—carbon reinforced grid, allowed to remain for 1½ minutes and then the excess is removed with a blotted.
3. The stain consists of a mixture of phosphotungstic acid, vanadium and molybdenum salts as proposed by Ball and Brakke. It is applied for 1½ minutes and the excess is again blotted off.
4. Depending on preference, the grids are either predried in a vacuum or taken immediately into the electron microscope for examination.
5. Particles of typical size and morphology have cubic symmetry, definite capsomeres and are 55-68 μ in diameter. Virus morphology may not be clear when feces are collected under optimal conditions. This may be due to a coating around the virions. Poorly preserved specimens which are not suitable for fluorescent antibody examination may give excellent results when examined by electron microscopy for the bacterial enzymatic digestion of cells apparently frees cell-bound virus and delineates viral structure.

REFERENCES

CHLAMYDIOSIS IN CALVES

by

A. K. Eugster, D.V.M., Ph.D.

Chlamydial agents were first isolated in 1951 by York and Baker from the feces of apparently normal calves in the state of New York. These agents were subsequently, also, isolated from calves in Indiana, Montana, California, Utah and Colorado. Most of the calves yielding these chlamydial agents were clinically normal. Storz first reported the isolation of chlamydia from calves with diarrhea. Some of the calves were only two days old. The incidence of these intestinal infections in calves is not known.

The study on which I would like to report was designed to assess the pathogenic events which take place in calves after oral inoculation with these chlamydial agents.

Nine Hereford calves ranging in age from 12 hours to 16 days were orally inoculated with a chlamydial agent originally isolated from the joint of a calf with polyarthritis. Some of these calves were colostrum deprived, others received a limited amount of chlamydial antibody containing colostrum.

All calves developed fever and diarrhea 1-3 days post inoculation. The feces were watery and of a yellowish color. Two calves excreted also some blood in their feces. Consistent patterns in the hemograms of the calves which would be helpful in the diagnosis could not be established. Colostrum deprived calves were in a moribund stage four to six days after oral exposure. Colostrum treated calves on the other hand revealed a less severe watery diarrhea and survived the initial intestinal infection. About one week after exposure these calves showed signs of polyarthritis.

The calves hesitated to move and tip-toed on the hind legs. The calves appeared to somewhat warm out of the stiffness after forced exercise.

The most prominent gross lesions consisted of petechial hemorrhages in the mucosa of the abomasum and all parts of intestines. The abomasum sometimes contained ulcers. In animals which survived longer the petechial hemorrhages were limited to the mucosa of the ileum and ileocecal valve.

All extremital joints contained increased amounts of turbid yellow synovial fluid with vast amounts of nonadherent fibrin plaques. Some synovial fluids contained up to 50,000 leukocytes per mm$^3$.

A characteristic lesion was the proliferation of the synovial villi.

Frequently the periarticular areas were edematous and the tendon and muscle sheets contained hemorrhages.

Chlamydial agents were traced by reisolation in chicken embryos, fluorescent antibody test and Gimenez staining of frozen tissue sections.

The direct FA test was employed. The intestinal tract in newborn calves contains a high number of eosinophils. The problem on nonspecific fluorescence was solved by absorbing the labeled antiserum with acetone dried tissue powder of the ilium of a newborn calf.
The epithelial cells on the tips of the villi in the ileum most frequently fluorced. Sometimes the intracytoplasmic inclusions had the typical helmet shape. Gimenez stained frozen intestinal sections revealed these inclusions to be accumulations of elementary bodies.

Isolation in chicken embryos seemed to be the most sensitive means of recovering the chlamydial agents from the tissues, followed closely by the FA test. The Gimenez staining method direct on tissues sections does not appear a sensitive diagnostic tool. The infection concentrates in the lower parts of the small intestine.

A flowsheet of pathogenetic events was constructed from results reported here and others which have been reported elsewhere. Chlamydial agents first multiplied in the epithelial cells of the gastrointestinal mucosa. Subsequently, fluorescing cells were also observed in the lamina propria of the ileum. Two routes of dissemination of the agents are postulated, namely the portal vascular system and the lymphatics. The agents were regularly isolated from the mesenteric lymph nodes. The agents were also frequently recovered from the liver before the chlamydemic phase, possibly pointing to an infection of the liver via the portal blood system. An interesting finding was the frequent isolation of the agents from the bile which could lead to a reinfection of the intestines. The intestinal infections never subsided after initiation of the systemic and joint infections. Chlamydial agents were always excreted in the feces, even in the presence of humoral antibodies although at lower concentrations than early after exposure.

Subsequent to the infection of the liver and mesenteric lymph nodes a chlamydemic phase was observed. This resulted in the infection of various organs, usually the lungs, liver, spleen and kidneys. The urinary tract was found to be another route of exit of these agents. A second chlamydemic phase was subsequently observed although there was most always overlapping with the first chlamydemia. However, two peaks in terms of concentration of the agent in the blood could be observed. Finally the joints became infected and the calves showed symptoms of polyarthritis 6-10 days after oral exposure.
HEALTH PROBLEMS
IN INTENSIVE VEAL CALF PRODUCTION UNITS IN NEW YORK STATE

by

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Cornell University, Ithaca, N. Y. 14850

One third of all postnatal bovine necropsies at the New York State Veterinary College Diagnostic Laboratory during the past year were calves from intensive veal calf production units. This seemed out of proportion to their numbers in the cattle and calf population and caused us to attempt to determine the significance and potential of the intensive veal calf feeding industry.

A brief review of the literature disclosed several articles and books relating diseases of young calves to animal husbandry methods. Multiple vaccinations of cows was reported by Kahrs\textsuperscript{3} to result in the birth of calves having a lowered incidence of septicemia and others\textsuperscript{2,4,5}, related resistance to septicemia to early feeding of colostrum. Serum immune globulin levels were found to be inversely proportional to the numbers of calves that died or were culled from a group of 293 veal calves\textsuperscript{2}. Sources of calves, their diets and housing were considered highly important factors relative to health and disease\textsuperscript{4,5,6}.

Information regarding numbers of veal calves being fed was obtained from personal communications\textsuperscript{7}. Those figures represent gross estimates but are related to published statistical data to indicate a potential for much greater growth of the veal feeding industry\textsuperscript{1}.

Intensive veal calf production units in New York State are estimated to be feeding veal calves at the rate of 60,000 per year. This is only about one tenth of the available dairy calves being sold off the farms and going to slaughter as lightweight "bob" vealers. With the average weight of calves slaughtered in New York being 113 pounds there is a great potential for increasing the tonnage of meat for human food without any increase in cattle numbers. Anticipation of health problems is the main factor that has kept many more thousands of those 100 pound calves from being bought by veal producers who prefer to market their higher-quality, finished calves at 240 to 300 pounds.

Although efforts are made to purchase strong calves which are then fed specially prepared commercial milk-replacer diets while they are housed in individual 2' by 5' stalls with elevated slotted floors (Illustration 1 & 2) in barns with controlled temperature and ventilation (Illustration 3 & 4), many calves are unable to survive and grow efficiently\textsuperscript{6}. Feed conversion ratios should be 1.5:1. While it is hoped that death losses will not exceed 2% of a group of calves, mortality has sometimes reached 20% or more and some entire units have been emptied by culling in an attempt to salvage some of the investment.
Sixty per cent of the veal calves examined at the NYS Diagnostic Laboratory at Cornell University during the past year have had septicemia. Most of that 60% were calves under three weeks of age but some were older than five weeks. *Escherichia coli,* usually nonhemolytic, has been isolated most often in culture but several cultures yielded no growth. In the latter cases it was suggested that extensive use of antibiotics in treatment of the calves may have interfered with bacterial growth on artificial mediums. An additional 20% of the calves had pneumonia. Culture of the lungs revealed *Pasteurella* spp., *Corynebacterium Pyogenes,* nonhemolytic *Streptococcus* spp., and *Escherichia coli.* Only two calves were diagnosed as having died of acute enteritis but the histories of most of the calves with septicemia or pneumonia implicated enteritis as an earlier disorder.

Salmonellosis was found in three separate groups of calves which were all past three weeks of age. One group was 13 weeks old and had only two deaths. In two younger groups the owners reported a total of 185 deaths, in these the cause of death was not confirmed by necropsy or culture after the initial diagnoses.

Nutritional myopathy, variously known as white muscle disease, muscular dystrophy, or vitamin E-selenium responsive disease was diagnosed in calves from two groups at 3 to 4 weeks of age, which had more than 10% deaths after having been healthy during their first 3 weeks. Inhalation pneumonia was ascribed to dystrophy of the muscles of the tongue and larynx.

Bloating occasionally affects older calves and has been reported to be occurring with increasing frequency just recently in calves fed one commercial milk-replacer. This has not been confirmed by laboratory investigation. However, one group of 110 calves had 6 calves, 10 weeks of age and weighing 200 pounds, bloat severely in one week. Two of the bloated calves died, 3 were marketed, and a sixth calf developed pneumonia following bloat but recovered. The affected calves had exhibited signs of “colds” and some scouring in addition to bloat. Antibiotics given orally and intra-muscularly were not curative. These calves were the first to occupy a newly constructed barn on the premises and had been fed the same lot number of feed which was fed to another but older group of 110 calves successfully marketed the previous week from another barn.

Urinary calculi were observed in the months of May and June, 1970, in calves from feeding operations located several miles apart but being fed the same commercial brand of milk replacer. Calves affected were the most thrifty ones in the groups and had been on feed between 5 and 8 weeks. Only a small number of calves were thought to have died but others were quickly marketed. No further occurrence of urinary calculi has been reported by those producers since the feed formulation was changed by reducing the levels of tricalcium phosphate and magnesium oxide.

Photographic illustrations are presented to demonstrate four different types of housing and feeding systems: a converted poultry house with maternal robot feeders and pen housing; a converted dairy bank-type barn with individual stalls typically used; and two newly constructed barns with different ventilation systems.

**SUMMARY**

Health problems remain the major obstacle to expansion of the intensive veal calf production units in New York State. In units with controlled environment and
improved milk replacer feeds there is continued extensive use of antibiotics, which often fail to accomplish the desired therapeutic effects.

The most frequently diagnosed cause of death in calves less than three weeks of age are: septicemia (usually only Escherichia coli is isolated in culture), enteritis, and pneumonia. In older calves the diagnoses have included salmonellosis, urolithiasis, and degenerative myophy (white muscle disease or vitamin E-selenium responsive disease). Clarification of the significance of viral infections requires further investigation.

BIBLIOGRAPHY


1. Individual stall with slotted, removable, elevated floor constructed of wood. Neck chain and feed bucket position at the front of the stall.

2. Ample room is provided for two-week-old calf in stall with slightly less than 2' by 5' inside floor space.
3. Interior of a well-ventilated barn with 700 calf capacity, 350 calves in each half of barn.

4. Calves in barn in illustration No. 3 relax after feeding. Space in stalls is barely adequate for these 13 week-old calves weighing 300 pounds.
A SURVEY OF FEE SYSTEMS IN VETERINARY MEDICAL DIAGNOSTIC LABORATORIES

by

L. G. Morehouse, D.V.M.,* K. K. Keahey, D.V.M.,**
and V. A. Seaton, D.V.M.***

INTRODUCTION

During the past several years there has been increased interest in the subject of fees for services of veterinary medical diagnostic laboratories. Many of these laboratories are tax supported either through state departments of agriculture or through universities. The latter often have an additional responsibility of teaching. Traditionally, many of these laboratories have given services on a non-fee basis. The reason for this are varied and complex, but comments from a number of diagnostic laboratory administrators indicate services have been offered on a non-fee basis in order that these laboratories can more effectively serve the mission of assessing and monitoring disease patterns in livestock and poultry in a state as well as rendering a meaningful service to these industries and to the public health in specific disease outbreaks. They have emphasized that diagnostic laboratories are a primary source of information on disease incidence and that charges for services in these laboratories discourage submission of specimens vital to this concern. It also has been pointed out in many instances that funds have been made available to some laboratories for diagnosis of disease associated with specific disease eradication programs and that charges for these services are therefore unjustified. Administrators of laboratories associated with universities have emphasized that, in education and research, the value of specimens far outweighs the cost of performing the service.

In the past decade, however, the soaring costs of equipment and supplies, as well as the increased demands for more sophisticated diagnostic techniques has placed many veterinary medical diagnostic laboratories in a critical financial position. Management practices in the livestock and poultry industry have changes dramatically in recent years. The increased concentration of livestock and poultry that has accompanied accelerated production methods has emphasized that rapid, accurate diagnosis is a must if devastating disease losses are to be prevented. This also has placed increasing pressures on diagnostic laboratories for highly skilled professional

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608
and technical personnel to perform these services.

During the past two years, officers of the American Association of Veterinary Laboratory Diagnosticians (AAVLD) have received an increasing number of questions from laboratory directors faced with the problem of how to continue operation of their laboratories in the face of increased service demands and either static or decreased budgets. Many were contemplating fees for their work and had questions concerning the position taken by other laboratories.

The authors of this paper were requested in July of 1971 by A.A.V.L.D. officers to gather information on the subject of fees in diagnostic laboratories and to report whatever information could be gathered to the fall meeting of the association.

METHODS

A questionnaire was prepared and mailed to 130 laboratories in 46 states. The questionnaire was designed to answer the following questions:

1. The number of veterinary medical diagnostic laboratories in various states that are charging for their services.
2. The number of laboratories that are anticipating a fee for their services.
3. The date of initiation of fees.
4. The major source of support for laboratories responding to the questionnaire.
5. The reason for initiating a fee system.
6. The level of fees assessed for various laboratory procedures.
7. Gross and net income realized from fees.
8. General comments concerning fee systems in veterinary medical diagnostic laboratories.

RESULTS

Questionnaires were received from 114 laboratories in 45 states. Table 1 outlines this information by states. It is shown in this table that 42 laboratories in 21 states were charging for their services whereas 72 laboratories in 35 states were operating on a non-fee basis.

Of thirty laboratories that listed the date of initiation of their fee systems, ten were prior to 1965, three were between 1965 and 1969 and seventeen had initiated fees between 1969 and 1971. Although it could not always be clearly established from the questionnaires whether or not a fee was charged for all services, it was apparent that at least ten of the forty-three laboratories with a fee system were charging only for selected services.

Table 2 lists information relative to fees in diagnostic laboratories according to their source of major support. It should be emphasized this information is of a general nature, since there are a variety of mechanisms for support of these laboratories and in some instances there are joint agreements between various agencies and institutions for their support.
A number of comments were received relative to why charges were initiated. The overriding reason cited by most laboratories was to help defray operational costs. Secondary to this objective was a desire to encourage greater selectivity of specimens and to assure state and university administrators that some attempt was being made to recover a part of the laboratory's cost of operation. Several laboratories whose support came through livestock sanitary boards or state departments of agriculture emphasized their charges were for pet animals only. Other laboratories pointed to a selectivity in charging for out of state specimens. Some public health laboratories indicated they were tax supported for their specific mission of diagnosis of animal disease in the public health interest and therefore did not levy a charge to the public for service. The reason for fees in non-tax supported profit oriented diagnostic laboratories is apparent, however, some diagnostic laboratories have been operated on a non-fee basis in the past by commercial companies manufacturing veterinary biologics and drugs.

Twenty laboratories responding to the questionnaire enclosed copies of their fee schedules. These varied in detail from a one paragraph statement announcing one standard fee for all entries submitted to the laboratory to a 6 page fee schedule with fees quoted for 150 different categories of laboratory service.

Although space prohibits a detailed listing of fees for the various laboratories and the circumstances under which they are charged, tables 3 through 8 summarize some of the information obtained from the various schedules that were submitted; the figures quoted in these tables are thought to be quite representative. Many of the individual laboratory charges carry minimum and maximum figures per consignment or per necropsy, i.e., all supportive laboratory charges on a given necropsy will not exceed $15.00 or, in the case of poultry, a minimum charge of $5.00 to $7.50 may be quoted.

In regard to gross and net income realized from their fees, 19 laboratories listed estimated gross income varying from under $5,000 to $90,000 per annum. Only 3 laboratories made an attempt to list a net income. This information is listed in table 9. Most laboratories emphasized the difficulty in arriving at a net income figure since in many instances all fee collections go to a general fund with appropriations later returned to the laboratory.

DISCUSSION

The return of 90% of the questionnaires submitted to laboratories across the country concerning fees charged in veterinary medical diagnostic laboratories reflects a high degree of interest in the subject. It is also interesting to note that of 30 laboratories listing the date of initiation of their fee systems, 20 had been initiated since 1965 and 17 of these 20 had been initiated in the past two years.

The principal reason for charging appeared to be an alleviation of operating costs. This was true regardless of whether laboratories received their major financial support through state departments of agriculture or through universities. The amount of fee charged for a wide variety of services was quite uniform in most of the
laboratories and would seem to represent a small fraction of the actual costs except in those instances where large numbers of routine tests are carried out.

A number of comments were received from individuals regarding the advantages gained versus the problems encountered in charging for services in veterinary medical diagnostic laboratories. A discussion of these is beyond the scope of a survey of this type since a wide variety of administrative structures and different laboratory goals were represented in the 114 laboratories responding to this questionnaire. It does seem apparent that careful consideration should be given to the primary mission of the laboratory in the initiation of fees and in the determination of the amount to be charged for the service.

SUMMARY

1. A survey of 114 veterinary medical diagnostic laboratories in 45 states revealed that 43 laboratories in 21 states had fees for their services.
2. Fifty-six percent of the laboratories reporting the date of initiation of fees had implemented charges in the past 2 years.
3. Five additional laboratories in 4 different states anticipate some type of fee structure in the coming two years.
4. The primary reason cited for adopting a fee system was for alleviation of operating costs.
5. Fee schedules were received from 20 laboratories; a high degree of uniformity in the amounts charged for specific services was noted.
### Table 1. GEOGRAPHICAL DISTRIBUTION OF LABORATORIES

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<th>No Fee</th>
<th>State</th>
<th>Number of Laboratories</th>
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### Table 2. SOURCE OF MAJOR SUPPORT TO LABORATORIES

AS RELATED TO FEES

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<th>State Departments</th>
<th>Universities</th>
<th>Public Health</th>
<th>Commercial</th>
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</tr>
<tr>
<td>19</td>
<td>41</td>
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<td>14</td>
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<tr>
<td>(31%)</td>
<td>(59%)</td>
<td>(44%)</td>
<td>(56%)</td>
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Table 3. CHARGES FOR NECROPSY AND HISTOPATHOLOGY

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<tr>
<th>Laboratory</th>
<th>Necropsy</th>
<th>Histopathology</th>
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<tbody>
<tr>
<td>1</td>
<td>$5.00 Per Animal</td>
<td>$4.00 Per Slide</td>
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<tr>
<td>2</td>
<td>$10.00 (Tumors)</td>
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<tr>
<td>3</td>
<td>$5.00 Per Animal (Charges doubled for out of state submissions.)</td>
<td>$7.50 - 15.00</td>
</tr>
<tr>
<td>4</td>
<td>$1.00 Minimum Small Animals $5.00 Minimum Large Animals</td>
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<tr>
<td>5</td>
<td>$7.50 Non-farm Animals</td>
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<td>6</td>
<td>$2.00 Small Animals and Poultry, per Consignment</td>
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<td>7</td>
<td>$3.00 Per Consignment for Poultry</td>
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<td>8</td>
<td>$10.00 Horse and Cow $5.00 Pig, Dog, Cat $5.00 Lab. Animals and Poultry</td>
<td>$5.00 - 10.00</td>
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<td>9</td>
<td>$5.00 - 10.00 Horses and Catt. (By $2.00 - 10.00 Weight) $2.00 - 4.00 Sheep and Pigs (By Weight) $5.00 All Pets $5.00 Avian (Per Consignment)</td>
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<td>10</td>
<td>$5.00 Under 300 lbs. $8.00 Over 300 lbs.</td>
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Table 4. CHARGES FOR BACTERIOLOGY

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<tr>
<td>7</td>
<td>$4.00</td>
<td>$2.00</td>
</tr>
<tr>
<td>8</td>
<td>$1.00 (Additional $2.00 for out of state)</td>
<td>$12.00 (Includes Culture)</td>
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<td>12</td>
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Table 5. CHARGES FOR VIROLOGY

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Virus Isolation and Identification</th>
<th>Serology (IBR, BVD, PI-3)</th>
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<tr>
<td>1</td>
<td>$10.00</td>
<td>$5.00 Per Test of Paired Sample</td>
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<tr>
<td>5</td>
<td>$2.00 - 10.00 (Maximum $15.00 Per Necropsy)</td>
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Table 5. CHARGES FOR CLINICAL PATHOLOGY

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### Table 7. CHARGES FOR SEPULOGY

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<th>Pullourn Tests</th>
<th>M. Gallisepticum Tests</th>
<th>HI Tests</th>
<th>Plate Agglutination</th>
<th>Tube Agglutination</th>
<th>Leptospirosis</th>
<th>Anaplasmosis</th>
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<tbody>
<tr>
<td>1</td>
<td>0.04 each (11.00 each)</td>
<td>5.05 each</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5 (- each)</td>
<td>5.05 each</td>
<td>$1.00 per tube</td>
<td>0.50 - 1.00</td>
<td>2.00</td>
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</tr>
<tr>
<td>3</td>
<td>6.12 each (6.00 Min)</td>
<td>3.10</td>
<td>0.20</td>
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<td>2.00</td>
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</tr>
<tr>
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<td>6.06 each</td>
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<tr>
<td>6</td>
<td>6.08 - 8.08</td>
<td>8.10 - 8.20</td>
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<td>1.00 - 2.00</td>
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<td>9</td>
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<tr>
<td>11</td>
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### Table 8. CHARGES FOR CLINICAL CHEMISTRY

<table>
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<tr>
<th>LAB</th>
<th>Ca and P</th>
<th>BUN</th>
<th>SGPT</th>
<th>SGOT</th>
<th>Phosphatase</th>
<th>BSP</th>
<th>LDH</th>
<th>Na and K</th>
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<tbody>
<tr>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>$3.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>$4.00</td>
<td>$3.00</td>
<td>$3.00</td>
<td>$3.00</td>
<td>$3.00</td>
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<td>$4.00</td>
<td>$4.00</td>
<td>$5.00</td>
<td>$4.00</td>
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*Three Other Laboratory Schedules Identical*
### Table 9. RANGES OF GROSS AND NET ANNUAL INCOME REPORTED

<table>
<thead>
<tr>
<th>GROSS</th>
<th>NET</th>
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<tbody>
<tr>
<td>$0-5,000</td>
<td>$0-5,000</td>
</tr>
<tr>
<td>$5,000-10,000</td>
<td>$5,000-10,000</td>
</tr>
<tr>
<td>$10,000-15,000</td>
<td>$10,000-15,000</td>
</tr>
<tr>
<td>$20,000-30,000</td>
<td>$20,000-30,000</td>
</tr>
<tr>
<td>$30,000-40,000</td>
<td></td>
</tr>
<tr>
<td>$40,000-50,000</td>
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</tr>
<tr>
<td>$90,000</td>
<td></td>
</tr>
</tbody>
</table>

Total Labs Reported: 19

3
PROTOCOL FOR THE COMPLEMENT-FIXATION TEST  
FOR EQUINE PIROPLASMSIS  

Equine Piroplasmosis Committee  
A. Holbrook, Chairman; D. E. Cooperrider, T. O. Roby, W. W. Kirkham,  
W. H. Martin.  

II. Equipment Required for Test  
The equipment required for the test consists of items usually present in all  
routine diagnostic laboratories. The following list includes all the equipment used in  
this laboratory for performing the test:  
Test tube racks, metal, 6 holes x 15 holes  
Test tube racks, metal, 2 holes x 10 holes  
Disposable serological test tubes, 12 x 75 mm  
Disposable serological test tubes, 13 x 100 mm  
Conical graduated centrifuge tubes, 50 ml  
Serological pipettes, 0.2 ml, 1.0 ml, 10.0 ml  
Cornwall automatic pipettes, 2.0 ml, 5.0 ml  
37°C waterbath  
56°C waterbath  
Centrifuges, IEC No. 1 or IEC No. 2  

III. Standard Serums  
Standard serums will consist of (1) high titered serums collected during the  
acute phases of infection with *B. caballi* or *B. equi*, (2) low titered serums collected  
from horses in the carrier state of *B. caballi* or *B. equi* infections, and (3) polled  
negative serums. These serums will be used during perliminary testing to establish  
the validity of testing techniques in individual laboratories. Determination of the  
antigenic unit is dependent upon the use of standard antiserums and to insure  
comparable results in different laboratories, the standard serums should always be  
tested at the same time that serums from the field are tested. If the results with the  
standard serums are reprodicible from test to test, greater reliance can be placed  
upon the results obtained with unknown serums.  

After preliminary testing has established the accuracy and reliability of results  
obtained in the individual laboratories, a set of coded test serums will be furnished  
upon request. These serums are to be tested and all reactor serums titrated  
according to the standard protocol for serum titrations. The results of the tests on  
the individual serums, as well as the titers obtained with the reactor serums, are to  
be forwarded to the Animal Health Diagnostic Laboratories for Veterinary Services.  
The unit values (or the results of parallel tests on known standard serums) for all  
test components should be included with the test results.  

IV. Complement Standard  
Commercially available, lyophilized complement may be used to establish  
values for hemolysin and antigen units. Parallel tests should be performed on the  
standard serums using complement produced in the individual laboratories and the
standard complement. In order for complement to be acceptable for equine piroplasmosis testing procedures, it must be comparable in sensitivity to that of standard complement as shown by identical reactions using the same serums. (For details concerning production of pooled complement, see VI-1-c, below.)

V. General Considerations
The total volume employed in this test is 1.25 ml, i.e., 0.25 ml each of suitable diluted serum, antigen, complement, hemolysin, and red blood cell suspension.

VI. The Standardized Complement-Fixation Test for Equine Piroplasmosis
A. General Information
1. The Antigen-Antibody-Complement System
   a. The antigen consists of the particulate material from lysed equine erythrocytes collected from acute experimental cases of equine piroplasmosis (pure B. caballi or B. equi infections). Lysed erythrocytes from a non-infected horse (normal antigen) should be included in the test to check for non-specific reactions. The antigens are diluted with veronal buffered saline solution (pH 7.3 - 7.4), and employed in the test at 2 antigenic units based on titrations with standard positive serums.
   b. Clear, non-hemolyzed serum of an animal, is tested for the presence of antibody to B. caballi or B. equi. Inactivated serum (56 C. for 30 minutes for horse serums, 59 C. for 30 minutes for donkeys and other Equidae) is diluted 1:5 in veronal buffered saline solution for the test. Blood samples should be collected under aseptic conditions and all precautions taken to avoid hemolysis.
   c. Normal guinea pig serum is used as complement. It is diluted in veronal buffered saline solution, so that each 0.25 ml contains 2 exact units. A pool of complement for use in a laboratory can be produced in the following manner. A minimum of 15 mature healthy guinea pigs fasted for at least 18 hours prior to bleeding are used. The guinea pigs are exsanguinated by severing the neck vessels, and the blood is collected in centrifuge tubes in which clotting is allowed to occur. The clot is loosened from the inside of the tube by "rimming" it with an applicator stick and spun down in a centrifuge (900 g). The clear, non-hemolyzed serum (complement) is poured off, sealed in ampules or vials, and stored at low temperatures (-50 to -70 C.) or lyophilized.

2. The Hemolytic System
   The hemolytic consists of equal volumes of 3% washed sheep red blood cells and hemolytic amboceptor (anti-sheep hemolysin) containing 2 hemolytic units per 0.25 ml. The amboceptor and sheep cells are mixed and held at 37 C for 10 minutes before use. In the presence of complement, i.e., if no antibody is present to complete the antigen-antibody-complement system described in A above, lysis of the erythrocytes occurs.

3. Since the total volume of reagents in this test is 1.25 ml, disposable test tubes or Pyrex test tubes 12 x 75 mm may be used in this procedure. A
standard serological tube rack (6 holes x 15 holes) is recommended, especially where titrations of reactor serums are involved.

B. Detailed Outline of the Procedure for the Complement-Fixation Test

1. Reagents
   a. Veronal buffer stock solution
      Preparation
      NaCl (C.P.) ........................................... 85.0 g.
      5, 5' diethylbarbituric acid ....................... 7.75 g.
      Sodium 5, 5' diethylbarbiturate .................. 3.75 g.
      Distilled water-q.s. ad. .......................... 2990 ml
      Dilute one part of the above stock solution with four parts distilled water on the day used. Final pH 7.3-7.4.
      This buffer serves as a diluent throughout the test — for serum, antigen, complement, amboceptor, and sheep cells.
   b. Alsever's Solution
      Preparation
      Sodium citrate ........................................ 12.0 g.
      Sodium chloride ..................................... 4.2 g.
      Dextrose .............................................. 20.5 g.
      Distilled water ...................................... 1000 ml
      (1) The sodium citrate and sodium chloride are dissolved in 800 ml. distilled water and autoclaved at 15 lbs. pressure for 15 minutes. The dextrose is dissolved in 200 ml. distilled water, and autoclaved separately, or sterilized by membrane filtration. The sterile dextrose solution is added aseptically to the cooled sterile saline-citrate solution.
      (2) Alsever's solution is dispensed aseptically into sterile flasks in suitable volumes so that the sheep erythrocytes collected in it can be used within a 2-week period after collection.
   c. Sheep Red Blood Cell Suspension - 3%
      Collection of Sheep Blood
      (1) A normal adult sheep is bled from the jugular vein into a sterile vessel containing Alsever's solution. The volume of blood collected is equal to the volume of Alsever's solution in the container. Bleeding should be performed using aseptic technique throughout.
      (2) The blood is thoroughly mixed with the Alsever's solution by gentle agitation of the flask, and the cell suspension is stored in the refrigerator at 4 C. This stock suspension of sheep red blood cells will keep satisfactorily for at least 2 weeks.
      Saline Suspension of Sheep Red Blood Cells
      (1) When a complement-fixation test is performed, the stock suspension of sheep red blood cells is mixed thoroughly by gentle rotation of the flask.
      (2) Sufficient cell suspension to meet the requirements of the test is poured into a conical graduated centrifuge tube and centrifuged for 10 minutes at 900-1000 g.
(3) The supernatant fluid is aspirated by suction through a capillary pipette; the buffy coat and upper layer of red blood cells are removed also. The centrifuge tube is refilled with veronal buffer (1:5 dilution of the stock buffer) and the cells resuspended by stirring.

(4) Centrifuge again at 900-1000 g for 10 minutes.

(5) Aspirate the supernatant fluid and upper layer of red blood cells and resuspend the cells in veronal buffer.

(6) Repeat steps (4) and (5) twice so that the cells have been washed 3 times in veronal buffered saline solution. If the supernatant fluid is not colorless after the last washing, the cells are too fragile for use in the complement-fixation test, and fresh sheep blood should be collected.

(7) After the final washing, resuspend the packed cells in sufficient veronal buffer to make a 3% suspension for use in the test.

d. **Amboceptor**

(1) Commercially available anti-sheep hemolysin approved by the Animal Health Diagnostic Laboratories, Animal & Plant Health Service, will be used.

(2) 2 units of hemolysin are used in the test. The hemolysin is diluted with veronal buffered saline so that this amount is contained in 0.25 ml. See Section C-2 for procedure of hemolysin titration.

e. **Antigen**

(1) Antigen for this test will be supplied by Animal Health Diagnostic Laboratories for Veterinary Services.

(2) The antigen is lyophilized and may be stored at 4 C. without loss of potency.

(3) The antigen is reconstituted to its original volume with veronal buffer, and diluted further so that 0.25 ml contains 2 antigenic units.

C. **Standardization of Components of the Complement-Fixation Test**

1. **Complement titration**

a. A 5% solution of complement is prepared by adding 0.5 ml of complement to exactly 9.5 ml of veronal buffer. The stock complement used for making this dilution may be stored at 4 C. until titration is complete, and then diluted for use in the test.

b. **Procedure**

The protocol in Table should be followed. When all components have been added to all tubes, the rack is placed in a 37 C. waterbath for 20 minutes. The test is read immediately at the end of this incubation period. The exact unit is the smallest amount of diluted complement which gives complete, sparkling hemolysis in 20 minutes at 37 C. Complement is diluted with veronal buffer so that each 0.25 ml contains two exact units. For example, if the first tube with sparkling hemolysis is 2 percent
complement, to obtain 4 percent complement, 4 ml of undiluted complement would be added to 96 ml of veronal buffer to have 2 units.

Table 1

<table>
<thead>
<tr>
<th>Complement Percentage (5% stock)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Complement</td>
</tr>
<tr>
<td>Complement 5%</td>
</tr>
<tr>
<td>Salline</td>
</tr>
<tr>
<td>Salline</td>
</tr>
<tr>
<td>Н. S.</td>
</tr>
</tbody>
</table>

Place tubes in incubator 37°C 20 min.
Lowest percent showing complete sparkling is considered 1 unit.
For piroplasmosis use 2 units.

2. *Amboceptor (Antisheep hemolysin) titration*
   a. 1:100 and 1:150 dilutions of amboceptor are prepared by (1) adding 0.1 ml of antisheep hemolysin to 9.9 ml of veronal buffer (1:100) and (2) adding 0.1 ml of the 1:00 dilution to 14.9 ml of veronal buffer (1:150).
   b. Complement is diluted so that 2.0 exact units are contained in 0.25 ml (See calculation above under C-1-b).
   c. Table 2 contains the protocol for titrating the amboceptor (anti-sheep hemolysin).

If the last tube showing complete sparkling hemolysis at the end of a 20-minute incubation period is Tube No. 10 (1:4800 dilution, Table 2), this dilution is considered to be the exact unit. Since the desired strength is 2 exact units, the required hemolysin dilution would be Tube No. 8, 1:2400. In this case, 1.7 ml of stock hemolysin would be diluted to 100 ml with veronal buffer.
Table 2
AMBOCEPTR TITRATION

Row 1

<table>
<thead>
<tr>
<th></th>
<th>1:100</th>
<th>1:200</th>
<th>1:400</th>
<th>1:800</th>
<th>1:1600</th>
<th>1:3200</th>
<th>1:6400</th>
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</thead>
<tbody>
<tr>
<td>Saline</td>
<td>9.9 cc</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Undil. Ambo.</td>
<td>.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambo. dil. carried over to next tube</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1 discard</td>
</tr>
<tr>
<td>5% Complement</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Saline</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sheep cells without amboceptor</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
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<td>5</td>
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</table>

Row 2

<table>
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<tr>
<th></th>
<th>1:150</th>
<th>1:300</th>
<th>1:600</th>
<th>1:1200</th>
<th>1:2400</th>
<th>1:4800</th>
<th>1:9600</th>
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</thead>
<tbody>
<tr>
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<td>14.9 cc</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Undil. Ambo.</td>
<td>.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambo. dil. carried over to next tube</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1 discard</td>
</tr>
<tr>
<td>5% Complement</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Saline</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sheep cells without amboceptor</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
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<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

After the 1:100 and 1:150 stock amboceptor dilutions are made 1 cc is carried over to the lowest dilution in its row and two-fold dilutions are made discarding 1 cc. at the end of the row. 1 cc. of 5% complement (excess complement) is added to each tube followed by saline and standardized sheep cells.

These are thoroughly mixed by shaking, incubated in the water bath 20 minutes at 37°C., then read immediately.

3. Antigen Titration

a. The reagents necessary for determining the antigenic unit are:
   (1) The antigen to be tested, diluted 1:4 with veronal buffer.
   (2) Complement diluted with veronal buffer to contain 2 exact units per 0.25 ml.
   (3) Hemolysin diluted with veronal buffer to contain 2 exact units per 0.25 ml.
   (4) Sheep red blood cells diluted with veronal buffer to make a 3% suspension.
   (5) Inactivated standard positive serums (high-titered and low-titered B. caballi antiserums and high-titered and low-titered B. equi antiserums) and standard negative serums.

The antigenic unit is defined as that amount of antigen which gives 50% fixation (a 2+ reaction) in the presence of 2 exact units of complement and a 1:5 dilution of standard low-titered antiserum. Two units ordinarily have no anti-complimentary effect under the test conditions employed.
b. **Procedure**

The following protocol is used for antigen titration (See Table 3). The amounts of veronal buffer listed in the table are added and then doubling dilutions of the antigen, beginning with 1:4 are made. Inactivated standard serums (1:5 dilution) are added to each series of tubes. Suitable diluted complement is added last. The rack is shaken thoroughly to effect complete mixing and placed in the 37 C. waterbath for 1 hour.

(B. caballi antigens are titrated against high-titered B. caballi antiserum, low-titered B. caballi antiserum, negative serum, and a high-titered B. equi antiserum.  

B. equi antigens are titered against high-titered B. equi antiserum, low-titered B. equi antiserum, negative serum, and a high-titered B. caballi antiserum.)

During this incubation period, the 3% sheep red blood cell suspension is mixed with an equal volume of hemolysin containing 2 hemolytic unit per 0.25 ml. Sensitization occurs during a 10-minute incubation period at 37 C. At the end of the first hour of incubation, 0.5 ml of the sensitized red blood cell suspension is added to each tube. The cells are dispersed by thorough shaking of the test tube rack, and the rack is returned to the 37 C. waterbath for a 30 minute incubation period. At the end of this period, the test is taken out of the bath, the individual tubes are centrifuged at 900 g so that any unlysed red blood cells form a compact button, and the test is read immediately. Alternatively, the racks may be placed in a refrigerator overnight and the test read the following morning.

### Table 3

<table>
<thead>
<tr>
<th>Antigen Titration</th>
<th>Sera Controls</th>
</tr>
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<tbody>
<tr>
<td><strong>Antigen</strong></td>
<td><strong>Controls</strong></td>
</tr>
<tr>
<td>U/ml.</td>
<td>1/2</td>
</tr>
<tr>
<td>Serum (Pos.)</td>
<td>.25</td>
</tr>
<tr>
<td>Compl. (2 units)</td>
<td>.25</td>
</tr>
<tr>
<td>H.S. Sensitive</td>
<td>.5</td>
</tr>
<tr>
<td>cells</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>Serum (one set for each serum)</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>.25</td>
</tr>
<tr>
<td>Compl.</td>
<td>.25</td>
</tr>
<tr>
<td>H.S.</td>
<td>.5</td>
</tr>
<tr>
<td>H.S.</td>
<td>.5</td>
</tr>
<tr>
<td>Hemolysin System</td>
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<tr>
<td>Saline</td>
<td>.75</td>
</tr>
<tr>
<td>H.S.</td>
<td>.3</td>
</tr>
</tbody>
</table>

Shake and incubate 60 min. at 37°C.

*Shake and incubate 30 min. at 37°C.

Antigen and saline solutions are mixed and carried over in tubes 2 through 8 only, the 0.25 surplus from tube 8 being discarded; a known specific inactivated hyperimmune horse serum of 1:5 dilution is used.

Tube 7 has 50% hemolysin in low-titered control serum which is a dilution of 1:64. Two antigen units would be 1:32.
c. Interpretation of titration

Table 4

Hypothetical Results of an Antigen Titration

<table>
<thead>
<tr>
<th>Serums</th>
<th>Tubes</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
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<tr>
<td></td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>32</td>
<td>64</td>
<td>128</td>
<td>256</td>
<td>512</td>
<td>SC₁</td>
<td>SC₂</td>
<td>AC₁</td>
</tr>
<tr>
<td>B. caballi HT</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>3+</td>
<td>-</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>B. caballi LT</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>2+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4+</td>
</tr>
<tr>
<td>Negative</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. equi HT</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4+</td>
<td></td>
</tr>
</tbody>
</table>

SC₁, SC₂ = Serum Controls
AC₁, AC₂ = Antigen Controls

D. The Test Proper

1. After all components have been properly standardized, the test itself can be performed. The components and their relative values are:
   (a) Antigen diluted in veronal buffer. The test dose is 2 exact units contained in 0.25 ml.
   (b) Complement diluted to contain 2 exact units in 0.25 ml.
   (c) Hemolysin diluted in veronal buffer to contain 2 exact units per 0.25 ml.
   (d) Washed sheep erythrocytes diluted with veronal buffer to a concentration of 3%.
   (e) Standard positive and negative serums for use as controls.

2. Procedure

A screening test is performed on each serum. The initial dilution of each serum is made by pipetting 0.5 ml of serum into 2.0 ml of veronal buffer. These diluted serums are inactivated for 30 minutes at 58 to 59 C. (depending on the species being tested) and then 0.25 ml is placed in each of 5 test tubes. Antigens (0.25 ml volumes os suitable dilutions) including B. caballi antigen, B. equi antigen, and lysed erythrocytes from a non-infected horse ("normal" antigen) are added to the first 3 tubes. The last 2 tubes are serum controls to test for possible anti-complimentary activity and are set up for each serum (See Table 3, SC1 and SC2). Complement (0.25 ml containing 2 exact units) is added to the four tubes for each serum, and the contents of the tubes mixed thoroughly by shaking. The rack containing the tubes is incubated at 37 C. for 1 hour. At the end of that time, 0.5 ml of the sensitized RBC suspension (3% sheep red blood cells and suitably diluted heomlysin) is added to each tube, and
the contents of the tubes mixed again by thorough shaking. The rack is returned to the 37 C. waterbath for a 30-minute period and the test is read immediately. Centrifugation at 900 g. for 2-3 minutes facilitates the interpretation of the test results. It is also possible to place the entire rack of tubes in the refrigerator overnight, and read the results the next morning.

Table 5

<table>
<thead>
<tr>
<th>Tube 1</th>
<th>Tube 2</th>
<th>Tube 3</th>
<th>Tube 4</th>
<th>Tube 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer - ml</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>.25</td>
</tr>
<tr>
<td>Test serum (1:5) - ml</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
</tr>
<tr>
<td>Antigen-1 unit/0.25 ml</td>
<td>.25 of B. caballi</td>
<td>.25 of B. equi</td>
<td>.25 of &quot;normal&quot;</td>
<td></td>
</tr>
<tr>
<td>Complement-2 units/0.25 ml</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
</tr>
</tbody>
</table>

Incubate for 1 hour at 37 C. in a waterbath

Sensitized RBC suspension - ml | .5 | .5 | .5 | .5 | .5 |

Incubate for 30 minutes at 37 C. in a waterbath

Tubes 4 and 5 serve as serum controls.

3. Interpretation of the Screening Test

Tube 4 should show complete hemolysis and Tube 5 should show no hemolysis and Tube 5 should show no hemolysis at all. If Tube 4 contains non-hemolyzed cells, the serum should be reported as anti-complimentary, at a dilution of 1:5, but the serum should be titrated anyway. Often, this activity disappears rapidly upon further dilution of the serum while complement-fixing ability specific for the parasites will remain at higher dilutions. Tube 3 also should show complete hemolysis, i.e., there should be no reaction to ordinary components of equine erythrocytes. Should there be any reaction in this tube, the comments concerning Tube 4 would be applicable. Depending on the status of the animal from which the test serum was derived, Tubes 1 and 2 may show reactions from complete hemolysis (negative) to no hemolysis (positive). Besides these extremes, intermediate reactions are recognized. The reactions are interpreted as follows:

- 4+ (no hemolysis) Positive
- 3+ (25% hemolysis) Positive
2+ (50% hemolysis)  Positive
1+ (75% hemolysis)  Suspicious
Trace (only a few cells  Negative
unhemolyzed)  
Complete hemolysis  Negative

Table 6

<table>
<thead>
<tr>
<th>Sera TITRATION</th>
<th>Controls</th>
<th>SC</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:5</td>
<td>1:10</td>
<td>1:20</td>
<td>1:40</td>
</tr>
<tr>
<td>Serum (1:5 dil.)</td>
<td>.25</td>
<td>.25 serum</td>
<td>.25 saline .25 saline .25 saline .25 saline .25 saline .25 saline .25 saline .25 saline .25 saline</td>
</tr>
<tr>
<td>Antigen (dil.)</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
</tr>
<tr>
<td>Controls</td>
<td>Antigen (one set for each antigen)</td>
<td>Compl.</td>
<td>Complement</td>
</tr>
<tr>
<td>Antigen</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
</tr>
<tr>
<td>Saline</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
</tr>
<tr>
<td>Compl.</td>
<td>.25</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>H. S.</td>
<td>.5</td>
<td>.5</td>
<td>.5</td>
</tr>
<tr>
<td>*</td>
<td>**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hemolytic System

Saline | .25 | .25 | .25 | .25 | .25 | .25 | .25 | .25 | .25 | .25 | .25 | .25 | .25 | .25 | None |
| H. S. | .5 | .5 | .5 | .5 | .5 | .5 | .5 | .5 | .5 | .5 | .5 | .5 | .5 | .5 |

*Shake and incubate 60 min. at 37°C.
**Shake and incubate 30 min. at 37°C.

The diluted inactivated sera are carried over in tubes 2 through 8 only, the 0.25 or surplus from tube 8 being discarded.

D. **Titration of Positive Serums**

The titer of a positive serum is strongly indicative of the stage of the infection whether the serum is from acutely infected animals or from carrier animals. The antibody level is determined by making doubling dilutions, beginning at 1:5, in veronal buffer. Ordinarily, a final dilution of 1:640 will give enough of a range to distinguish between animals in the late carrier state (1:20 or less) and those in the acute stages (1:160 or above).

Read the results of the test immediately after centrifuging the tubes at 900 g. for 2-3 minutes or after allowing non-hemolyzed cells to settle out in a refrigerator overnight.

The serum titer is defined as the highest dilution showing a 50% fixation of complement (2+ reaction). If Tubes 1 through 4 have 4+ reactions and Tube 5 has a 2+ reaction, the titer of the serum is 1:80. If the serum was not anti-complimentary in the screening test, no serum controls are necessary for the test serums during this titration.

Every time unknown serums are titrated, standard positive serums (homologous and heterologous) with known antibody titers and standard negative serum should be included as controls for the test proper. Two racks of tubes set up as described in Table 7 are needed for these control tests.
Table 7

Protocol for Controls

Rack 1

<table>
<thead>
<tr>
<th>Serum</th>
<th>Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8</td>
</tr>
</tbody>
</table>
| High-titered  
B. caballl | R.  
caballl | antigen |
| Low-titered  
B. caballl | in | tube |
| Negative | (0.25 ml) | 15 16 |
| High-titered  
B. equi | (2 units/.25 ml) | 17 18 |

Tubes 1 through 8 contain doubling dilutions of the serums listed, starting at 1:5 and ending at 1:640. The other test reagents are the same as those listed in Table 6 and are added in the same order. Tubes 9 and 10 are B. caballl antigen controls; tube 9 should show complete hemolysis and tube 10 should show none. Tubes 11 and 12, 13 and 14, 15 and 16, 17 and 18 are serum controls for the standard serums. The odd-numbered tubes should show complete hemolysis and the even-numbered ones should have no hemolysis.

Rack 2

<table>
<thead>
<tr>
<th>Serum</th>
<th>Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8</td>
</tr>
</tbody>
</table>
| High-titered  
B. equi | R.  
equi | antigen |
| Low-titered  
B. equi | in | tube |
| Negative | (2 units/0.25 ml) | 11 12 |
| Low-titered  
B. caballl | (2 units/.025 ml) | 15 16 |
| High-titered  
B. caballl | 17 18 |

Tubes 1 through 8 contain doubling dilutions of the serums listed at the left, beginning at 1:5 and ending at 1:640. The test reagents are the same as those listed in Table 6, and are added in the same order. Tubes 11 and 12 are serum control tubes for the new serum not present in Rack 1. Tubes 9 and 10 are antigen control tubes for B. equi antigen; tube 9 should show complete hemolysis, while tube 10 should have no hemolysis. Tubes 13, 14, 15, 16 represent 5 rows of 4 tubes each containing doubling dilutions of the 5 standard serums (1:5 through 1:60), the "normal" antigen, complement and the sensitized RBC suspension added in the usual manner. Tubes 17 and 18 are the antigen controls for the normal antigen. These 22 tubes are included to show that normal horse components are not influencing the test. Tubes 19 through 22 are the complement titration controls. Tube 19 contains 0.25 ml (2 exact units) of complement. The next three tubes contain doubling dilutions of this material. 0.5 ml volumes of veronal buffer are added to each of the tubes, and after the primary incubation, 0.5 ml of sensitized RBC suspension is added to each tube. The first tube should show complete hemolysis, the second tube should show a few non-hemolyzed cells, the third tube should show a 2+ to 3+ reaction and the last tube should show a 4+ reaction. Tube 23 contains 0.75 ml of veronal buffer and 0.5 ml of the sensitized RBC suspension to determine if there is any spontaneous hemolysis in the sheep cells alone. There should be none.
With serums positive to *B. caballi* antigen, those with titers of 1:160 or above are considered to have come from horses in the acute stage of the disease. At this time, many of these serums will show reactions to *B. equi* antigen at dilutions of 1:5 and 1:20; this cross-reaction disappears sometime during the second month of an infection. Serums with titers of 1:80 or less are considered to have come from animals in the carrier state of this disease.

With serums positive to *B. equi* antigen, those with titers of 1:160 or above are considered to have come from animals in the acute stage of the disease. Usually, these serums will show little or no cross-reaction with *B. caballi* antigen, but if they do so, the comments in the paragraph above would apply. Serums with titers of 1:80 or less are considered to have come from carrier state animals.

Very rarely, a serum will show fixation of complement at 1:5 dilution with the "normal" antigen. This reaction may be disregarded if the specific titer with either of the other 2 antigens is at least 3 dilutions higher.

The Microtiter\(^{(R)}\) system has been tested using the procedures described above for the tube test and the dilution techniques recommended by the manufacturer. Titration results were in close agreement when serums were tested in parallel with the Microtiter system and the standard tube test.
RECOMMENDED STANDARD LABORATORY TECHNIQUES FOR DIAGNOSING INFECTIOUS BOVINE RHINOTRACHEITIS, BOVINE VIRUS DIARRHEA, AND SHIPPING FEVER (PARAINFLUENZA-3)

Supplement I. Microtiter Serum Neutralization Test for Infectious Bovine Rhinotracheitis (Kindly provided by Dr. D. G. McKercher)

Supplement II. Fluorescent Antibody, Tissue Section Technique for the Detection of Infectious Bovine Rhinotracheitis Virus Antigen in Aborted Fetuses (Kindly provided by Dr. D. E. Reed)


INTRODUCTION

The diagnosis of infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD), or parainfluenza-3 (PI-3) infection (shipping fever) is difficult when based only on clinical signs and postmortem lesions. Laboratory confirmation by virus isolation or detection of a convalescent increase in antibody titer has become an essential feature of good veterinary service. Standard techniques assembled for this purpose by the members of the committee are presented here along with two supplemental techniques.

I. Virus Isolation Identification

A. Cell cultures

The cell cultures most suitable for propagation of IBR, BVD, and PI-3 viruses are those derived from bovine tissues. Primary cell cultures or passaged cell lines may be employed depending on the availability of fresh tissue usually from a nearby abattoir. Primary cell cultures propagated from the kidneys of fetuses obtained from slaughtered pregnant cattle are quite susceptible to the viruses. However, the embryonic bovine kidney (EBK) cell cultures are frequently found infected with non-cytopathogenic strains of BVD virus due to the prevalence of this agent in cattle.

As an alternative bovine cell lines may be employed that are equally susceptible to virus infection. These cell lines have been derived from some primary bovine tissue source and adapted to growth on the sides of glass or plastic bottles and tubes. The lines are usually supplied at about the 20th passage level and seem to lose susceptibility and refuse to grow at about the 70th passage. It is an advantage to have a low temperature freezer or liquid nitrogen cannister for preservation of seed stock so
that it is unnecessary to obtain a new supply of the cell line when it reaches a high
passage level. Unless accidentally contaminated with virus from fetal serums these
cell lines are more reliable and consistent in their growth and susceptibility charac-
teristics than primary kidney cell cultures.

Two cell lines are recommended for use in isolating and identifying IBR, BVD, and
PI-3 viruses:

**Bovine Turbinate Cell Line (BT).—**The BT cell line was initiated from a section
of bovine turbinate obtained from a 2 year old Hereford. The culture was started
in Hank’s medium with 20% specific-pathogenfree (SPF) calf serum and passaged in
Eagle’s minimum essential medium*(MEM) with 10% SPF calf serum. The BT cell
line may be obtained from Diagnostic Services, Animal Health Division, NADL,
Box 70, Ames, Iowa 50010.

**Embryonic Bovine Tracheal Cell Line (EBTr).—**The EBTr cell line, code CCL-
44, was initiated from minced, whole trachea of a male fetus (9” long).8 The cells
have been grown in Eagle’s MEM plus nonessential amino acids and 10% fetal calf
serum. The EBTr cell line may be obtained from the American Type Culture Col-
lection, Cell Repository, 12301 Parktown Drive, Rockville, Maryland 20852.

B. Culture Mediums, Nutrient Serums and Solutions

The following mediums and solutions are recommended for the propagation
of primary and cell line cultures. Hank’s balanced salt solution (BSS) plus 0.5%
lactalbumin hydrolysate (LAH) and 10% SPF calf serum free of antibodies against
IBR, BVD, or PI-3 virus is suitable for initiating the growth of primary EBK cell cul-
tures. A suggested formula is as follows and others are available with minor modifi-
cations: 6,7

<table>
<thead>
<tr>
<th>Hank's BSS with LAH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>80.0 gm.</td>
</tr>
<tr>
<td>KCl</td>
<td>4.0 gm.</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1.4 gm.</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>2.0 gm.</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>0.6 gm.</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.6 gm.</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.0 gm.</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>3.5 gm.</td>
</tr>
<tr>
<td>LAH</td>
<td>50.0 gm.</td>
</tr>
<tr>
<td>Phenol Red 5% soln.</td>
<td>5.0 ml</td>
</tr>
<tr>
<td>Triple dist. or ion exchange</td>
<td></td>
</tr>
<tr>
<td>H₂O q.s.</td>
<td>10.0 L</td>
</tr>
</tbody>
</table>

After the cell cultures have grown to more than 50% confluency or when the medium
has become acidic may be replaced with Earle’s BSS plus 0.5% LAH and 5% SPF calf
serum:

*Eagle’s MEM, F15, available in powdered form from Grand Island Biological Co.,
Grand Island, New York 14072.*
BOVINE RHINOTRACHEITIS, VIRUS DIARRHEA, SHIPPING FEVER

Earles's BSS with LAH

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>80.0 gm.</td>
</tr>
<tr>
<td>KC1</td>
<td>4.0 gm.</td>
</tr>
<tr>
<td>CaCl2</td>
<td>2.0 gm.</td>
</tr>
<tr>
<td>MgSO4. 7H2O</td>
<td>2.0 gm.</td>
</tr>
<tr>
<td>NaH2PO4. H2O</td>
<td>1.4 gm.</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.0 gm.</td>
</tr>
<tr>
<td>Phenol Red 5% soln.</td>
<td>5.0 ml.</td>
</tr>
<tr>
<td>NaHCO3 fresh 7.5% soln.</td>
<td>100.0 ml.</td>
</tr>
<tr>
<td>LAH</td>
<td>50.0 gm.</td>
</tr>
<tr>
<td>H2O q.s.</td>
<td>10.0 L</td>
</tr>
</tbody>
</table>

For the cultivation of bovine cell lines the appropriate medium for propagation and maintenance is Eagle's MEM with nonessential amino acids, Napyruvate, LAH, and 10% calf serum. The EBTr cell line requires fetal calf serum while the BT cell line will grow with SPF calf serum. Great care must be taken to avoid using fetal calf serum that contains BVD virus. Each batch of fetal calf serum should be filter sterilized through an asbestos pad and tested for the presence of BVD and other viruses. All serums including fetal serums should be tested for antibodies since prenatal infection of the calf may result in either persistent infection or recovery with an immune response. Since the formula of Eagle's medium is somewhat long and complicated a commercial source such as F15 powdered medium previously mentioned is recommended.

To prepare a 5 L batch of Eagle's medium, F15, the preweighed package is dissolved in slightly less than 5 L of glass distilled or ion exchange water; and 11 gm. of NaHCO3 and 25 gm. of LAH dissolved in water are added. Then add 50 ml. of Napyruvate 100x solution (GIB Co., Cat. No. 136),* bring the volume to 5 L with additional water, and sterilize by filtration.** The medium may be stored at 4 C. and the pH should be adjusted to 7.4 to 7.6 after the addition of 10% calf serum.

A set of solutions is required for the removal and dispersal of the cell line cultures from the sides of the culture flasks for transfer to new containers.

Saline A 10x

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>80.0 gm.</td>
</tr>
<tr>
<td>KC1</td>
<td>4.0 gm.</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.0 gm.</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>3.5 gm.</td>
</tr>
<tr>
<td>Phenol Red 5%</td>
<td>2.0 ml.</td>
</tr>
<tr>
<td>H2O q.s.</td>
<td>1.0 L</td>
</tr>
</tbody>
</table>

Trypsin 1% (Difco+)

* Grand Island Biological Co., Grand Island, N. Y. 14072
** Millipore Filter Corp., Bedford, Mass. 01730
+Difco Laboratories, Inc., Detroit 1, Michigan 48200
Dissolve 10 gm. in 1 L of H₂O. Let stand overnight at 4 C. and sterilize by filtration.

Versene 1% (ethylene dinitrilo tetracetic acid, disodium salt)†

Dissolve 10 gm. in 1 L of H₂O and steam sterilize.

The above cultures are combined as follows to prepare the STV solution employed for subculture of the cell line:

**STV Solution**

- Trypsin 1% 10.0 ml.
- Versene 1% 10.0 ml.
- Saline A 10x 100.0 ml.
- H₂O, sterile 900.0 ml.

Combine the solutions and adjust the pH to 7.2 to 7.4 with sterile 7.5% NaHCO₃.

All of the culture mediums and the STV solution should be fortified by the addition of suitable concentrations of antibiotics. For the preparation of either 10 L of medium or 1 L of 10x medium concentrate the following antibiotics are recommended:

- Penicillin G, potassium 1.0 gm.
- Dihydrostreptomycin sulfate 0.5 gm.
- Fungizone 0.03 gm.
- Kanamycin 1.0 gm.

**C. Cleaning and Sterilization of Cell Culture Vessels**

All glassware employed should be of good quality or disposable glassware suitable for use in cell culture. Sterile plastic bottles and dishes to be used once and discarded are recommended.* After use glassware should be soaked immediately in detergent solution** and kept immersed until washed. Adequate washing will involve brushing the surfaces of individual tubes and bottles and boiling the detergent solution for at least 10 min. Final rinsing with distilled water after at least 12-14 rinsings with tap water will be necessary. Clean glassware should be covered with aluminum foil and sterilized by dry heat. Stoppers should be made of nontoxic silicone rubber and may be wrapped in paper and sterilized in an autoclave. Coverslips for cell culture growth may be placed in small stainless steel holders and rinsed in boiling water or transferred to the tubes with forceps directly from the package.

**D. Cell Culture Propagation**

The propagation of primary cell cultures requires more time than that of cell lines since a fresh organ such as a fetal kidney must be dissected, minced, and dispersed in the culture medium. The following outline may be used as a general guide.

---

†Eastman Organic Chemicals, Rochester, N. Y. 14600.

* Falcon Plastics Co., Los Angeles, Calif. 90000.

** 7X, Linden Chemicals, New Haven, Conn., or Micro-Solv, Microbiological Assoc., Inc., Washington, D.C. 20000.
and additional details may be found in the literature.\textsuperscript{5,6}

1. Using aseptic technique strip the capsule from the kidney, remove the cortex and place it in a sterile petri dish.
2. Mince the cortex by stroking with a scalpel or cutting repeatedly with scissors.
3. Place the minced cortex in a special flask with indented sides (trypsinizing flask) and wash three times with Saline A solution by stirring the contents of the flask with a magnetic mixer.
4. Add warm 0.25% trypsin solution (37 \textdegree C.) to the washed tissue, stir as before for 20 min., and discard the supernate after allowing the tissue pieces to settle.
5. Repeat the above step for 20 to 60 minutes and filter the supernate through sterile gauze.
6. Chill the filtrate in an ice-water bath and repeat step 4 until all of the tissue has been dispersed by the action of the trypsin. As an alternative, the tissue may be trypsinized overnight at 4 \textdegree C.
7. Centrifuge the combined filtrate in a refrigerated centrifuge\textsuperscript{+} at 1000 RPM for 5 min. and discard the supernate.
8. Repeat step 7 with a centrifuge speed of 600 RPM and a third time at 400 RPM.
9. Measure the volume of the packed cells and dilute to an appropriate concentration for seeding with Hank's BSS with 0.5% LAH, 10% SPF calf serum and antibiotics. Usually a ratio of 1 volume of packed cells to 200 volumes of medium is employed.
10. The cell suspension may be dispersed in tubes, Leighton tubes with cover-slips, plastic or glass bottles. If a CO\textsubscript{2} incubator is available cultures may be propagated in glass or plastic petri dishes.
11. The cultures are placed at 37 \textdegree C. and the medium is changed when it develops an acid pH usually after 2 to 4 days depending on the growth rate of the cells. The maintenance medium is usually Earle's BSS with 0.5% LAH and 5% calf serum.
12. For virus propagation the cell cultures are most suitable for use just after producing about 70\% confluent growth on the sides of the bottles or tubes.

For convenience the cell suspension may be stored frozen. Before the last centrifugation the cells are washed with Saline A enriched with 10\% calf serum. The packed cells are suspended in this medium plus 10\% dimethyl sulfoxide; 1 ml. packed cells per 40 ml. medium. The suspension is placed in a low temperature freezer and held at below -70 \textdegree C. Cells may be stored in this manner for several months. The frozen cells are reconstituted by rapid thawing in a water bath.

Primary cultures may also be passaged in the manner of cell lines and secondary and

tertiary cultures prepared. These cultures may not have the same characteristics as the primary cell cultures.

The cell lines require less effort for the production of cultures and are more consistent in their susceptibility to the viruses. The cell lines are obtained from the source laboratory either in the frozen state or as confluent monolayers in flasks. The frozen cells should be reconstituted according to the directions furnished by the supplier. The EBTr and BT cell lines may be initiated with Eagle's medium with 10% fetal or SPF calf serum, respectively. As the cell cultures approach confluency the medium may be changed to Eagle's with only 2-5% serum.

The cell line cultures as well as the primary EBK cultures should be passaged as soon as the cultures are confluent so that the growth rate is maintained at a high level. To remove the cells the medium is poured off and the cell sheet is washed with a small volume of STV solution, 2 ml. for a 120 ml. flask. Then add sufficient STV solution about 8 ml. for a 120 ml. flask, and incubate for 5-15 min. at 37 C. During this period gently agitate the bottle to help detach the cells. The cell suspension is pipetted gently to disperse the cells and centrifuged at 600 to 800 RPM for 10 min. The supernate is discarded and the cells resuspended by gentle pipetting in medium. Usually one flask will provide sufficient cells for 3 new flasks; however, Leighton tube cultures require heavier seeding.

Cell line cultures should be used for virus inoculation as soon as the cultures are at least 70% confluent. Cultures maintained for more than 3 or 4 days after becoming confluent are less sensitive to virus infection and should be reserved for neutralizing tests rather than virus isolation attempts.

After the laboratory has the cell lines growing an effort should be made to store some cells in liquid nitrogen or low temperature freezer (-70 C.) for seed stock. A suspension of the cells prepared as if for passage is resuspended at 1/10 the usual seeding volume in medium with 20% glycerin. The suspension is frozen slowly overnight on the special cap provided with the liquid nitrogen cannister and then immersed in the nitrogen for storage. When reconstituting, thaw rapidly at 37 C. in water bath and dilute 1 to 8 in medium. Centrifuge the suspension, discard the supernate, resuspend in medium at higher than usual concentrations of cells, and place in culture vessels.

E. Specimen Selection, Shipment and Preparation
For the isolation of IBR, BVD, and P1-3 viruses the appropriate specimens to be collected are as follows:
Infectious Bovine Rhinotracheitis
a) Tissue- lung, bronchial lymph nodes, or section of trachea
b) fluid-if the nasal exudate is copious it may be collected or sterile cotton sw-
abs may be taken from both the nasal orifice and the conjunctival sac and immersed in tryptose phosphate broth.

Bovine Virus Diarrhea
a) Tissue - spleen, mesenteric lymph nodes, Peyer's patches, section of femur for bone marrow.
b) Fluid - urine, heparinized whole blood, feces and nasal exudate. Sterile cotton swabs of the nasal orifice and rectum may be taken and immersed in tryptose phosphate broth.

Parainfluenza-3
a) Tissue - lung, bronchial lymph nodes or sections of trachea
b) Fluid - nasal exudate or cotton swabs of the nasal orifice and conjunctival sac may be taken and immersed in tryptose phosphate broth.

The specimens should be packed with dry ice or ice cans, depending on the distance to be shipped, and sent to the laboratory in insulated styrofoam containers. For in transit periods of more than 24 hours, dry ice should be used.

On receipt at the laboratory the specimens should be identified and logged into the laboratory accession series. The tissue specimens should be minced with scissors and triturated in a chilled homogenizer or with mortar and pestle so as to minimize exposure of laboratory personnel to pathogens. A 20% suspension is prepared in Earle's or Hank's BSS containing antibiotics and then centrifuged (2900 RPM, head No.269, PR-2 refrigerated centrifuge for 20 min.) to remove tissue debris. The supernate is employed in the inoculation of cell cultures.

Blood, urine or broth from the tubes containing the swabs is centrifuged to remove bacteria and debris in a similar manner as above.

F. Virus Culture

Coverslip cell cultures are prepared in Leighton tubes and are inoculated with the specimen suspensions or fluids as soon as the cell sheets are at least 70% confluent. A minimum of 4 coverslip cultures is employed for each specimen. The medium is discarded and 1.0 ml. of the tissue suspension, whole citrated blood, broth or urine (diluted 1-5) is added to each of the 4 tubes. The tubes are restoppered and placed in the incubator at 37 C. for 1-2 hours.

After incubation the specimen material is discarded and the coverslip cultures are washed 3 times with at least 2.0 ml. of Earle's BSS per washing. The coverslip should be shaken gently each time to facilitate washing.

Fresh medium with 10% serum is added to the tubes and the cell cultures are placed at 37 C. The cultures are examined daily for cytopathic effects. From the appearance of the cytopathic changes it may be possible to select the appropriate fluorescent antibody conjugate for staining the coverslip. Experience will be of value but
the cytopathic effects of viruses are often inconsistent in appearance depending on
the strain of the virus or other variables. The cytopathic effects of IBR may be de-
tected as early as 18-24 hours.

If no cell destruction has been observed, then at 48 hours after inoculation 2 cover-
slip cultures should be harvested. The coverslips are washed with PBS (pH 7.2), fixed
in acetone for 5 min. and dried thoroughly. The coverslips are then placed in a moist
chamber and one is covered with a conjugate prepared against IBR virus and the
other with PI-3 virus conjugate. The coverslips covered with conjugate are incubated
at 37°C for 30 min., washed in PBS, dipped in distilled water, dried thoroughly, and
mounted on glass slides with a medium consisting of 50% glycerin in PBS. Separate
glassware for the specimens from each accession should be employed to prevent cross
contamination with detached infected cells.

At 72 and 96 hours after incubation the remaining cultures are harvested and the
medium is frozen for future passage work or subpassaged immediately on fresh cul-
tures. The 72 and 96 hour coverslips should be fixed and stained with BVD conjuga-
te as above with special care for the detection of noncytopathogenic strains of
BVD virus. However, if cytopathic effects are observed in these cultures it may be
advisable to use IBR or PI-3 conjugate on one of the coverslips. Second and third
blind passages with coverslip cultures may be employed in a similar manner. Sub-
passage is of value when there is a low concentration of virus in the original specimen.

The examination of the coverslips by fluorescence microscopy may be performed as
described in the standards for hog cholera virus isolation and identification. Although there is an excellent selection of microscopes on the market the Orthoplan* micro-
scope with the dry darkfield condenser has been found of value for coverslip
culture examination. The detection of virus infected fluorescing cells on the cover-
slip is enhanced by the cytopathic effects of the virus. This makes the location of the
escence do not coincide with those of cell destruction another coverslip should be
stained with a different conjugate. In addition, the presence of other cytopathic
agents such as adeno or enteroviruses must be considered.

G. Source of Reagents

The sources of the EBTr and BT cell lines have been mentioned. The following
virus strains, antiseraums and conjugates may be obtained from Diagnostic Services,
Animal Health Division, National Animal Disease Laboratory, Ames, Iowa 50010.1

Virus Strains: IBR (Colorado 1)11
BVD (Draper) noncytopathogenic
BVD (Singer) cytopathogenic

* E. Leitz Co., 468 Park Ave. S., New York, N. Y. 10016
Antiserums and Conjugates:

- PL-3 (SF4)\textsuperscript{11}
- IBR
- BVD
- PI-3

Other sources are available and laboratory personnel may wish to prepare their own reagents. Training assistance and consultation are also available from Diagnostic Services as well as limited supplies of SPF and fetal calf nutrient serums. These BVD virus strains were selected rather than reference strains because of their special characteristics in cell culture.

II. Neutralization Tests.

A. Specimens

The detection of neutralizing antibodies in the sera of cattle is conclusive proof that the animals have been infected previously or received passive immunity through colostrum. However, the most reliable information based on serology is acquired by collecting paired samples, acute and convalescent, at least 3 weeks apart. A rise in titer of at least 2 dilutions against a specific virus is evidence of recent infection.

Serums should be collected from at least 10 to 20 cattle during the acute phase of the disease and stored in a freezer, locally. After 3-4 weeks the same cattle should be bled and the samples paired with the first set for shipment to the laboratory. The serums should be packed with dry ice or an ice can, depending on the shipping time to the laboratory, in an insulated polystyrene container.

Neutralization tests for antibodies against IBR and BVD virus are performed with cytopathogenic strains of these viruses on cell cultures. Antibody titers against PI-3 virus are more easily detected with a hemagglutination inhibition (HI) test that utilizes the hemagglutinating properties of this myxovirus.

B. Cell Cultures

Either primary or cell line cultures may be employed for neutralization tests with cytopathogenic strains of IBR and BVD. The cells are propagated as described under "Virus Isolation and Identification" and are seeded in disposable glass culture tubes maintained in the standard slanted cell culture rack. Uninoculated control cell cultures must be maintained throughout the test period and remain confluent without signs of cell degeneration. The serum used to supplement the medium must be free of antibodies against the test virus.

There will be a marked reduction in the cytopathogenic titer of BVD virus if the cell cultures are inadvertently infected with a noncytopathogenic strain of BVD virus from fetal calf serums or infected tissue donors.
C. Virus Strains for Antigen

Virus strains of IBR, BVD and PI-3 employed in the neutralization or hemagglutination tests should be recognized type strains or strains that have been confirmed by comparison with type strains such as the virus strains listed under "Virus Isolation and Identification."

To expedite the testing of serums it is an advantage to prepare a large batch of test virus. This may be done by inoculating several flask cultures with the virus suspension and harvesting the fluid from the cultures just as the cytopathic effect becomes extensive. The flasks are frozen and thawed and the contents are pooled. The harvested pooled. The harvested pool is frozen down in small convenient aliquots and placed in screwcap, vaccine or sealed vials. After the titer of the virus has been determined a fresh vial may be used each time to prepare the correct dilution of virus for the neutralization test.

To determine the titer of the virus a fresh vial is thawed rapidly and a tenfold serial dilution is prepared using Earle’s medium, pH 7.2 - 7.4, as diluent. Separate pipettes should be used to transfer and mix each dilution. Sufficient tube cultures of cells should be prepared so that each virus dilution can be inoculated into 4 tubes. An inoculum of 0.1 ml per tube should be employed. The virus titration tubes should be examined every day for cytopathic effects. The final reading should be made by 5 to 6 days postinoculation. An adequate number of uninoculated tubes of cell cultures should be maintained in the same rack with the virus titration as a control on the quality of the cell sheet and as an aid in determining the highest dilution of virus that caused destruction of the cells. Each tube should be recorded as positive or negative for cytopathic effects.

The 50% endpoint of the virus titer may be calculated by the method of Kärber or Reed and Meunch. An example of the Kärber method is given below:

<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>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>$10^1$</td>
<td>$10^2$</td>
<td>$10^3$</td>
<td>$10^4$</td>
<td>$10^5$</td>
<td>$10^6$</td>
</tr>
<tr>
<td>Positive CPE</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

If $t_{50} = 50\%$ endpoint

$t^* = \text{any tube number below the } 50\% \text{ endpoint having 100}\% \text{ positive tubes}$

$\Delta t = \text{increment to } 50\% \text{ endpoint}$

Then the formula is:

$$t_{50} = t^* + \Delta t$$
As an approximation:

\[ \Delta t = \frac{\text{Sum of the positive tubes at and above } t^*}{\text{Number of tubes per dilution}} - \frac{1}{2} \]

\[ \Delta t = \frac{4 + 3}{4} - \frac{1}{2} \]

\[ \Delta t = 1.25 \]

\[ t^* = 3 \]

\[ t_{50} = t^* + \Delta t = 3 + 1.25 = 4.2 \]

Since a tenfold dilution scheme was used the tube number corresponds to the logarithm of the virus dilution and the 50% endpoint of the virus was produced when the virus was diluted $1:10^{4.2}$ and 0.1 ml was inoculated per dilution.

To determine the number of 50% tissue culture infective doses in the 0.1 ml of inoculum calculate the antilogarithm of 4.2.

\[ \log_{10} N = 4.2 \]

\[ N = 16,000 \]

The virus suspension contains 16,000 tissue culture infective doses (50%), (TCID$_{50}$), per 0.1 ml.

D. Neutralization Test Method

The beta method should be employed in which a constant virus inoculum is titrated against different serum dilutions. The serums should be heat inactivated at 56 C for 30 min. or 60 C for 20 min.

First, the virus suspension is diluted with Earle’s BSS so that the test inoculum will contain 100 - 1000 TCID$_{50}$ per 0.1 ml. For instance, the virus is the example which contains 16,000 TCID$_{50}$ per 0.1 ml if diluted 1:100 would then contain 160 TCID$_{50}$ per 0.1 ml.

The serums to be titrated for antibody are diluted in a fourfold scheme in Earle’s BSS and each serum dilution is mixed with an equal amount of the test inoculum of virus. A suggested protocol is as follows:

Take a series of 5 sterile stoppered tubes. Place 1 ml of Earle’s BSS in the first tube and 1.5 ml in the other five tubes. Pipette 1.0 ml of serum into the first tube, discard the pipette, take a fresh pipette, mix thoroughly, and transfer 0.5 ml of this
tube to the next tube in the series. Discard the pipette, take another pipette, mix thoroughly, and transfer 0.5 ml to the next tube. Repeat the procedure until the last tube when 0.5 ml is removed and discarded. Now each tube will contain 1.5 ml of serum dilutions 1:2, 1:8, etc.

Add 1.5 ml of the test inoculum of virus to each tube and incubate for 30 minutes at 37 C. The final dilutions after the addition of an equal amount of the virus dilution will be 1-4, 1-16, 1-64 through 1-1024. In table form the titration may be expressed as follows:

<table>
<thead>
<tr>
<th>Serum or Aliquot Transferred ml.</th>
<th>Diluent ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 (serum)</td>
<td>1.0</td>
</tr>
<tr>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>0.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Preliminary Dilution</th>
<th>Final Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>1-4</td>
</tr>
<tr>
<td>1-8</td>
<td>1-16</td>
</tr>
<tr>
<td>1-32</td>
<td>1-64</td>
</tr>
<tr>
<td>1-128</td>
<td>1-256</td>
</tr>
<tr>
<td>1-512</td>
<td>1-1024</td>
</tr>
</tbody>
</table>

After incubation, 0.2 ml of each serum dilution-virus mixture is inoculated into 4 tube cultures. The cultures should have confluent cell layers and receive a change of medium before inoculation.

The inoculated tubes are placed in the incubator at 37 C and observed daily. Cytopathic changes are first observed in 2-3 days and final readings are made on the 4th or 5th day of the test. The neutralization titer of the serum will be the highest dilution that protects the cell sheet from the cytopathic effect of the virus.

As controls of the test a positive serum of known titer and a negative serum should be tested. The test virus inoculum should also be titrated.

The 50% endpoint titer of the serum may be determined in a similar manner to the calculation of the virus titer as follows:
<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>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>4</td>
<td>16</td>
<td>64</td>
<td>256</td>
<td>1024</td>
<td>4096</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\[ \Delta t = \frac{4+1}{4} - \frac{1}{2} \]
\[ \Delta t = 0.75 \]
\[ t^* = 3 \]
\[ t_{50} = t^* + \Delta t = 3.75 \]

To calculate the log\(_{10}\) of the endpoint, multiply \(t_{50}\) by log\(_{10}4\), the factor of the dilution scheme

\[ \log_{10} t_{50} = (t_{50}) (\log_{10} \text{dilution factor}) \]
\[ \log_{10} t_{50} = (3.75) (0.6) \]
\[ \log_{10} t_{50} = 2.2 \]

The 50% endpoint titer of the serum was 2.2 against 100-1000 TCID\(_{50}\) of the virus.

A table has been prepared for convenient determination of the serum titers (Table 1).

E. Interpretation

Any neutralization of IBR or BVD virus at the 1-4 dilution or higher by a bovine serum is considered an indication that the sampled animal has either had an infection with virus, been vaccinated, or acquired passive immunity through the ingestion of colostrum. Passive immunity titers against BVD virus of 1-1024 may be detected in calves and it may take five months before a passive antibody titer of this magnitude will recede to negative at 1-4.

The most reliable information obtained from the serum neutralization test is through the use of paired samples. The first serum sample should be collected as soon as possible after the disease is detected, preferably during the first 6 or 7 days following the onset of signs. The second sample may be collected 3 or 4 weeks later. After an acute infection the peak antibody titer occurs in about five weeks. A rise in titer of two dilutions as from 1-4 to 1-64, is considered a significant indication that the animal was recently infected. If the interval between the paired samples was only one or two weeks a four fold rise in titer in several cattle tested may be equally valid.

The standard deviation of most serological titrations is approximately plus or minus...
Serums may be routinely screened at the 1:4 dilution to save them. After eliminating the negative serums in this manner the serums with antibody titers may be tested in higher dilutions to determine endpoint titers.

Neutralization titers following infection with IBR are of lower magnitude than those found after BVD infection. Antibody titers after IBR infection will range from log\textsubscript{10} titers of 1.2 to 1.8 (1-16 to 1-64) with occasional high titers of 2.1 (1.128). Titers associated with BVD infection range from log\textsubscript{10} 1.8 to 2.4 (1-64 to 1-256) with occasional high titers encountered of 2.70 (1-512).

III. Hemagglutination-Inhibition Test for Serum Antibodies Against Parainfluenza-3 Virus (Shipping Fever)

The hemagglutinating properties of the paramyxovirus group are the basis for the development of a simple, rapid serological technique. A suspension of PI-3 virus propagated in tissue culture will agglutinate bovine erythrocytes. Serums from cattle previously infected with PI-3 virus will contain antibodies that inhibit this hemagglutination. However, some bovine serums contain substances which prevent the agglutination of the red blood cells by the virus. It was found that adsorption with kaoline removed these nonspecific inhibitors permitting the application of a hemagglutination inhibition (HI) method with PI-3 virus.\(^3\)

A. Serum samples may be collected as described for IBR and BVD with emphasis on paired samples.

B. Virus strain for antigen-virus is propagated in monolayer cultures of primary EBK, BT or EBTr cell cultures. The medium is harvested after they cytopathic effect of the virus is well marked and stored frozen.

C. Hemagglutination-Inhibition Test Method.

Heparinized cattle blood is collected aseptically by venipuncture into heparin at a concentration slightly in excess of 0.3 mg per ml of blood. The erythrocytes are separated by centrifugation at 1800 rpm for 10 min. (head radius 20 cm) and washed 3 times with 3 parts of saline (0.85% NaCl) per packed volume of cells. The erythrocytes may be stored at 4 \(^\circ\)C in 3 volumes of PBS per packed volume of cells for up to 5 days. The stock suspension of erythrocytes is diluted for use in the technique by adding 1.0 ml to 59 ml of saline.

A special rack with a mirror for viewing the hemagglutination may be constructed. Pipettes, flasks, test tube racks, centrifuge, refrigerator, waterbath, and glass test tubes (12 x 75 mm) are required.

The acid-washed kaolin employed in the test is commercially available.*

* Fisher Scientific Co., Fairlawn, New Jersey 07410
The PI-3 virus is titrated as follows:

1. Place a row of 10 clean test tubes in a rack and pipette 0.8 ml of BBS into the first tube and 0.5 ml into the rest of the tubes.
2. Pipette 0.2 ml of stock virus suspension into the first tube, mix with a fresh pipette and transfer 0.5 ml to the next tube.
3. Continue the dilution system transferring 0.5 ml until the 9th tube is reached at which point the last aliquot of 0.5 ml is discarded. The 0.5 ml saline in the 10th tube will be used for a saline control.
4. Pipette 0.25 ml of erythrocyte suspension into each tube including the saline control and shake to disperse the cells.
5. Place tubes at 4°C overnight.
6. Observe for hemagglutination by examining the bottom of the tubes (handle gently). In negative tubes the erythrocytes will be arranged in a sharply demarcated disc or button. In positive tubes the red blood cells will be spread out in a thin layer on the bottom of the tube.
7. Employing the dilution scheme described, the virus was diluted 1-5, 1-10 through 1-280 and 0.5 ml of each virus dilution was mixed with 0.25 ml of the erythrocyte suspension. The highest dilution of the virus that produced hemagglutination is considered to contain 1 unit of hemagglutination. Since 0.5 ml of each virus dilution was employed, the number of hemagglutination units calculated for the virus suspension will correspond to a volume of 0.5 ml. For instance, hemagglutination at 1-80 indicates that 0.5 ml of the original virus suspension contained 80 hemagglutination units and, therefore, the hemagglutination activity of the suspension is 160 hemagglutination units per 1.0 ml.

The stock virus suspension is diluted for use in the hemagglutination inhibition test so that each ml of antigen contains 16 hemagglutinating units. If the stock virus hemagglutinated at the 1-80 dilution then a 1-10 dilution will be employed in the hemagglutination-inhibition test as antigen against each serum dilution.

The Hemagglutination-Inhibition Test is performed as follows:

1. Prepare virus antigen by diluting the stock virus suspension so that each ml contains 16 HA units per 0.25 ml., the volume employed in the test. Prepare a virus control with PBS and erythrocytes.
2. Mix 0.3 ml of each serum to be tested with 1.2 ml of PBS in a test tube, and place in a waterbath at 56°C for 30 min. or 60°C for 20 min.
3. Remove diluted serum from waterbath and add 0.1 gm of acid-washed kaoline and agetate. (A volume of kaoline easily picked up on a small narrow spatula with a blade area measuring 5 x 12 mm). Allow the adsorption to take place for 10 min. at room temperature. Centrifuge at 1500 rpm for 10 min. (20 cm. head radius). Remove the serum from above the packed kaoline with a pipette.
4. Place 10 clean test tubes in a rack for each serum. Leave tube no. 1 empty and pipette 0.25 ml of saline into tube no. 2 through tube no. 10.
5. Pipette 0.25 ml of the inactivated serum, previously diluted 1-5, into tube no. 1 and tube no. 2, and 0.25 ml into the last tube in the row which will serve as a serum control.

6. Mix the contents of tube no. 2 with a pipette and transfer 0.25 ml to tube no. 3. Continue the dilution process and discard 0.25 ml from tube no. 9. The dilutions will be 1-5 in tube no. 1, 1-10 in tube no. 2, through 1-280 in tube no. 9.

7. Pipette 0.25 ml of the standard antigen which contains 16 hemagglutinating units per ml into all tubes except the last tube, the serum control, and agitate vigorously.

8. Allow the reaction to proceed for 1 hr. at room temperature.

9. Pipette 0.25 ml of erythrocyte suspension into each tube and agitate.

10. Place the tubes at 4°C overnight and examine for hemagglutination the following day. Serums containing antibodies against PI-3 virus will inhibit or prevent the hemagglutination observed in the antibody control. No hemagglutination should be observed on the serum control tube containing serum and erythrocyte suspension. The highest dilution completely inhibiting hemagglutination is the HI titer of the serum.

D. Interpretation

Hemagglutination-inhibition titers as high as 1-40 have been observed in the serums of cattle following a minimal exposure to PI-3 virus which did not produce clinical signs of disease. When cattle had a clinical response following infection, HI titers of from 1-80 to 1-640, and higher were found.

Titers in the range of 1-10 to 1-40 indicate previous exposure to PI-3 virus but may not be related to a recent illness. Since the HI titers have been observed to increase about 2 weeks after infection, the best utilization of the technique will be obtained by collecting acute and convalescent serum samples. A fourfold increase or higher in the HI titer may be considered confirmation of a recent infection with PI-3 virus.
Table 1. Fifty percent endpoint neutralization titers with 4 tubes per dilution (Kärber Method).

<table>
<thead>
<tr>
<th>Dilution*</th>
<th>Positive for CPE**</th>
<th>Negative for CPE</th>
<th>Endpoint Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4</td>
<td>4</td>
<td>0</td>
<td>Negative 0.4†</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1-16</td>
<td>3</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
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<td>3</td>
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<td>1.5</td>
</tr>
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<td>1-64</td>
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<td>1</td>
<td>1.6</td>
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<td>2.2</td>
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<td>2</td>
<td>2</td>
<td>2.4</td>
</tr>
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<td>1-1024</td>
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<td>3.0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>3.3</td>
</tr>
</tbody>
</table>

*This table should be used only when the dilutions above and below the breakpoint have a 100% effect. Otherwise, the endpoint should be calculated as described in the test.

**Cytopathic effect

†Rounded off to two significant figures
REFERENCES


Supplement I. Recommended Standard Laboratory Techniques for Diagnosing Infectious Bovine Rhinotracheitis, Bovine Virus Diarrhea, and Shipping Fever. (Parainfluenza-3).
MICROTITITER SERUM NEUTRALIZATION TEST FOR INFECTIOUS BOVINE RHINOTRACHEITIS

Medium: Eagle’s MEM + fetal calf serum (FCS) 10%
Cells: Embryonic Bovine kidney cell cultures (BEK), 1-5 passage
Plates: Flat bottom

1. Inactivate serums at 56°C for 30 min.
2. Place one drop (0.025 ml) of medium in all cups from no.3 to no 12 rows on microtiter plates.* Plates can be used either length-or width-wise depending on the number of dilutions required for a given serum. Therefore, with a 2-fold dilution scheme, and using the first row for serum controls, dilutions of 1-2 to 1-128 or 1-2 to 1-2048 are possible.
3. Place one drop (0.025 ml) of undiluted serum in first cup and 2 drops (0.05 ml) in second cup. Using 0.025 ml microdiluters starting with second row, make serial 2-fold dilutions. Six to 12 serums can be diluted at one time depending on the workers ability to handle the microdiluters.
4. Add 0.025 ml of virus suspension containing 100 TCID50. Titer of the stock virus should be determined previously using the microtiter method and titrated each time the test is performed to check the titer.
5. Seal plates with transparent tape and incubate at room temperature for 90 min.
6. Trypsinize BEK cells from young (2-5 days old), 2nd to 5th passage cultures. Perform cell count. Dilute the suspension with Eagle’s MEM + 10% FCS to a concentration of 400,000 cells/ml.
7. Add one drop (0.025 ml) of cell suspension to all cups.
8. Incubate at 37°C in 5% CO2 atmosphere.
9. Test can be read at 3 to 6 days. Examine cell sheet for cytopathic effects using an inverted microscope.
10. The findings can be marked on the tape directly over the cups as + or – and transposed to the test record later.
11. Tests are usually set up in duplicates. Therefore, 4 or 6 samples can be performed on one plate depending on how many dilutions are carried out. Each plate can be numbered, dated and lines drawn to separate each set of serum dilutions for easy identification.


* Cooke Engineering Co., 735 St. Asaph St., Alexandria, Va. 22314.
Supplement II. Recommended Standard Laboratory Techniques for Diagnosing Infectious Bovine Rhinotracheitis, Bovine Virus Diarrhea, and Shipping Fever (Parainfluenza-3)

FLUORESCENT ANTIBODY, TISSUE SECTION TECHNIQUE FOR THE DETECTION OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS ANTIGEN IN ABORTED FETUSES¹

1. Kidney is the preferred tissue from the aborted fetus; however, liver, lung, and spleen may also be used.
2. Freeze tissues on sectioning blocks in a 70°C freezer, or immerse in a 70°C acetone bath.
3. Cut sections with a cryostat at a thickness of 8 microns, mount on slides, place in acetone at −20°C.
4. Spread IBR conjugate over the section and place in a humid chamber for 20 min. at 37°C.
5. Prepare a set of control sections with normal bovine serum conjugate in a similar manner.
6. Rinse sections twice in 0.01 M phosphate buffered saline (PBS), pH 7.2 and once with distilled water.
7. Apply coverslip with mounting medium made of equal parts PBS and glycerin.
8. Examine stained sections with a darkfield fluorescence microscope employing a BG 12 exciting filter and a K530 barrier filter.
9. The presence of IBR virus antigen is confirmed by the presence of bright focal areas of fluorescence on the sections stained with the IBR conjugate. This specific focal staining is not observed in the control sections stained with the normal bovine serum conjugates.

REFERENCES


* Slee International Inc., 8010 U.S. Highway 130, Pennsauken, N. J.
INTRODUCTION

The immunodiffusion test for the diagnosis of equine infectious anemia (EIA) was described by Coggins and Norcross in 1970. The technique has since been evaluated and described in more detail. The test has proven to be reliable and is widely accepted.

A degree of expertise is required to perform and accurately interpret the immunodiffusion test. The basis of the test is concurrent migration of antigen and antibody toward each other through an agar gel. As the antigen and antibody come in contact, they combine specifically to form a precipitate which is trapped in the gel matrix and produces a visible line. There are a variety of physiochemical conditions which affect the reaction. The precipitin line forms where the ratio of the concentrations of antigen and antibody is optional. Any extreme variation in this ratio will alter the location of the line or may render it difficult to observe. Electrolyte concentration, buffer system and pH also affect precipitate formation. Higher temperatures cause faster migration but lower temperatures will often produce more complete reactions with sharper, more distinct lines. Temperature changes during migration may lead to artifacts. High levels of lipid or protein in the reagents may affect migration and formation of precipitate lines. The technique described by Dr. Coggins and outlined here has been found to produce accurate and dependable results. Several modifications have been attempted with no improvement in the procedure.

Antigen and Control Positive EIA Antiserum

The antigen and control positive antiserum will be supplied by a laboratory designated by the Animal and Plant Health Service, USDA. It is imperative that a control positive antiserum matched with the specific antigen be used. With storage
and use the strength of the antigen may change slightly. To correct for this change
the dilution of the antiserum can often be varied so that a clear sharp band is pro-
duced which is about midway between the antigen and serum wells. A higher dil-
ution of antiserum will cause the line to move away from the antigen well. If the
control line is not distinct or if a second line is observed between antigen and con-
trol antiserum, the reference laboratory supplying the reagents should be contacted.
The antigen and antiserum should be kept frozen at -20 C. or lower. Any part of
of bottles that are left after setting up tests can be refrozen for later use. If only a
few tests are anticipated, large quantities of reagents should be divided into smaller
amounts and frozen.

Immunodiffusion Test.

I. Preparation of Agar Gel
   A. Borate buffer is prepared by mixing:
      2 gm NaOH
      9 gm H₃BO₃
      1 liter distilled water
   The resulting pH should be approximately 8.6 The antimicrobial properties
   of borate buffer will inhibit growth of bacteria and fungi.

   B. One and two percent solutions of Noble's special agar* are prepared
   in the borate buffer. Some lots of the agar have contained very coarse gran-
ules which are difficult to dissolve. Agar which still has undissolved granules
   after proper heating should not be used.

   C. The agar is dissolved by boiling or autoclaving without pressure
   (100 C.) for 5 minutes. Autoclaving under pressure or excessive boiling may
   result in discoloration.

   D. Either 100 mm. or 60 mm. diameter petri dishes may be used. The
   bottom of the dishes should be free of scratches. The 100 mm. diameter
   petri dish allows the use of 4 seven-well patterns per plate while only 1 pattern
   can be used with the 69 mm. plate.

   E. A 1.3 mm. thick base layer of 2% boiling agar is poured. This thick-
   ness is obtained by pouring 5 ml. of agar in the 100 mm. plate and 2 ml. in
   the 60 mm. plate. This base layer is allowed to harden and will prevent see-
   page of the serum under the agar.

   F. The plates with 2% agar base layer or the agar dissolved in buffer can
   be stored at 4 C. for up to 2 weeks if in an airtight container.

   G. A layer of 1 percent agar is poured over the hardened 2% layer. The
   1% agar is maintained at 60 C. until poured. Fifteen ml of 1% agar is used in
   the 100 mm. plate and 4 ml. in the 60 mm. plate.

   H. The plates are allowed to cool with the lids off to permit the escape
   of water vapor.

II Procedure for Cutting Wells in the Agar.

   A. A seven-well pattern is used with one center well and 6 wells in a
   circle around it. The wells are 3 mm. apart and 7 to 8 mm. in diameter. A

* Difco Laboratories, Detroit, Michigan.
template with cutters for the wells can be constructed (Fig. 1) or purchased.* A plastic template that will fit over an uncovered petri dish with cylindrical openings for a no. 4 (8mm.) cork borer can be used (Fig.2) A less accurate technique is to use a sheet of paper with the pattern drawn on it under the petri dish and cut the wells with a cork borer. The cork borer should be sharp with no ragged edges.

B. The top layer of agar (1%) is cut within 1 hour after pouring and before it has completely hardened. The bottom layer is not cut. The agar must be sufficiently hardened so that the edges of the wells do not break down when agar plugs are removed.

C. The agar plug is removed from the well using a metal or glass cannula drawn to a small opening (1-2mm. in diameter), connected to a vacuum line. Avoid removing the bottom layer of 2% agar in the well or separation of the two layers by lifting the 1% layer. If moisture is observed in the wells the plates should not be used unless moisture is siphoned out or allowed to evaporate.

III. Procedure for Filling Wells and Incubation of Agar Plates.
A. It is recommended that tests be set up in duplication. There is sometimes a slight variation between plates and duplication may be sufficient enough to establish a definite diagnosis in a questionable case. Duplicate patterns or plates are also recommended for retests of suspicious samples.

B. The antigen will be supplied as a liquid, lyophilized extract or raw splenic pulp. The liquid material is placed in the well with a Pasteur pipette. The splenic pulp should be teased from the stroma or well minced, then packed in the well with sharp pointed forceps being careful not to leave air pockets. The well should be filled slightly less than level full. The splenic pulp may adsorb moisture, swell, and bulge out of the well if overfilled.

C. Control positive serum is placed on each side of the serum to be tested. This arrangement provides a positive control line on each side of the test serum, thus facilitating accurate determination of lines of identity. A total of 3 samples is tested with each pattern.

D. Plates are incubated at room temperature (20-24 C.) in a moist chamber. An inverted beaker, dessicator jar, or tight pan can be used with a damp paper towels or sponge as a source of moisture.

IV. Reading the Immunodiffusion Test.
A. A strong narrow beam of light* will provide good illumination. It should be adjustable for varying intensities and positions. The reaction should be observed against a black background (Fig.3). A magnifying lens is helpful in some cases. Viewers made for observing stained immunodiffusion reactions are generally not suitable for reading this test.

* A commercial source for template and cutters is: Grafar Corp., P. O. Box 7788, Detroit, Michigan 48207.

* Possible commercial source: Model No. 653 Universal Microscope Illuminator, American Optical Company, Buffalo, New York.
B. The lines will usually appear within 24 hours and can be read at 48 hours. The patterns rarely change after 72 hours. Weak positive reactions may not be visible at 24 hours.

C. The type of reaction will vary with the strength of the serum being tested. The control positive serum line is the basis for reading the test; if this serum does not produce a distinct line the test must be repeated. The following types of reactions are observed:

1. Negative—The control lines continue into the test serum will without bending or with a slight bend toward the positive control serum (Fig. 4, 5, 6, 7, and 8).

2. Positive—Control line joins with and forms a continuous line with the line between the test serum and antigen (Fig. 4, 6, and 8).

3. Weak positive—The control line bends slightly toward the antigen well but does not continue on to form a complete line between antigen and test serum (Fig. 5 and 6). These reactions are the most difficult to detect and are easily overlooked. It is recommended that they be run in duplicate or repeated before reporting the results. A weak positive can occur if there has been seepage of positive control serum between or under the agar layers into a negative test serum well.

4. Very strong positive—The control line turns toward the antigen well before it reaches the well containing the test serum and continues on as a hazy or broad line between test serum and antigen (Fig. 7). This line is situated very near the antigen well. This reaction should not be confused with those obtained by use of a weak control serum which produces only a short control line extending an equal distance on each side of the antigen. A more distinct line will often form if these samples are diluted 1:4 or 1:8 and retested.

5. Non-specific lines—These lines will either cross the control line or fail to join the control line smoothly as they continue on into the test serum well (Fig. 8). These lines are formed by antigen-antibody reactions other than EIA. Specific EIA and non-specific lines may occur with the same test serum.

6. Haze around well—Occasionally a haze due to lipids or other material in the serum will form around the test serum well that may obscure the lines (Fig. 6). If the test is read at 24 hours results can often be determined because the control line usually forms before the haze.

7. Double lines—Occasionally double specific lines are observed. Their significance has not been determined; however, the test is considered positive (Fig. 9).

D. The results should be recorded by accurate drawing on a printed pattern such as Fig. 10. Photographs can be taken to record results; however, it is difficult to get the consistently accurate photographs needed for recording results.

V. Interpretation of the Test.

It is generally accepted that EIA infected horses are virus carriers for life. It also has been observed that many horses may not exhibit clinical signs
for months or years but still have EIA virus in the blood. Therefore, any adult horse that is positive on the immunodiffusion test should be considered to be infected.

Week immunodiffusion reactions have been observed in 3 types of cases:

1) Foals nursing infected mares have weak to fairly strong reactions which may persist for up to 5 months due to antibodies in the colostrum. If the mare is negative, a positive reacting foal can be declared infected. If the mare and foal are both positive the foal should be retested 1 month after weaning or at 6 months of age to determine if infected.

2) Weak positives have been observed during the incubation period up until 45 days after infection. If a second sample is obtained 2 to 3 weeks later, the reaction should become stronger.

3) Inapparent carriers that have exhibited no clinical signs of EIA for long periods of time occasionally have weak antibody reactions to this test. In this case retesting rarely alters the reaction strength.

Any questionable sample should be sent to a reference laboratory for verification. The reference laboratory will supply check coded samples to verify reagents and competency. When the antibody status of a horse cannot be resolved, horse inoculation may be employed.

ACKNOWLEDGEMENTS

The assistance of Mr. R. M. Glazier and his staff for photographic services is gratefully acknowledged.

REFERENCES

Fig. 1. Two views of a metal template containing 4 patterns for use with 100 mm. diameter petri dishes. (Photograph courtesy of Dr. L. Coggins.)

Fig. 2. Plastic template for use with 60 mm. diameter petri dishes. The template has cylindrical holes to insert a cork borer which is used to cut the wells in the agar.
Fig. 3. Viewing the immunodiffusion reaction over a strong narrow beam of light against a dark background.

Fig. 4. Immunodiffusion test which has antigen in center well; control positive serum in wells A, C and E; negative serum in well B; positive serum in wells D and F.
Fig. 5. Immunodiffusion test which has antigen in center well; control positive serum in wells A, C and E; weak positive serum in well B; and negative serum in wells D and F.

Fig. 6. Immunodiffusion test which has antigen in center well, control positive serum in wells A, C and E; weak positive serum with reaction partially obscured by a haze in well F; positive serum in well B and negative serum in well D.
Fig. 7 Immunodiffusion test which has antigen in center well; control positive serum in wells A, C and E; strong positive serum in well F; negative serum in wells B and D.

Fig. 8 Immunodiffusion test which has antigen in center well; control positive serum in wells A, C and E; weak positive serum in well B; negative serum in wells D and F, and antigen.
Fig. 9. Immunodiffusion test which has antigen in center well; control positive serum in wells A, C and E; the same positive serum in wells B, D and F, with a double specific line.
Figure 10. Patterns for recording results.
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