PROCEEDINGS

SEVENTY-SEVENTH

ANNUAL MEETING

of the

UNITED STATES ANIMAL
HEALTH ASSOCIATION

and

SIXTEENTH ANNUAL CONFERENCE
OF AMERICAN ASSOCIATION OF
VETERINARY LABORATORY
DIAGNOSTICIANS

SHERATON-JEFFERSON HOTEL
St. Louis, Missouri
October 14, 15, 16, 17, 18, 19, 1973
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ANNUAL MEETING

of the

UNITED STATES
ANIMAL HEALTH
ASSOCIATION

Sheraton-Jefferson Hotel
St. Louis, Missouri

October 14, 15, 16, 17, 18, 19, 1973
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B. S. Pomeroy, St. Paul, Minn.
W. C. Schofield, St. Louis, Mo.
J. A. Smiley, Augusta, Me.
A. E. Decoteau, Waltham, Mass.
J. B. Thomas, Columbia, S.C.
J. B. Roberts, Muldrow, Okla.
T. B. Ryan, Cary, N.C.
S. A. Moore, Beltsville, Md.
C. L. Pfow, Wash., D.C.
H. W. Towers, Dover, Del.
I. L. Peterson, Beltsville, Md.
E. S. Bryant, Storrs, Conn.
A. S. Rosenwald, Davis, Calif.
A. H. Dardiri, Greenport, L.I., N.Y.
N. O. Olson, Morgantown, W. Va.

Committee on Rabies—1974

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

L. N. Butler, Jr., Phoenix, Ariz.
E. M. Joneschild, Pierre, S. Dak.
M. T. Goff, Ames, Iowa
A. L. Brown, Lincoln, Neb.
Mel Abelseth, Albany, N.Y.
J. E. Frank, Hull, Que, Canada
D. L. Huxsoll, Wheaton, Neb.
Alfred Strating, Ames, Iowa
E. H. Willers, Honolulu, Hawaii

Committee on Transmissible Diseases of Swine—1974

E. A. Butler, Chairman, Des Moines, Iowa
R. D. Ledgerwood, Co-Chairman, West Plaines, Mo.

E. H. Bohl, Wooster, Ohio
J. E. Fox, Ashland, Ohio
J. B. Nance, Alamo, Tenn.
Miodrag Ristic, Urbana, Ill.
John Villari, Wemonah, N.J.
E. O. Haelterman, Lafayette, Inc.
Norman Kruse, Lincoln, Neb.
Taylor Woods, Jefferson City, Mo.
T. F. Zweigart, Raleigh, N.C.
H. W. Dunne, University Park, Pa.
D. P. Gustafson, Lafayette, Ind.
Gary Combs, Hyattsville, Md.
Don Brothers, Padueah, Tex.
W. C. Stewart, Ames, Iowa
Donald Larson, Brookings, S. Dak.
Committee on Diseases of Sheep and Goats—1974

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

W. A. Hickman, Pierre, S. Dak.
R. E. Simmons, Boise, Idaho
L. R. Barnes, Indianapolis, Ind.
H. E. Metcalf, Lakewood, Colo.
T. A. Kincaid, Jr., La Vernia, Tex.
M. E. Macheak, Ames, Iowa.
W. W. Hawkins, Bozeman, Mont.
Ward Van Horn, Buffalo, S. Dak.
A. L. Klingsporn, Bowie, Md.
J. E. Pearson, Ames, Iowa
R. F. Hall, Caldwell, Idaho
T. B. Snodgrass, Dallas, Tex.
G. E. Reynolds, Corvallis, Ore.
V. E. Terrill, Beltsville, Md.

E. L. Drake, Reno, Nev.

Committee on Tuberculosis and Paratuberculosis—1974

P. L. Smith, Chairman, Sacramento, Calif.
A. R. McLaughlin, Co-Chairman, Madison, Wisc.

C. E. Boyd, Columbia, S.C.
G. R. Snyder, Washington, D.C.
J. G. Flint, St. Paul, Minn.
Rodney Larson, Fruitdale, S. Dak.
G. W. Spangler, Des Moines, Iowa
A. F. Kaufmann, Atlanta, Ga.
A. B. Larsen, Ames, Iowa
W. L. Mallman, East Lansing, Mich.
A. P. Schneider, Boise, Idaho
Charles Thoen, Ames, Iowa
D. M. Weinland, Lafayette, Ind.
C. S. Duncan, Albany, N.Y.
A. E. Lewis, Ottawa, Ont., Canada
Lloyd Konyha, Hyattsville, Md.
Jamie White, Beaumont, Tex.
Bruce Pipkin, Beaumont, Tex.
J. L. Blair, Washington, D.C.
Neal Black, St. Paul, Minn.
A. N. Carey, Beltsville, Md.
John Dick, Harrisburg, Pa.
Alfred Karlson, Rochester, Minn.
V. L. Branch, Boston, Mass.
H. E. Nadler, Albany, N.Y.
R. J. Stadler, Hartford, Conn.

Committee on State-Federal Relations

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

H. E. Goldstein, Columbus, Ohio
A. E. Janswicz, Montpelier, Vermont
W. L. Bendix, Richmond, Va.
J. C. Shook, Mechanicsburg, Pa.
D. H. Spangler, Olympia, Wash.
T. A. Ladson, College Park, Md.
H. Q. Sibley, Austin, Texas
O. H. TIMM
President

J. F. ANDREWS
President-Elect

A. E. JANAWICZ
Second Vice-President

W. L. BENDIX
Secretary

H. E. GOLDSTEIN
First Vice-President

J. C. SHOOK
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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<td>Date</td>
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<tr>
<td>Dec. 2-4, 1925</td>
<td>Chicago, Ill.</td>
<td>Dr. J. H. McNeil, Trenton, N.J.</td>
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<tr>
<td>Dec. 1-3, 1926</td>
<td>Chicago, Ill.</td>
<td>Dr. John R. Mohler, Wash., D.C.</td>
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<tr>
<td>Nov. 30-Dec. 1-2, 1927</td>
<td>Chicago, Ill.</td>
<td>Dr. L. Van Es, Lincoln, Neb.</td>
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<td>Dec. 5-7, 1928</td>
<td>Chicago, Ill.</td>
<td>Dr. C. A. Cary, Auburn, Ala.</td>
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<td>Dec. 4-6, 1929</td>
<td>Chicago, Ill.</td>
<td>Dr. Chas. G. Lamb, Denver, Colo.</td>
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<tr>
<td>Dec. 3-5, 1930</td>
<td>Chicago, Ill.</td>
<td>Dr. A. E. Wight, Wash., D.C.</td>
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<tr>
<td>Dec. 2-4, 1931</td>
<td>Chicago, Ill.</td>
<td>Dr. J. W. Connaway, Columbia, Md.</td>
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<tr>
<td>Nov. 30-Dec. 1-2, 1932</td>
<td>Chicago, Ill.</td>
<td>Dr. Peter Malcolm, Des Moines, Iowa</td>
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<td>Dec. 6-8, 1933</td>
<td>Chicago, Ill.</td>
<td>Dr. E. T. Faulder, Albany, N.Y.</td>
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<td>Dec. 5-7, 1934</td>
<td>Chicago, Ill.</td>
<td>Dr. T. E. Robinson, Providence, R.I.</td>
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<td>Dec. 4-6, 1935</td>
<td>Chicago, Ill.</td>
<td>Dr. Edward Records, Reno, Nev.</td>
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<td>Dec. 2-4, 1936</td>
<td>Chicago, Ill.</td>
<td>Dr. Walter Wisnicky, Madison, Wis.</td>
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<td>Dec. 1-3, 1937</td>
<td>Chicago, Ill.</td>
<td>Dr. R. W. Smith, Concord, N.H.</td>
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<tr>
<td>Nov. 30-Dec. 1-2, 1938</td>
<td>Chicago, Ill.</td>
<td>Dr. D.E. Westmoreland, Frankfort, Ky.</td>
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<tr>
<td>Dec. 6-8, 1939</td>
<td>Chicago, Ill.</td>
<td>Dr. J. L. Axyb, Indianapolis, Ind.</td>
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<td>Dec. 4-6, 1940</td>
<td>Chicago, Ill.</td>
<td>Dr. H. A. Port, Cheyenne, Wyo.</td>
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<td>Dec. 3-5, 1941</td>
<td>Chicago, Ill.</td>
<td>Dr. E. A. Crossman, Boston, Mass</td>
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<td>Dec. 2-4, 1942</td>
<td>Chicago, Ill.</td>
<td>Dr. I. S. McAdory, Auburn, Ala.</td>
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<td>Dec. 1-3, 1943</td>
<td>Chicago, Ill.</td>
<td>Dr. W. H. Hendricks, Salt Lake City, Utah</td>
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<td>Dec. 6-8, 1944</td>
<td>Chicago, Ill.</td>
<td>Dr. J. M. Sutton, Atlanta, Ga.</td>
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<td>Dec. 5-7, 1945</td>
<td>Chicago, Ill.</td>
<td>Dr. C. U. Duckworth, Sacramento, Calif.</td>
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<td>Dec. 4-6, 1946</td>
<td>Chicago, Ill.</td>
<td>Dr. William Moore, Raleigh, N.C.</td>
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<td>Dec. 3-5, 1947</td>
<td>Chicago, Ill.</td>
<td>Mr. Will J. Miller, Topeka, Kan.</td>
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<tr>
<td>Oct. 12-14, 1949</td>
<td>Columbus, Ohio</td>
<td>Dr. T. O. Brandenburg, Bismarck, N.D.</td>
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<tr>
<td>Nov. 14-16, 1951</td>
<td>Kansas City, Kan.</td>
<td>Mr. F. E. Mollin, Denver, Colo.</td>
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<tr>
<td>Sept. 23-25, 1953</td>
<td>Atlantic City, N.J.</td>
<td>Dr. T. Childs, Ottawa, Canada</td>
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<tr>
<td>Nov. 10-12, 1954</td>
<td>Omaha, Neb.</td>
<td>Dr. T. C. Green, Charleston, W.Va.</td>
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<tr>
<td>Nov. 16-18, 1955</td>
<td>New Orleans, La.</td>
<td>Dr. H. F. Wilkins, Helena, Mont.</td>
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<tr>
<td>Nov. 28-30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. A. L. Brueckner, Baltimore, Md.</td>
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<tr>
<td>Nov. 13-15, 1957</td>
<td>St. Louis, Mo.</td>
<td>Dr. G. H. Good, Cheyenne, Wyo.</td>
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<tr>
<td>Nov. 5-7, 1958</td>
<td>Chicago, Ill.</td>
<td>Dr. A. E. Wysocki, Buffalo, N.Y.</td>
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<tr>
<td>Dec. 3-5, 1959</td>
<td>Chicago, Ill.</td>
<td>Mr. E. E. Mullins, Niceville, Fla.</td>
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<td>Date</td>
<td>Place of Meeting</td>
<td>President</td>
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<td>Nov. 4-6, 1958</td>
<td>Miami Beach, Fla.</td>
<td>Dr. John G. Milligan, Montgomery, Ala.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
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<tr>
<td>Dec. 15-18, 1959</td>
<td>San Francisco, Calif.</td>
<td>Mr. F. G. Buzzell, Augusta, Me.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
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<tr>
<td>Oct. 3-Nov. 1-3, 1961</td>
<td>Minneapolis, Minn.</td>
<td>Dr. A. P. Schneider, Boise, Idaho</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
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<tr>
<td>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>
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<tr>
<td>Oct. 10-14, 1966</td>
<td>Buffalo, N.Y.</td>
<td>Dr. C. L. Campbell, Tallahassee, Fla.</td>
<td>Dr. R. A. Hendershott, Trenton, N.J.</td>
</tr>
<tr>
<td>Nov. 5-10, 1972</td>
<td>Miami Beach, Fla.</td>
<td>J. C. Shook, Mechanicsburg, Pa.</td>
<td>Dr. W. L. Bendix, Richmond, Va.</td>
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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

H. E. GOLDSTEIN, D.V.M.

Heavenly Father, We ask your blessings on this Seventy-Seventh Annual United States Animal Health Association Meeting.

Grant the Officers, Committee Chairman, and all our members the ability to communicate in productive programming, as we conduct the business of this Association.

We respectfully ask Thee to grant us the ability to provide leadership in developing new programs, and continuing existing programs, that will provide lasting beneficial effects upon animal health, as well as the socio-economic well being of this nation.

We beseech Thee, Dear God, to direct us in all our activities with Thy gracious favor, that we may please Thee and glorify Thy Holy Name in transacting the business of this Seventy-Seventh Convention of our Association.

Amen . . . . . . .
MEMORIAL SERVICE

HARRY E. GOLDSTEIN, D.V.M.
COLUMBUS, OHIO

President Tobin, Distinguished Guests, Ladies and Gentlemen—

Each year the United States Animal Health Association takes time to recognize and pay tribute to members who have passed on since our last meeting.

We the living sometimes fail to properly evaluate the works that came before us. There are no words, phrases or even paragraphs that can adequately convey properly the true sentiments of recognition for those who are no longer on our roster.

Their deeds, feats, and contributions speak for themselves. So that the memory of our departed members may become recorded, let the record show:

PAUL L. TAYLOR (TUS 55) Died November 10, 1972
C. CREED E BICKLEY (KSU 50) died January 17, 1973
MARTIN H. ROEPHE (KSU 28) died January 25, 1973
ED POPE (IOWA 49) died May 11, 1973
EVERETT A. TUNICLIFF (KSU 21) died May 13, 1973

SILENT PRAYER

May we bow our head for a moment of silent prayer.

.................. Amen

It is impossible for us the living to properly evaluate the life and work of these men. We would need volumes to even abstract the many contributions to the science of disease control, to the livestock and poultry industries, to public health, to research, to education and related fields of endeavor.

These contributions have in no small way contributed to the overall agri-business effort in providing bountiful amounts of animal protein for the nation’s food supply and food reserve. These contributions have provided adequate consumer protection in providing services assuring safe wholesome products.

We all feel a deep sense of personal loss for these departed colleagues, but let us be thankful that we had the opportunity of knowing and working with them. Let us pledge ourselves to carry out and help complete the links of the chain in the ever moving progress in our chosen field.
MESSAGE OF PRESIDENT ELECT

Olin Henry Timm
Dixon, California

Mr. President, Distinguished Guests, Ladies and Gentlemen:

Before I get into the main part of my speech I would like to tell a story that emphasizes the interrelationship among all branches of science. My son and daughter-in-law are both practicing veterinarians. A few weeks ago after paturition—it was a boy—my daughter-in-law, Karen, who is a lactating mother felt pain in one breast. She used the California Mastitis Test. It was positive. Then she called her doctor and told him what was wrong. After she explained CMT, his only comment was, "You learn something every day." The doctor's culture proved the CMT correct.

I have been working in this organization for many years, but only after my acceptance of my new responsibilities did I start to ponder just what the U.S.A.H.A. is and what makes it go. Certainly the devotion to the organization is not due to a search for prestige. The typical reaction after mentioning U.S.A.H.A. is a blank stare.

I think it is appropriate, therefore, to describe the U.S.A.H.A. as I see it. Article II of the Constitution and By-Laws outlines the purpose and functions of the organization, a fact which I discovered after I organized my own description, so I will give you mine.

The various members and groups of the association are brought together by a marriage of necessity, a common law relationship for the care of a common responsibility, the health of our creatures and our people. But from this relationship there has developed an extreme loyalty to the association's purposes and to the association itself. For within its structure, people of various interests meet and express their agreements and disagreements. Animal diseases and their related human impacts are the provence of the U.S.A.H.A. At our meetings, animal disease control and eradication programs are established and efficiently organized. The potential threats and prevention of exotic disease are discussed and methods of preventing their entrance into our country outlined. Papers ranging from descriptions of new diseases to new diagnostic techniques and new treatments of old diseases are presented here.

I believe that the strength of this association lies in the devotion of the committee chairmen. The committees essentially operate autonomously. When Hog Cholera broke out two years ago that committee was assembled with the executive committee to establish causes and prescribe prevention of future outbreaks. The hard working committee on Foreign Animal Diseases is assembling on its own, a new book on exotic diseases, to replace the present Grey Book. The Brucillosis Committee starts early in the week of this convention and barely finishes its report by Wed-
nesday. The report will contain the current revisions of the uniform rules and regulations of the eradication program.

It is a loose organization held together by a minimum of centralization. The above description is idyllic. The convention is actually bedlam. And our office staff must be complimented for keeping the program rolling. The hotel facilities have not always been the best, but the executive committee picks the hotels. There seem to be more stories about one’s inconveniences than about our satisfactory accommodations.

I would like to present now some problems in the various areas in which the U.S.A.H.A. operates. The first is scientific input. We will have some excellent research papers presented to us during this convention. Much of the funding for these papers has been with federal monies. For several years the Office of the Budget and Management has been headed by an administrator unsympathetic toward scientific research and I believe the appropriation for animal and human research have been cut unduly because of the basic antipathy towards these fields.

Of interest to us all is S1388, a bill introduced by Senator Talmage and others to authorize the Secretary of Agriculture to allot moneys as appropriated by Congress to States for animal health research. This bill in itself carries no money. I do not know what the future of this bill will be, but some congressmen recognize this need for research.

Another area of concern is in the regulation of our therapeutic products. There are continual revisions in the interpretation of the Virus-Serum Toxin Act of 1913 which calls for the elimination from interstate commerce of all biological products to be used on animals, which are worthless, contaminated, dangerous or harmful. The reinterpretations come to livestock producers and veterinarians as bolts from the sky. Yet when we question the reasons for these revisions in licensing requirements we are accused of inadvertently adopting the views of the biologics manufacturers without giving the Veterinary Services opportunity to present the facts. I believe that we should help decide the facts if facts are up for revision.

Now the F.D.A. has threatened to remove the sulfonamides as drugs for use until they can be proven for efficacy. For those of us who have controlled pneumonia and coccidiosis in lambs through sulfamethazine these actions seem redundant. I realize that these drugs were accepted before the standards were revised but the consumer is caught in the middle between the F.D.A. and the drug producers.

The criteria for clearing drugs and biologics as safe may vary according to who is affected. One death of a human may label a drug as dangerous. The chance of killing a sheep as against saving a whole band of sheep may permit a drug to be labeled safe.

Benefit and the determination of efficacy may be judged by many standards. Our attitude toward insecticides may be irrelevant in a malaria infected country. Our standards for drugs and biologics used in animal husbandry should once again be established by a cost benefit calculation. Livestock producers and veterinarians should participate in these deter-
minations.

Another problem lies in our programs for animal disease eradication. We believe that sheep ticks have been eradicated in the United States but this success has not been duplicated elsewhere. This lack of success in other programs is caused by failure in surveillance and ability to identify source flocks. Those states which are not brucellosis free contain approximately one-half of the cattle in the United States. Our monitoring at packing plants, and this includes Federal Plants, is far from complete and livestock identification for traceback is inadequate. Now there is constant danger to animals unprotected by vaccination. If these conditions continue the brucellosis program will be a bust.

Another of our problems is exotic diseases. Surveillance at our ports of entry which now include all of our international airports is one of our lines of defense. Several years ago when there was an effort to establish more free movement of people from country to country, our inspectors were about to be instructed to examine baggage on a sample basis. But providence was on our side. The efforts to keep out narcotics intensified our customs inspection and the efficacy of our inspections benefitted from this change in emphasis. But even now screening and examination of peoples clothing is far less than in other countries.

Our ability to control the entrance of certain animals into the United States is prevented by multiple authority over these species. Three departments have the authority to control the importation of foreign animals. Asiatic Newcastle disease devastated thousands of birds in wholesale pet shops for several years. But until a declaration was made by the Secretary of Agriculture that these birds endangered our farm animals the Secretary could do nothing.

We are prohibited by law from manufacturing vaccines for diseases not existant in the United States. But Venezuelan Equine Encephalomyelitis was contained to our southern border because we had such a vaccine stored in this country for national security reasons. The D.O.D. had this stockpile as a defence against biological warfare. It would have been illegal for the Department of Agriculture, any state, or the pharmaceutical house that manufactured this vaccine to have held it. We lucked out.

And now the problem of Foot and Mouth. What will be our program if it reappears in the United States? If it is an isolated instance, we will probably go on a kill program with quarentines. But if it appears in a feedlot with tens of thousands of cattle I doubt public opinion would allow the destruction of these animals. The cost would not be $60,000,000 as it was for Asiatic Newcastle eradication but much more. With thousands of cattle moving in and out of a feedlot daily the problem of quarentine would seem insurmountable. A non-specific vaccine was used in the Asiatic Newcastle explosion. Without a vaccine for V.E.E. what would
we have done? I believe we should think ahead and have available vac-
cines of various strains of F and M disease to use against an outbreak. We
should also have available commitments that it will be used.

It is the responsibility of the U.S.A.H.A. to the livestock industry in-
cluding poultry to define and propose solutions for its problems. And
what the livestock industry needs as I see it is a rebuilding of our re-
search, rapid action to clean up our existing eradication programs, con-
sumer participation in the continual reinterpretation of what are safe
dependent drugs, new policies toward exotic diseases which conform with
the realities of our capacity to control them.

The committees of U.S.A.H.A. will have positions and recom-
mandations on the above topics and many more livestock problems.
These positions and recommendations should be communicated to our
members, the livestock associations and livestock publication and the
public. These communications should be done with alacrity.

My last item is the organization of the U.S.A.H.A. itself. At the Buffalo
Meeting in 1966 Grant Kaley proposed that, applications for membership
be authorized to be granted immediately. He also suggested a newsletter
be mailed to each member four times a year with information in the June
and September letters on the convention. He suggested short
biographical notes on new members. He also suggested that an editor be
appointed to produce this publication. These suggestions need to be ac-
tivated.

Dr. Tobin and I wish to apologize for the tardiness of the programs
and proceedings. This problem we realize must be rectified.

I am well aware of the internment of the papers read before the
association. The indexing is in progress. I will push this program.

I have had self addressed penny postcards printed. These the com-
mittee members will have, I will have some and there will be some at the
desk. If you wish to be on a committee, or reassigned, use them. If you
have any other suggestion use them for this purpose also.

And now President Tobin, may I say that I could not have worked with
a better president. You have let us, who are officers in on all your
thoughts and problems, as well as your hospitality. We wish you well at
this meeting and will do our best to make this meeting go.

It is my privilege to present you with the official momento of this
association for outgoing presidents. This last year you have been diligent
in your efforts to make this a better association.

It is with admiration and deep friendship that I present this key and
certificate of service on behalf of the organization.

I would like to suggest that an officer of this association be invited to
the regional meetings of the U.S.A.H.A. Such attendance would be more
certain if a travel allowance accompanied the invitation.
PRESIDENT'S MESSAGE

W. C. TOBIN, D.V.M.

Members of United States Animal Health Association, American Association Veterinary Laboratory Diagnosticians, distinguished guests, ladies and gentlemen—first, I would like to thank the organization for the momento, you can rest assured that I will wear it with pride.

The past year has proven to be one of the most interesting of my lifetime. It has been a tremendous honor to serve this organization as its president. During the past year, I have been honored to represent USAHA at the meetings conducted by the National Association of State Department of Agriculture at St. Croix; the American National Cattlemen's Association at San Antonio; National Livestock Dealers Association at Albuquerque; as well as several meetings with officials of Animal Plant Health Inspection Service-USDA, in Washington, D.C. In all instances, I was gratified with the respectful and cordial treatment afforded to the representative of this organization—USAHA. In this same area, I am very pleased to report that the American National Cattlemen's Association, Livestock Conservation, Inc. and National Livestock Dealers Association have all expressed their desire to become officially affiliated with USAHA. This cooperation and interest by the livestock industry cannot help but strengthen the effort toward the common goal of disease control and eradication.

In my remarks as president-elect last year, I referred to my firm conviction that leaders of the livestock industry should be encouraged to become more active in USAHA functions and activities. Tonight, I can point with pleasure to the fact that our president, Olin Timm, is the first true representative of the livestock industry to hold this position since 1951.

Certainly he is deserving of the complete support of every member of our organization—with proper publicity, communications and recovery from our recent lethargy, I have every confidence that Mr. Timm will be able to lead this organization to an even greater position of prominence than ever enjoyed.

Every indication is evident that the secretary's office, as well as the local arrangements committee have progressed extremely well in making plans for a fine meeting here in St. Louis. I would admonish each of you to get with the task at hand and help make this one of the outstanding USAHA meetings in history.
REMARKS BY CHAIRMAN-ELECT

D.E. COOPERRIDER

JOINT SESSION AAVLD-USAHA

President Tobin, President-Elect Timm, Dr. Quinn, Honorable Commissioner Boillot, Members of the Laboratory Diagnosticians and Animal Health Associations:

It is indeed a pleasure to be in this fair city and to be holding our scientific meetings in such surroundings.

Our two organizations have progressed jointly for the past 16 years to the mutual good of both. I have been a member of the USAHA for over 25 years and was fortunate enough to be a part of the birth and growth of the AAVLD.

We are both now facing the problems of further readjustment to one another’s problems and these will work themselves to finality soon, probably in the coming year. Our own diagnosticians are again reworking our constitution and by-laws to facilitate processing the work of the Association. We have progressed from a small group of people interested in the learning of other laboratories’ better techniques to an organization of over 500 active participants and one that is consulted and requested to put forth accepted and “official” techniques. This has given our Association support and acceptance that has benefitted both associations. I shall do my best to see that these mutual benefits continue.

I have no “clarion call” to issue to this group, only repeating that I am happy to be here, happy to help if I can during the meeting and pledge my best for the coming year.
REPORT OF THE SECRETARY-TREASURER
FOR FISCAL YEAR ENDED SEPTEMBER 30, 1973

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

The Secretary wishes to advise the membership that for the year 1973 we had in our association 722 paid members. We have 56 paid official and allied-organization memberships, and one junior member. In addition, between the time the books were closed, as of September 30, 1973, and the present the Secretary’s office received 1973 dues from an additional ten individual members. Our individual membership should be at least twice what it is.

During the year, discussions were inaugurated between the United States Animal Health Association and the American Association of Veterinary Laboratory Diagnosticians with a view, hopefully, toward some form of ultimate merger of the two organizations. Put briefly, in the face of a spiraling inflation, coupled with the increasing needs that exist to service each organization, neither group can continue much longer alone and on its own. Certainly, some form of operation out of a central office can be at least the beginning of a solution to some of our problems. The Secretary hopes that the ad hoc committee appointed by our President to study the situation will point us in the direction we should attempt to go.

The Secretary apologizes for the delay in delivery to the membership of the 1972 Proceedings. We have become accustomed to delays resulting from proof corrections, rewrites, and this sort of thing. This year, we had to add to that mechanical breakdowns and labor problems; and just about the time we thought everything was ready, we ran into the paper shortage. The type of paper we counted on was not available, and what we substituted was considerably delayed before delivery. You can see for yourselves, each of you, the size of the current Proceedings. It is an inch and three-quarters thick, and it contains more than 100 pages over what our average annual Proceedings have been running. It has a sewn binding, glued for strength. Quite frankly, with existing equipment, it is about the maximum size we can achieve in a single volume unless the page size is materially increased. I mentioned herein previously some of the problems being shared by our organization and the laboratory diagnosticians’ group. Not the least of these is the matter of publication, because here again, the Proceedings of the 1972 AAVLD meeting consumes nearly 300 of the 872 pages in this volume. I do not believe we can go, nor do I believe it is wise to go, to a greater physical size with this book. This problem, too, cries for solution. The indexing of neither of the Proceedings contained in this one volume is adequate, nor has it been in our Proceedings for a long time. The need for cross-indexing and similar
changes to make any given subject, paper, report, etc., immediately available or findable and to have this done on a continuing basis year by year is becoming more urgent. I think the expertise to do this is available to the association, but this too will add to the sheer physical size of the volume and, consequently, to the cost.

I would like to depart from the routine of the Secretary’s report and go on to our financial position. There is much more that can be said about the needs of our association and about its place in the field of veterinary medicine and animal health, but if we are to fulfil our obligations to the industry and enlarge our services, certainly some serious consideration must be given to our finances. We started the fiscal year just ended with a net worth of $25,392.17. Our financial statement, as prepared by our accountant, indicates that our net worth at the end of the fiscal year, September 30, 1973, was $33,718.30. This shows a gain, on paper anyway, of $8,326.13. The financial statement also shows cash receipts of $36,015.12, and our expenditures for the fiscal year were $30,448.06. This indicates that we had receipts in excess of expenditures of $5,567.06. There is one other figure that I can give you: We had a cash balance as of September 30, 1973, in the amount of $9,308.78.

Because of the lateness in the delivery of the Proceedings, the books were closed prior to receiving the printer’s bill for printing and mailing. This bill is now in hand, and it puts an entirely different complexion on the financial position of our association. The contract was awarded at the end of last year’s meeting to the Spencer Printing Company of Richmond, Virginia, on the basis of competitive bidding and because the Spencer bid for the base contract was $10,750, or $1,000 below its competition. A base bid is on the printing of the book on the basis of the average number of pages it had been running in previous years plus extra charges for pages over the average, for halftones, and for proof correction at an hourly rate. Reprints are another matter. We negotiate with the printer for reprints for so much a page, with or without covers, and these are charged directly to the person ordering them and paid for out of moneys collected specifically from the sale of such reprints. We do make a little out of reprint sales, and therefore the more reprints the merrier; but, quite frankly, collecting for reprints is a rather difficult problem, and we are usually faced with considerable accounts receivable due to not having received payment for reprints delivered. At the end of the fiscal year, our accounts receivable in this category amounted to $7,490. To get back to the matter at hand, we have the printer’s bill for the current Proceedings in the amount of $13,300.56, which has not been paid because it was just received. From this you can see that we now have a single account payable that is some $4,000 more than we had cash on hand at the end of the fiscal year.

We are not insolvent in that we have assets that more than offset our liabilities. However, the association is facing the assumption of additional responsibilities. It is facing some form of accommodation with our sister group in the laboratory diagnosticians’ field. It will have to accommodate...
itself to the changing needs not only of the times but of our two groups and certainly increase its services, and it will have to find a new or an increased source of revenue.

We occupy an extremely important and prestigious position in the animal-health field in our country. We cannot stand still. We are certainly not going backward, and so we must go forward. The ways and means must be found, hopefully at this meeting, to accomplish this.

As is his custom, the Secretary wishes to advise the membership that a copy of our financial statement is available to any member who desires it for the asking. It will of course be in the Proceedings of this meeting, but if you wish one before that, just drop a card to our office.

W. L. Bendix, D.V.M., Secretary-Treasurer
UNITED STATES ANIMAL HEALTH ASSOCIATION
2810 Buford Road
Richmond, Virginia, 23235

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR
PERIOD OCTOBER 1, 1972 THROUGH SEPTEMBER 30, 1973

CASH BALANCE—October 1, 1972:

<table>
<thead>
<tr>
<th>Account Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cash on Hand—October 1, 1972</td>
<td>$390.00</td>
</tr>
<tr>
<td>Southern Bank and Trust Company</td>
<td>$2,926.33</td>
</tr>
<tr>
<td>Richmond, Virginia (Savings)</td>
<td></td>
</tr>
<tr>
<td>Southern Bank and Trust Company</td>
<td>$286.39</td>
</tr>
<tr>
<td>Richmond, Virginia (Checking)</td>
<td></td>
</tr>
<tr>
<td>Southern Bank and Trust Company</td>
<td>$137.00</td>
</tr>
<tr>
<td>Richmond, Virginia (Local Arrangements Acct. Savings)</td>
<td></td>
</tr>
<tr>
<td>Trevose Savings and Loan Association</td>
<td>$1.00</td>
</tr>
<tr>
<td>Morrisville, Pennsylvania</td>
<td></td>
</tr>
<tr>
<td>Sandia Savings and Loan Association</td>
<td>$1.00</td>
</tr>
<tr>
<td>Albuquerque, New Mexico</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$3,741.72</strong></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>$14,440.00</td>
</tr>
<tr>
<td>Official Dues</td>
<td>5,600.00</td>
</tr>
<tr>
<td>Junior Membership Dues</td>
<td>3.00</td>
</tr>
<tr>
<td>Proceedings, Reprints and Foreign Animal Books</td>
<td>3,025.22</td>
</tr>
<tr>
<td>Registration Fees</td>
<td>12,189.00</td>
</tr>
<tr>
<td>Interest Income</td>
<td>757.22</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>.68</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>36,015.12</strong></td>
</tr>
</tbody>
</table>

TOTAL BEGINNING BALANCE AND RECEIPTS $39,756.84
# UNITED STATES ANIMAL HEALTH ASSOCIATION

2810 Buford Road  
Richmond, Virginia 23235

**STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR**  
**PERIOD OCTOBER 1, 1972 THROUGH SEPTEMBER 30, 1973**

## DECEASED BY EXPENDITURES:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual Meeting</td>
<td>$4,469.60</td>
</tr>
<tr>
<td>Printing</td>
<td>$5,264.32</td>
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<tr>
<td>Office Supplies</td>
<td>$1,834.34</td>
</tr>
<tr>
<td>Salaries</td>
<td>$11,100.00</td>
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<tr>
<td>Social Security Tax</td>
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</tr>
<tr>
<td>Communication</td>
<td>$2,154.74</td>
</tr>
<tr>
<td>Travel:</td>
<td></td>
</tr>
<tr>
<td>Dr. W. C. Tobin</td>
<td>$681.04</td>
</tr>
<tr>
<td>Olin H. Timm</td>
<td>$658.56</td>
</tr>
<tr>
<td>Dr. Joseph L. O'Harra</td>
<td>$312.74</td>
</tr>
<tr>
<td>Dr. W. L. Bendix</td>
<td>$286.84</td>
</tr>
<tr>
<td>Mrs. Ella R. Blanton</td>
<td>$124.55</td>
</tr>
<tr>
<td>Other Meetings</td>
<td>$537.67</td>
</tr>
<tr>
<td>Rent—Office Space</td>
<td>$650.00</td>
</tr>
<tr>
<td>Furniture and Fixtures</td>
<td>$894.52</td>
</tr>
<tr>
<td>American Association of Veterinary</td>
<td></td>
</tr>
<tr>
<td>Livestock Diagnosticians</td>
<td>$600.00</td>
</tr>
<tr>
<td>Virginia Unemployment Insurance</td>
<td>$111.00</td>
</tr>
<tr>
<td>Miscellaneous Expense</td>
<td>$95.29</td>
</tr>
<tr>
<td>Bank Service Charge</td>
<td>$38.59</td>
</tr>
</tbody>
</table>

**$30,448.06**

## CASH BALANCE—SEPTEMBER 30, 1973:

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cash on Hand—September 30, 1973</td>
<td>$1,028.00</td>
</tr>
<tr>
<td>Southern Bank and Trust Company</td>
<td></td>
</tr>
<tr>
<td>Richmond, Virginia</td>
<td></td>
</tr>
<tr>
<td>Checking Account</td>
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</tr>
<tr>
<td>Savings Account</td>
<td>$7,947.04</td>
</tr>
<tr>
<td>Local Arrangements</td>
<td>$143.23</td>
</tr>
<tr>
<td>Trevose Savings and Loan Association</td>
<td></td>
</tr>
<tr>
<td>Morrisville, Pennsylvania</td>
<td>$1.00</td>
</tr>
<tr>
<td>Sandia Savings and Loan Association</td>
<td></td>
</tr>
<tr>
<td>Albuquerque, New Mexico</td>
<td>$1.00</td>
</tr>
</tbody>
</table>

**$9,308.78**
UNITED STATES ANIMAL HEALTH ASSOCIATION
2810 Buford Road
Richmond, Virginia 23235

SUMMARY OF OPERATIONS
FOR PERIOD OCTOBER 1, 1972 THROUGH SEPTEMBER 30, 1973

REVENUE:

Total Cash Receipts $36,015.12
Less—Expenditures 30,448.06
Excess of Receipts over Expenditures $ 5,567.06*

NET WORTH—SEPTEMBER 30, 1973

Cash on Hand—September 30, 1973 $ 1,028.00
Accounts Receivable 7,490.00

Balance:
Southern Bank and Trust Company, Richmond, Virginia
Checking Account 188.51
Savings Account 7,947.04
Local Arrangements 143.23

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 5,500.00
U.S. Treasury Bond 10,000.00
Furniture and Fixtures 1,294.52

NET WORTH—SEPTEMBER 30, 1973 $33,718.30

* The cost of printing the Proceedings and Reprints has not been paid in this fiscal year. We do not know the exact amount at this time.
ANALYSIS OF CHANGE IN NET WORTH:

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net Worth, September 30, 1972</td>
<td>$25,392.17</td>
</tr>
<tr>
<td>Increased by:</td>
<td></td>
</tr>
<tr>
<td>Excess of Receipts over Expenditures</td>
<td>$5,567.06</td>
</tr>
<tr>
<td>Purchase of Additional Furniture and Fixtures</td>
<td>894.52</td>
</tr>
<tr>
<td>Inventory—Supplies and Proceedings</td>
<td>500.00</td>
</tr>
<tr>
<td>Accounts Receivable</td>
<td>1,364.55</td>
</tr>
<tr>
<td><strong>NET WORTH, SEPTEMBER 30, 1973</strong></td>
<td>$33,718.30</td>
</tr>
</tbody>
</table>

Henry H. Budd  
Accountant
A NEW WORLD OF ANIMAL HEALTH PROGRAM FINANCING

E. E. Saulmon*

We have all heard the philosophical quotation: “Everything changes but change” (Zangwill). One needs only to think back a few years to recognize the truth of that statement. Whether we are talking about outer space exploration, mass transportation, or milk production, the changes affecting this country are enormous. Changes vary in the way they affect the surroundings—some good—some bad. The common denominator, it appears to me, is the challenge they present. We all recognize that, according to our own concepts, change is a challenge.

The Challenge of Change.

I think that the livestock industry, and the regulatory agencies, whose job is to protect the industry from the devastations of disease, are being confronted with a serious situation, and it is time that we bring some thought to bear on ways and means to meet the challenge of finding a new method, or methods, to finance animal health programs in the future.

Change.

For many years, the operational costs of disease eradication programs far exceeded the indemnity costs. (See Chart #1) Nonemergency programs have generally been financed from about 50 percent Federal and 50 percent State funds.

This is no longer so. Indemnity costs soared upward in dealing with the viscerotropic velogenic Newcastle disease emergency. We had to change our thinking from the family flock to a multithousand, even a multimillion unit business. The same situation will face us in dealing with, say foot-and-mouth disease in the Colorado cattle feeding area, or in a Florida or California dairy shed. The impact of a successful disease eradication endeavor in the future will affect the whole business and labor market of an affected community.

A similar situation developed during the hog cholera outbreak last fall in Indiana and New Jersey.

Producers were not satisfied with indemnity for animals affected with or exposed to disease. They became concerned with “down time,” and business losses resulting from quarantine actions. In fact, legislation was introduced in Congress which would have provided “down time” indemnity from appropriated funds.

Obviously, the cost of disease eradication, already high, is going to continue to climb. The high cost of such programs coupled with the reluctance of urban oriented legislative bodies to further subsidize agriculture, and add to this the competition for tax dollars by all sorts of social, defense, environment, and other programs. It demonstrates the

* Deputy Administrator, Veterinary Services Animal and Plant Health Inspection Service U.S. Department of Agriculture Washington, D.C.
problem that will be encountered in the development of future animal health programs. A case in point is the ongoing difficulty of financing even a relatively small program such as swine tuberculosis.

Since there is, and will continue to be, limited Federal money for new or expanded programs, it appears that State or private monies will be needed to finance significant additional disease control activities. However, most States face funding problems very much like those affecting Federal funds.

So, where do we go from here?

We in the Department of Agriculture have given this problem a good deal of thought in the past year, and let me stress, have made no decisions nor reached any conclusions as to how to solve the problem. We do have some ideas for consideration by industry and cooperating State officials.

In early September, we met with a group of persons representing the livestock and poultry industries, and apprised them of the problem. Their reaction was, as you would expect, not entirely enthusiastic. Nevertheless, we do not believe we would be fulfilling our responsibilities to you if we did not call this situation to your attention now rather than wait until an emergency arises. If we waited until an emergency arose and then had to haggle over the details, as I mentioned earlier, it might be too late to properly administer an eradication program. When the industry really wants to eradicate a disease such as swine tuberculosis, we must have a way to finance it, and industry may wish to help do it.

The concept is not new or unique. In fact, I doubt that screwworms would ever have been eradicated in the United States if the Southwest Animal Health Research Foundation had not raised a few million dollars to add to the Federal and State funds.

Other industries have developed systems for promotion, research, or protection from certain losses, i.e., the Cotton Promotion and Research Program which is financed from a $1.00 per bale assessment.

Grasshopper control on rangeland has long been partially supported by producer contributions.

These programs are based upon certain laws. If there were to be a checkoff system for animal health programs, it would require some laws that do not now exist. But, this is one way to get at the problem. Perhaps a law could be enacted, whereby industry could develop a plan for the collection of assessment on a given product, say eggs, to be used in a Newcastle disease program or on milk for mastitis control and eradication.

How to prepare financially for an emergency that would involve several species is quite a different problem. Perhaps a mandatory checkoff system for a given period of time starting with the declaration of an emergency would be one method.

Another approach might be a voluntary checkoff system with industry collecting funds, and also expending them in indemnity payments, while
Federal and State funds would be used for program operations only.

These are only ideas that piggyback on existing programs in other industries and are only suggested as a starting point for consideration.

There is a reasonable basis for this concept. While such programs are initiated because of economic or health threats to the general public, the immediate beneficiaries of these programs are members of the affected industry. The reason livestock producers attempt as individuals to control whatever diseases or pests affect their herds or flocks is that it improves their competitive position by lowering their cost of production with respect to other individuals in the industry.

Similarly, the basis for industry wide support for disease eradication programs is that they improve the competitive position of the affected industry relative to the position of producers of other food products both within the United States and abroad.

In the short run, while an emergency disease of livestock or poultry is being eradicated by quarantine and slaughter, it is obvious that producers outside the eradication zone are the immediate primary beneficiaries. Under past financing practices, their costs do not change on account of the disease, and they benefit not only by being protected against the disease, but also in being able to operate without restriction in competing for the market previously supplied by producers under quarantine.

In the long run, control or eradication of significant economic diseases prevents the producers of competing products from filling a gap in the nation's food supply which would follow the increased production costs resulting from uncontrolled spread of the disease. As a result of disease eradication, markets are preserved and potential profits increase for the industry as a whole.

In our thoughts and discussions, we do not see a plan for industry to shoulder the entire cost of a program. The control and eradication of animal diseases is of great public benefit, and it is reasonable to expect Government funding of a reasonable share of the costs in most cases. It is also reasonable to expect the industry to provide financial support in proportion to the direct benefit it receives, and for an unaffected portion of a benefitting industry to contribute to its own protection. For example, in the case of V.V.N.D. eradication, not only was the public protected from a shortage of eggs, but the poultry industry of Georgia, or of the Delmarva Peninsula, or of northern California, benefitted directly from the emergency program in southern California. Should they not be willing to pay a portion of the costs incurred?

The advantages to the industry affected are not insignificant.

1. It will have a direct means of bringing about prompt initiation of a disease control or eradication program, when such action is needed, without going through State and Federal appropriation processes to obtain all the funds required.

2. The industry will have a more direct input into the conduct of a program, and because of their direct financial support, will have a
greater sense of responsibility to ensure its rapid completion.
3. The industry will be in a position to better protect production
efficiency, and the markets for its products, through its own initiative.
4. By its own decision and under controls it recommends, the in-
dustry could increase or expand indemnity payments for losses other
than those resulting from the disposition of the affected animals.
5. The image of the livestock or poultry producing industry will be
enhanced in public opinion; and
6. Since producers may suffer cash losses from non-program diseases
in the forms of mortality, reduced gain, and medical expenses, contri-
butions to disease eradication programs would provide a direct means to
eliminate such costs.

I have outlined the problem as we see it. The solution will come only
through the input of all of the interests represented here today. I hope
the U.S. Animal Health Association will establish a group to work on this
problem. We will be happy to work with such a group and will welcome
their advice and counsel.
REPORT OF THE COMMITTEE ON LIVESTOCK COMMERCE 1973

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


Mr. Chairman:

The Committee on Livestock Commerce met as scheduled with twenty-three members and guests in attendance.

The Committee reviewed several recommendations made in previous years.

The Uniform Health Certificate, originally recommended in 1964, has been adopted by some 30 states. A certificate printed with a standard format is easier to read with specific items located where they can be found. The revised Veterinary Certification recommended by the AVMA, and concurred in by this Committee, should be considered so that reprints can be ordered with an up to date and standard format. We recommend that other states consider the adoption of the Uniform Health Certificates and Veterinary Certification.

A survey was made of the possible use of the Livestock Transportation Certificate recommended by this Committee last year. Twenty-six States indicated that they would be willing to accept such a certificate. We recommend that the states review this certificate as printed in the 1972 proceedings and consider its use for the appropriate classes of Livestock.

Procedures were implemented in 1971 for states to submit their regulation changes to the Senior Staff Veterinarian, Program Services, APHIS, 870 Federal Center Building #1, Hyattsville, Maryland, 20782. Some states are not yet taking advantage of this opportunity and we urge them to do so. This is the most expedient way to get changes into the hands of interested persons.

The format for submitting these changes will be issued by APHIS. Additional copies of the book, Pub. 91-17-6, have been printed and distributed.

The proposal to revise marketing laws prepared by the Livestock Laws Reform Commission was discussed.

That portion of the proposal referring to livestock health, which is of interest to this Committee, is a commendable declaration of principle.

Several states are implementing regulations regarding Equine Infectious Anemia. Wide variations are appearing in these regulations. This committee urges that states contemplating EIA regulations follow
the recommendations of the committee on Infectious Diseases of Horses. This is urged in the interest of uniformity and adequacy of regulations.

There was a brief discussion of state quarantines and embargoes, particularly when an entire state is embargoed and the disease is confined to a small area of the state. No conclusion was reached.

This constitutes the report of Livestock Commerce Committee. I respectfully submit the report for approval by the Executive Committee.
ECONOMIC BENEFITS OF
ANIMAL DISEASE CONTROL AND ERADICATION

by K. M. Weinland, D.V.M.
Extension Veterinarian
Purdue University

Mr. Chairman, Collegues, and guests of the 77th Annual meeting of
the U.S. Animal Health Association. There has to be something a little
bit wrong with any one who would agree to discuss the cost of animal
disease and parasites in this country, because there is sure to be someone
waiting to shoot him down by asking just what do you have to back up
those figures and what do you have to prove that those animals would not
have died or recovered in spite of you and veterinary medicine. In the
past year we have made some attempt to put together some of the figures
that have been in print. Most of these we have documented or referenced
in the handout that you can pick up later. Some of this information is from
our own personal experience.

I have been concerned for some time that the economic importance of
the control and eradication of animal disease has not had more publicity.
(Quote from folder—"Lots of people think there's plenty of beef no
farther away than their favorite food store. But it's not that simple to get
high quality beef where people want it and when they want it.

Beef supply is no accident. Actually it's thousands of miles and 20-24
months from conception to consumption ... from ranges, to feedlots, to
packing plants until that steak or roast is finally cut, wrapped and ready
at your neighborhood food store. In between are countless management
decisions resulting in profits, losses, successes, failures, huge invest-
ments and months of long hard work.

There's no quick way to a T-bone steak. Nine months of a cow's room
and board until the calf is born . . . six or seven months with cow and calf
on pasture, plus 330 lbs. of grain, 70 lbs. of protein and 10,000 lbs. of hay,
silage and grass, just to grow the calf to weaning age of 450 lbs.

Then follows another 4-6 months in the feedlot . . . 2,200 lbs. more of
grain, 360 lbs. of protein supplement and 2,300 lbs. of hay, silage and
pasture before the steer is feedlot-finished at 1,000 lbs. plus and sold
to a packer." This I believe is good publicity for the beef industry.

A few months ago we visited a dairy farm in Indiana where they were
having a problem with sore teats and mastitis. The local veterinarian was
working with the dairyman but was unable to convince him that his
problem was as serious as it was and was one that he could not control by
just treating cows. The history that we received on the herd was that
they had experienced an outbreak of IBR earlier in the year, had had a
bout with downer cows all of which they felt they had under control at
the present time. The herd had gone thru an epidemic of sore teats which
resulted in a number of cases of mastitis. Cows with lesions on their
teats did not respond to treatment, some of them improved for a time and
then seemed to relapse. Ninety percent of the cows were or had been

1. "A Steer's Not All Steak" by Beef Industry Council, National Live-
stock and Meat Board, 35 South Wabash Ave., Chicago, Ill. 60603.
involved with the condition. The owner stated that when he added 35 head of cows to the herd they soon had lesions on their teats and a number of cows in his original herd had a return bout with the condition. Milk production was down to about 30 pounds of milk per animal per day. On the way to the farm I read a newspaper article written by a farm editor on this particular farm. It stated that, the owner, was having a difficult time making money due to the high cost of feed and labor. It said nothing about the problems and low production.

The facilities were excellent as it was an environmentally controlled building for 120 cows with automatic cleaning into a manure pit, 3 Harvestor silos with automatic mixing and feeding. They had a herring bone milk parlor with 8 on each side. Automatic udder washers and milking machine with automatic vacuum control; bulk tank room and office attached. The facilities and equipment had not been over used as they had only been in the installation a year and a half. They had spent $250,000.00 in the installation with 120 cows at $600.00 per cow he would have invested another $72,000.00 for a total of $320,000.00. This does not include feed or replacement animals.

We ran the CMT test on 116 cows and found 24% of the quarters with a 2 or 3 reaction for a total of 111 quarters. The 111 quarters involved 59 cows. Taking figures by Dr. George Marx of Minnesota in which he said that a new infection would decrease production in the amount of $110.00 per cow with milk at $5.00. This would add up to about $6,490.00 annual cost to the dairyman. From about 25% of the infected quarters streptagalatae was cultured. We recommended some culling and treatment and inside of 30 days production had increased 10 lbs. per cow per day or over a 1,000 lb. total. Figure this milk at $7.00 per 100 it means $70.00 per day. We can repeat this same picture many times in Indiana and hundreds of times throughout the country.

The United States produces 26.5 million feeder calves a year; 1.6 million of these calves are lost each year during and shortly after moving to the feed lot. One year ago this represented a 5 million dollar loss to the beef industry. That was when feeder calves were selling for 45c per pound. Today the same calf is costing the feeder 60c to 65c per pound, which would increase that 5 million dollars about 30%. In other words today the beef industry is suffering about $6,500,000 loss. Also we should keep in mind the increase in the cost of the major feeds over what they were a year ago. There is a 100% increase in the price of corn and almost a 300% increase in the price of bean meal going into these more expensive cattle. The increased costs should be of great concern to the cattle feeder.

I would like to quote from some more recent information on feeder cattle. This was in a News Release by LCI and was on a survey of “Shipping Fever” for its National Committee on Feeder Cattle Health. This committee is chaired by Dr. Don Gill, Oklahoma State University. Quote from news release, “Cattle deaths attributed to shipping fever in 1972 reduced the potential supply of carcass beef by 22,000,000 lbs. or enough to supply the average annual U.S. beef consumption of 1,900.00
This death loss cost livestock feeders $76,000,000.00 plus 19,000,000 for treating sick animals. This represents a total out-of-pocket cost to the beef industry of $95,000,000.00 in 1972.

In a report in the 1970 proceedings of U.S. Animal Health Associations by the Committee on State and Federal Relations they stated that "Losses due to disease and parasites are annually in the vicinity of 15% of the total livestock marketed or in the area of $450,000,000.00." From what I can gather with the increase in cattle and hog numbers and the increased market prices, this figure would be around 1 billion dollars at today's prices.

This same thing would apply to the figure that was tossed about quite readily in 1963 of what hog cholera was costing the swine industry. At that time we were quoting a figure of $50,000,000.00 annually. At today's costs and prices the figure would be close to $100,000,000 annually. A report from Dr. C. J. Callahan one of the ambulatory clinicians at Purdue on an outbreak of leptosperosis in a 72 cow dairy herd cost the dairyman, $4,785.00 in the loss of calves, milk production and veterinary costs. A report from the AVMA Journal, December 1972, about a Guernsey breeder with 110 cows that Johne's disease (Paratuberculosis) cost him $110,000.00. "Economic Loss from an acute outbreak of IBR in a Diary Herd". Modern Veterinary Practice, May 1971 reports; death of 3 cows at $500.00 each, production losses at 100 lbs. milk per day for 33 days and veterinary costs all totaled to $1,881.20.

Another report from LCI proceedings of their annual meeting 1971 stated that a survey of 16 slaughtering plants on grub damage to carcasses for the year 1971 was $6,700,000.00 plus $3,500,000.00 damage to the hides and another 30 or 40 million dollars lost in feedlot efficiency. Predicted an estimated savings from grub control program of 17 million dollars on carcasses and hides alone.

We are talking about large sums of money and anything we can do to shave any of the death losses and losses due to inefficient production will mean millions of dollars in the pockets of our food animal producers. There have been reports from Michigan, New York and Indiana that Health Programming in the dairy herd can give a return of from 300 to 640% on monies invested in these programs. The programs point out that pregnancy diagnosis, decreasing the calving interval, mastitis control and effective methods of raising calves can increase profits for the dairyman as well as decrease the losses in inefficient production. At Purdue we are drawing up plans for Health Programming for the swine producer to reduce the incideous infections that cut back efficiency in growth and development of market hogs.

We have the same plans on the drawing board for the beef cow calf herds in Indiana. We are growing in beef cow numbers each year. We believe that management practices to prevent disease will be the means to control some of these tremendous losses in the future.

We mentioned the great loss experienced each year in the feeder calf movement into the feed lot. Pre-conditioning or back-grounding for calves before they start the hazardous road to the feed lot is the answer
to most of this problem. We believe this type of programming is a must for the livestock producer and will be a great help to the veterinary practitioner.

During our problem with hog cholera in Indiana a year ago, our local press was not too kind to the regulatory people. They took the plight of the swine owners on the number of swine producers being put out of business, etc. After it was over an editorial titled “After the Epidemic”, appeared. They pointed out the amount of indemnity paid for hogs slaughtered, all the time and effort that had gone into the outbreak, and what have we accomplished? This got to me and I answered the editorial. I would like to read you part of my “letter to the editor”.

“It is now more than 100 days since the last positive case of Hog Cholera in Indiana, the quarantines have been lifted and both feeder and market hogs are moving freely. We don’t see one printed word or figure on how much these Cholera-free days are saving the swine producers and the consumer!

Indiana swine producers received a little over $430 million for hogs marketed in 1972, this consisted of about 7 million market hogs. Last fall everyone wanted to vaccinate, why can’t we vaccinate? I would like to point out a couple of reasons: Suppose we had a vaccine that was 100 per cent effective and we vaccinate 100 per cent of the swine population, the cost would be 75¢ up to $1 per head, the total cost to the swine producers to vaccinate just the market hogs would be in the area of $7 to $8 million or about 7 to 8 times the cost of the epidemic in Indiana. As a result of the nationwide eradication program, states surrounding Indiana, Michigan, Ohio, Illinois, Minnesota, and Iowa reported no cases of Hog Cholera in 1972. They collectively produce about 6 times as many hogs as Indiana or about 48 million. The cost to have vaccinated them plus breeding animals, would have been close to $60 million. Those of us, the consumers, who buy pork chops, ham, and bacon should remember the cost of production is inevitably reflected in the price of the finished product. We can see very readily the cost of the outbreak in Indiana last fall was very minimal when compared to vaccinating swine and living with the disease as we did for over 60 years.

Recent information from Canada points out that their Hog Cholera Eradication Program for more than 50 years cost them 2 cents per each hog marketed; while the United States, living with the disease, over the same period cost our swine producers $1 per hog marketed.

The control of disease and parasites makes more money for the livestock producer than any other phase of production, thus making our meat supply one of the greatest of any country in the world. The average American consumed 240 pounds in 1971 or about 5 lbs. per week.

We would like the veterinary profession to receive credit for having a large part in the wholesomeness and production of America’s meat supply and when there is another outbreak, and there is sure to be, some encouragement for the Regulatory Animal Disease people instead of criticism, would be very helpful. They are scientists and using the best known methods to cope with animal diseases today.
VETERINARY SERVICE FOR REMOTE AREAS

Presented by:
Howard J. Neely, D.V.M.,
Extension Veterinarian,
Veterinary Services Branch,
Ontario Ministry of Agriculture & Food,
Guelph, Ontario, Canada.

The problem of providing veterinary service in remote areas is not a new one to many jurisdictions in Canada, and indeed throughout the world. It is my understanding that there are many states that have faced this problem. However, I am not aware of the systems that may have been used to solve these problems in the U. S. A.

Ontario is one of 10 Provinces in Canada. It is Canada's second largest Province and is its most highly populated. Roughly one third of Canada's 22 million people live in the Province of Ontario. It is interesting to note that most of Canada's population live within 200 miles of the United States border in a strip about 3,000 miles long.

The areas of Northern Ontario that are sparsely settled extend from the Ottawa River on the East to the Manitoba border on the West. There are two major railways and two major highways running through these areas. As one would expect most of the communities have evolved in close proximity to the major rail lines and highways.

The Veterinary Assistance Policy for Northern Ontario Objectives

There are three objectives to the program:

1. To encourage veterinarians to practice in the more sparsely settled areas of the province where distances are frequently great and weather conditions and roads may leave something to be desired.

2. By the provision of veterinary service to encourage the development of livestock production in remote areas of the province that have a limited capability for other types of agricultural production.

3. To provide veterinary service to livestock owners at a price that is reasonable and is equalized when compared to the costs of veterinary service in the heavily populated areas of Southern Ontario.

Origin

Prior to World War II there was no organized program for providing service in the sparsely settled areas of Ontario. The program had its beginnings in 1945. The veterinary and agricultural leaders at that time saw an opportunity to initiate a new program and were able to sell the idea to the Provincial Government. The pressures that made the program feasible and possible were twofold. There were many young veterinarians returning from war who were anxious to locate and there were several government programs to encourage young men returning from
the armed forces to go into farming. Land was cheap and plentiful in the remote areas and thus there was increased emphasis on livestock production where cheap land was available.

The program of assistance for veterinary service remains today, very similar to the original plan.

Essentially the motivation for having an area created for the Veterinary Assistance Program is at the local level. The farm community forms a committee. The committee defines its boundaries and applies to the Provincial Government for assistance. The Government authorities examine the application and make the final decision. The financial structure is very simple and is as follows:

| Funding: | Community annual grant | $1,000. |
| | Government annual grant | $7,000. |
| Total | | $8,000. |

Veterinarian receives 7¢/mile from Government for calls. The farmer pays on the basis of Ontario Veterinary Association Fee Schedule plus drug charges.

**Livestock Population**

<table>
<thead>
<tr>
<th>District or County</th>
<th>Cattle</th>
<th>Horses</th>
<th>Pigs</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algoma</td>
<td>12,829</td>
<td>329</td>
<td>1,032</td>
<td>1,233</td>
</tr>
<tr>
<td>Cochrane</td>
<td>10,367</td>
<td>283</td>
<td>1,194</td>
<td>1,754</td>
</tr>
<tr>
<td>Kenora</td>
<td>3,027</td>
<td>138</td>
<td>575</td>
<td>914</td>
</tr>
<tr>
<td>Renfrew</td>
<td>77,848</td>
<td>2,070</td>
<td>10,590</td>
<td>5,088</td>
</tr>
<tr>
<td>Muskoka</td>
<td>3,985</td>
<td>257</td>
<td>425</td>
<td>393</td>
</tr>
<tr>
<td>Parry Sound</td>
<td>13,549</td>
<td>438</td>
<td>1,612</td>
<td>1,341</td>
</tr>
<tr>
<td>Sudbury</td>
<td>8,723</td>
<td>391</td>
<td>1,281</td>
<td>185</td>
</tr>
<tr>
<td>Nipissing</td>
<td>15,061</td>
<td>251</td>
<td>2,421</td>
<td>697</td>
</tr>
<tr>
<td>Manitoulin</td>
<td>25,398</td>
<td>238</td>
<td>3,313</td>
<td>8,837</td>
</tr>
<tr>
<td>Rainy River</td>
<td>21,211</td>
<td>387</td>
<td>956</td>
<td>3,250</td>
</tr>
<tr>
<td>Timiskaming</td>
<td>25,807</td>
<td>594</td>
<td>5,443</td>
<td>3,029</td>
</tr>
<tr>
<td>Thunder Bay</td>
<td>12,092</td>
<td>272</td>
<td>2,965</td>
<td>422</td>
</tr>
<tr>
<td>Bruce</td>
<td>40,170</td>
<td>419</td>
<td>21,916</td>
<td>1,624</td>
</tr>
<tr>
<td>Total</td>
<td>270,067</td>
<td>6,067</td>
<td>53,723</td>
<td>28,767</td>
</tr>
</tbody>
</table>

Grand Total, All Livestock, Northern Ontario 358,624

The total livestock population is 358,624. This figure will be brought into discussion later in this presentation.

**The Veterinarian**

The veterinarian signs a contract with the Ministry of Agriculture and Food, Veterinary Services Branch. In the contract the veterinarian guarantees to provide veterinary service to the community under the following conditions:
1. Provide veterinary services to all eligible livestock owners on a priority basis.
2. Provide veterinary services to livestock owners under agreed on conditions to those residing outside the designated area.
3. The veterinarian is entitled to 3 weeks vacation each year.
4. The veterinarian may attend a course or courses relating to the practice of veterinary medicine—the time for such courses shall not exceed one week per year.
5. The veterinarian may attend association meetings related to the practice of veterinary medicine for a period not exceeding 3 days each year.
6. The veterinarian shall be licensed to practice in the Province of Ontario.

There are other clauses pertinent to the agreement that are not mentioned that deal with the protection of all parties concerned.

The administrative structure is composed of three separate parties, each with its own area of responsibility.

1. **The Local Committee**
   This committee is made up of livestock owners and the Agricultural Representative (county agent) serves as the secretary. The committee defines the boundaries, compiles a list of all eligible owners and is the sounding board for complaints.

2. **The Director of the Veterinary Services Branch of the Ontario Ministry of Agriculture and Food**
   is responsible for the selection of and the contract with the participating veterinarian. The local committee, the Director, and the veterinarian are required to meet together at least once a year. Such meetings deal with any complaints that may arise; alterations in boundaries, etc. In recent years the meetings have resulted in provisions for a second veterinarian and an additional grant for some of the communities. On very rare occasions a veterinarian has been removed from his participation in the program and a replacement has been necessary.

3. The veterinarian is obligated to attend the annual meeting and provide a report on his services. The veterinarian must give priority to serving the large animal practice and in most areas it is this phase of his practice that provides the bulk of his income.

*Computerized Disease Reporting Services*

As a sidelight to this program, each participating veterinarian is required to submit a specially designed form on each call that is made. This is processed for computer purposes, and quarterly and annual computerized printouts are available for all three parties concerned. In actual fact the data collection system has provided the basic experience for what will become very shortly a province wide program in Animal Disease Statistics.

*Special Practice*

There are 25 veterinarians involved in the Veterinary Assistance Pol-
icy for Northern Ontario. One of the veterinarians operates under special circumstances. This practice is located in the most remote of the areas that have been designated. It covers a sparsely settled area nearly 200 miles long and not very wide.

In this practice the practising veterinarian is a full time civil servant. His office and equipment, drugs, supplies, automobile and lay help are all paid for by Government. Calls are made and the farmers are charged the standard fees plus medicines. All revenue is turned in to the Government. The practice had an operating deficit of approximately $12,000 in 1972. However, it provides a service to an expanding livestock industry that would not otherwise be available.

Costs

<table>
<thead>
<tr>
<th>Veterinary Assistance Policy</th>
<th>Costs 1972</th>
<th>Revenue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Designated Areas</td>
<td>205,184.86</td>
<td>21,587.21</td>
</tr>
<tr>
<td>Special Practice</td>
<td>38,894.65</td>
<td>26,822.36</td>
</tr>
<tr>
<td></td>
<td>244,079.51</td>
<td>48,409.57</td>
</tr>
</tbody>
</table>

Net Cost of Program - 195,669.94

The net cost to Government for this program is $195,669.94. The total number of animals in the area serviced is 358,624. In round figures the cost to Government to provide this insurance to livestock owners is 54¢ per animal.
The following summary provides a detailed tabulation from the computer records of the number of calls and number of animals examined.

**Veterinary Assistance Policy for Designated Areas**

*A summarized report of Veterinary Services*

<table>
<thead>
<tr>
<th>Name of Unit</th>
<th>All areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months covered by report</td>
<td>January—December 1972</td>
</tr>
<tr>
<td>Number of calls for reporting period</td>
<td>462,588</td>
</tr>
<tr>
<td>Total mileage for reporting period:</td>
<td>22,789</td>
</tr>
<tr>
<td>No. Sick</td>
<td>No. Operations</td>
</tr>
<tr>
<td>Number of CATTLE examined</td>
<td>18466</td>
</tr>
<tr>
<td>Number of HORSES examined</td>
<td>1399</td>
</tr>
<tr>
<td>Number of SHEEP examined</td>
<td>370</td>
</tr>
<tr>
<td>Number of GOATS examined</td>
<td>42</td>
</tr>
<tr>
<td>Number of SWINE examined</td>
<td>551</td>
</tr>
<tr>
<td>Number of OTHERS examined</td>
<td>317</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>21145</strong></td>
</tr>
</tbody>
</table>

Total number of animals examined 41,238

*Operations include vaccinations*

The total number of animals treated last year was 41,238. Thus if we examine the statistics in another way we find that by dividing the number of animals treated into the net cost of Government of roughly $200,000. (actual $195,669.94) that the Government paid approximately $5. per animal treated. The farmer paid a reasonable fee to the veterinarian for his services but he did not have to pay a mileage rate to his farm. It makes no difference to the farmer whether the call is 4 or 40 miles from the office of the veterinarian.

The number of calls and animals treated varies a great deal. The following chart provides information on the high volume and low volume practices operating under the program.

**Total Animals/Calls/Averages**

<table>
<thead>
<tr>
<th>High</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total # of calls</td>
<td>157</td>
</tr>
<tr>
<td>Total # of animals treated</td>
<td>313</td>
</tr>
<tr>
<td>Total Mileage</td>
<td>4,675</td>
</tr>
<tr>
<td>Average Miles/call</td>
<td>10.8</td>
</tr>
</tbody>
</table>

**Other Areas in Canada**

There are other areas in Canada that provide veterinary service programs which should be mentioned.
The Province of New Brunswick

This province instituted a veterinary service program many years ago that involves all veterinarians in farm animal practice. All veterinarians are civil servants and are paid an annual salary. All costs are paid by Government and the farmer pays set fees for the services rendered. The veterinarian buys all drugs from a central depot and the prices are set by the Government. In effect the whole Province has a socialized veterinary medicine program. The plan has insured that veterinary service is available throughout the Province.

The Province of Nova Scotia

Nova Scotia, an adjoining Province, chose a program that leaves the free enterprise incentive in place. In this province all veterinarians in agricultural practice receive a basic grant for which they must provide service. Beyond that they carry out their practice just as any other private practitioner would.

The Province of Quebec

Two years ago the Province of Quebec instituted a "Veticare" program which is unique. Stated simply, the farmer pays $4 and the Government pays $6 for every call within a certain radius of the office of the veterinarian. As the radius extends the Government pays the increased charges with the farmer payment remaining constant at $4. This program is costing something over two million dollars a year. It appears that it has solved the problem of a shortage of Large Animal Practitioners.

The Province of Manitoba

The Province of Manitoba is encouraging veterinarians into large animal practice by a system of funding veterinary clinics throughout the province. The reports that I have heard indicate that their system is working well.

The Province of Saskatchewan

This province has a program somewhat similar to the program in Ontario.

The Province of Alberta

The Province of Alberta has a pilot program in operation where the Government provides a clinic for veterinarians to work from.

Conclusions

The program in Ontario has worked well since its inception in 1945. Essentially each practitioner operates as any private practitioner would. The free enterprise system is insured and the grant system provides for financial protection to the livestock owner in areas that would not support an independent veterinary practice. The structure or organization insures that there is input from all agencies concerned. One comment might be appropriate at this time. The structure and organization would imply that it is the veterinarian who received the subsidy of $8,000. plus mileage. In actual fact it is the livestock producer that is being subsidized. The participating veterinarians are very quick to point out this discrete difference.
COMMITTEE ON PROFESSIONAL RELATIONS

Chairman: R. C. Hammond, Silver Springs, Md.
Co-Chairman: N. B. Haynes, Ithaca, N.Y.


The Committee on Professional Relations met on Tuesday afternoon in the Baroque Room of this hotel. Actions resulting from the committee meeting are as follows:

1. The committee is pleased to have sponsored the paper on "The Veterinary Care of Remote Animals" by Dr. Neely from Ontario, and the paper on "Economic Rewards of Disease Eradication" by Dr. Weinland. They have discussed two of the special concerns of the committee.

The committee recommends that the USAHA make every effort to emphasize to the public the benefits of animal disease control as related to food products, public health and preventative medicine. Additional support would be forthcoming from a membership and a public well informed in these areas.

2. The committee views with concern the long interval between the annual meeting and the publication of the proceedings. Since the value of the proceedings is greatly reduced by late publication, the committee recommends that the Executive Board investigate the causes for the delay and take immediate action to correct them.

3. The committee recommends that the association publish a quarterly news letter to be sent to all members of the USAHA. In addition, a news packet should be sent to all state public relations officers, state VMA secretaries and extension veterinarians, plus the officers of specialty groups associated with animal health.

4. The committee recommends that the Federal Extension Service immediately fill the position of Federal Extension Veterinarian. This position has been vacant for over two years. Its being vacant has resulted in a serious break in animal health communications to the cooperative extension services of the various states.

5. The committee recommends that the USAHA commend the University of Missouri Veterinary continuing education system for their 1973 Symposium, designed specifically to meet the needs of public service veterinarians.
6. The committee recommends that the USAHA commend Livestock Conservation, Inc. for the excellent job they are doing in educating the public about animal disease programs.

7. The committee recommends that the chairman of each committee invite the officers of associated livestock groups to send someone to officially represent their organization in the committee meetings. We anticipate that they would participate in the committee meeting and take the recommendations back to their parent organization. In this way, ideas and knowledge can be quickly disseminated to allied groups. This would create new interest in the USAHA and would result in new members.

Mr. Chairman, these recommendations and decisions constitute the action of the committee on professional relations and they are respectfully submitted. We recommend their adoption.
REPORT OF THE COMMITTEE ON RABIES

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


The National Rabies Problem

There were 4427 laboratory confirmed cases of animal rabies in the United States during the past year. This incidence represents an increase of 14% above the preceding 5 year average for the United States, (Table 1 and Fig. 1). The disease was reported from Alaska and all the contiguous states for the second consecutive year (Fig. 2). Only Hawaii remains free from this disease. The stringent 4 month quarantine for all dogs and cats imported into the Hawaiian Islands has been a major deterrent to the introduction of rabies in Hawaii.

Wildlife, especially skunks, foxes, bats, and raccoons remains as the major reservoir of infection in the United States. Skunks accounted for 2,095 (47%) of all cases, foxes for 645 (15%), bats for 504 (11%), and raccoons for 162 (4%).

There were only 232 cases of dog rabies and 184 cases in cats. Cattle who are attacked primarily by rabid skunks and foxes accounted for 474 (11%) of the total number of rabid animals during 1972.

There were two humans who died of rabies in the United States during 1972. Only one of these cases developed from an exposure inside the United States, that in a 56 year old veterinary microbiologist from Temple, Texas who was apparently exposed by the aerosol route while working with a fixed strain of rabies virus. The other was in a 70 year old resident of California who was bitten by a rabid dog in the Philippines approximately one month before symptoms of rabies developed. Approximately 30,000 people who were bitten by proven or suspicious rabid animals received antirabies treatment during the year.

The continuing problem of rabies in the wild animals in the United States makes it clear that we cannot expect this disease to be self-limiting over a long period of time. Unless drastic changes in land use and conservation practices are implemented there is no reason to expect any real change in the incidence of rabies among the wildlife reservoirs in this country.
ACTIVITIES AND ACCOMPLISHMENTS

A. Follow-up on 1972 recommendations

1. A letter containing the Committee's three 1972 recommendations was mailed to each of the 50 state veterinarians. They were requested to discuss these recommendations with their staff and with their states public health officials and advise the Rabies Committee Chairman of their willingness to cooperate in these matters. The essence of these recommendations was to request: (a) Each state to adopt the uniform vaccination procedures as stipulated in the "Compendium of Animal Rabies Vaccines" by January 1, 1974, so vaccination procedures will be standardized throughout the United States. (b) To adopt a standard rabies vaccination certificate when developed by this Committee, hopefully by January 1, 1974. This certificate would be proposed as the only certificate required for interstate shipment of dogs and cats. (c) A final recommendation was that the "Compendium of Animal Rabies Vaccines" be revised at least biennially or as often as indicated by the development of new vaccines.

Generally, the state officials who responded agreed to cooperate with the Committee's recommendations. The specific comments of these state officials and members of the Committee were discussed and a revised "Official Rabies Vaccination Certificate" was presented for consideration by the Rabies Committee members. After discussion on the details of this certificate, it was decided that a new revision include relevant matters omitted from the previous one. When finalized, the Committee has requested the certificate be mailed to each state veterinarian and public health veterinarian asking for their comments. It now appears that the earliest date this final version of a standard rabies vaccination certificate can be adopted in the states will be January 1, 1975. As requested by the Michigan Public Health and Environmental Quality Committee, the USAHA Rabies Committee delegated the authority for developing this certificate to the U. S. Department of Health, Education and Welfare, Public Health Service. Dr. W. G. Winkler, Chief of the Rabies Control Unit, CDC, and a member of this Committee has accepted this responsibility. (Note: The Rabies Committee acknowledges the official endorsement that the USAHA Livestock Committee gave to this recommendation that the Official Rabies Vaccination Certificate be used in lieu of a health certificate.)

2. A revision of the Compendium of Animal Rabies Vaccines was presented for discussion in the 1973 Committee meeting. A final revision to include necessary changes, as brought out by the Committee, will be incorporated by CDC officials. This final version will be published as soon as possible, probably by January 15, 1974. This compendium is recommended by the USAHA Rabies Committee to each of the 50 states as a basis for standardizing animal rabies vaccination.
B. New Activities: Discussions and Recommendations

1. The Committee discussed some of the administrative problems of strengthening rabies and animal control programs at the state and local level. Getting the support of local veterinarians to implement the vaccination procedures as outlined in the compendium is a very difficult problem. Provisions for getting canine vaccination acceptance for longer than one year with accompanying certificates and tags to show this acceptance for longer than one year is likewise a problem. Further, proper legislation for updated rabies and stray animal control needs to be enacted in every state and local community. In order to solve these problems, the Committee recommends: (a) That each state and local veterinary medical association be asked to adopt these compendium recommendations which are also the same as those of the AVMA; (b) That public education to the veterinarians and to the public be undertaken by state and public health veterinarians and the state association's public health committees in order to standardize the vaccination and control practices in ever state; (c) That efforts be made to obtain state rabies control legislation in every state and that each state develop a model set of ordinances or rules and regulations to assist the communities in each state.

2. Discussions were held concerning a letter from Dr. Paul Doby, Illinois Department of Agriculture, to Dr. Sikes suggesting the Rabies Committee and Animal Welfare Committee of the USAHA be combined and the name "Companion Animal Population Committee" be designated. Reasons for and against such a change were presented. The Committee appointed a 3-man subcommittee to discuss this in more detail with Dr. Doby, Dr. Bendix, and others. Their findings will be presented and final action will be taken at the 1974 meeting of this Committee.

3. A new product, *Rabies Immune Globulin (Human Origin)*, will be available within a very short while. The Rabies Committee wants all veterinarians as well as physicians to be aware of this new product which can be used in post exposure Antirabies treatment. Use of this product will prevent serum sickness in people who must receive passive antirabies treatment.
REPORT OF THE COMMITTEE

Table 1
INCIDENCE OF RABIES IN THE UNITED STATES BY TYPE OF ANIMAL
1953–1972

<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>1,031</td>
<td>319</td>
<td>8</td>
<td>119</td>
<td>14</td>
<td>8,837</td>
</tr>
<tr>
<td>1954</td>
<td>4,083</td>
<td>62</td>
<td>1,023</td>
<td>1,026</td>
<td>347</td>
<td>4</td>
<td>118</td>
<td>8</td>
<td>7,282</td>
</tr>
<tr>
<td>1955</td>
<td>2,657</td>
<td>343</td>
<td>794</td>
<td>1,223</td>
<td>580</td>
<td>4</td>
<td>98</td>
<td>5</td>
<td>5,824</td>
</tr>
<tr>
<td>1956</td>
<td>2,592</td>
<td>371</td>
<td>794</td>
<td>1,281</td>
<td>631</td>
<td>4</td>
<td>126</td>
<td>10</td>
<td>5,846</td>
</tr>
<tr>
<td>1957</td>
<td>1,756</td>
<td>382</td>
<td>714</td>
<td>1,031</td>
<td>775</td>
<td>31</td>
<td>115</td>
<td>6</td>
<td>4,802</td>
</tr>
<tr>
<td>1958</td>
<td>1,643</td>
<td>353</td>
<td>737</td>
<td>845</td>
<td>1,005</td>
<td>68</td>
<td>157</td>
<td>6</td>
<td>4,814</td>
</tr>
<tr>
<td>1959</td>
<td>1,119</td>
<td>252</td>
<td>751</td>
<td>920</td>
<td>789</td>
<td>80</td>
<td>126</td>
<td>b</td>
<td>4,083</td>
</tr>
<tr>
<td>1960</td>
<td>697</td>
<td>277</td>
<td>645</td>
<td>915</td>
<td>725</td>
<td>88</td>
<td>108</td>
<td>2</td>
<td>3,457</td>
</tr>
<tr>
<td>1961</td>
<td>594</td>
<td>217</td>
<td>482</td>
<td>664</td>
<td>1,254</td>
<td>186</td>
<td>320</td>
<td>3</td>
<td>3,470</td>
</tr>
<tr>
<td>1962</td>
<td>565</td>
<td>232</td>
<td>614</td>
<td>594</td>
<td>1,449</td>
<td>157</td>
<td>114</td>
<td>2</td>
<td>3,727</td>
</tr>
<tr>
<td>1963</td>
<td>573</td>
<td>217</td>
<td>531</td>
<td>632</td>
<td>1,462</td>
<td>393</td>
<td>224</td>
<td>1</td>
<td>3,933</td>
</tr>
<tr>
<td>1964</td>
<td>408</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>4,704</td>
</tr>
<tr>
<td>1965</td>
<td>412</td>
<td>289</td>
<td>625</td>
<td>1,038</td>
<td>1,582</td>
<td>484</td>
<td>153</td>
<td>1</td>
<td>4,584</td>
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<tr>
<td>1966</td>
<td>412</td>
<td>252</td>
<td>587</td>
<td>864</td>
<td>1,222</td>
<td>377</td>
<td>183</td>
<td>1</td>
<td>4,198</td>
</tr>
<tr>
<td>1967</td>
<td>412</td>
<td>293</td>
<td>691</td>
<td>979</td>
<td>1,568</td>
<td>414</td>
<td>250</td>
<td>2</td>
<td>4,609</td>
</tr>
<tr>
<td>1968</td>
<td>296</td>
<td>157</td>
<td>457</td>
<td>801</td>
<td>1,400</td>
<td>291</td>
<td>210</td>
<td>1</td>
<td>3,613</td>
</tr>
<tr>
<td>1969</td>
<td>256</td>
<td>165</td>
<td>428</td>
<td>888</td>
<td>1,156</td>
<td>321</td>
<td>307</td>
<td>1</td>
<td>3,522</td>
</tr>
<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>
<tr>
<td>1971</td>
<td>235</td>
<td>222</td>
<td>484</td>
<td>677</td>
<td>2,018</td>
<td>465</td>
<td>289</td>
<td>2</td>
<td>4,392</td>
</tr>
<tr>
<td>1972</td>
<td>237</td>
<td>184</td>
<td>547</td>
<td>645</td>
<td>2,095</td>
<td>504</td>
<td>218</td>
<td>2</td>
<td>4,427</td>
</tr>
</tbody>
</table>

*a Data prior to 1958 from USDA, ARS; subsequent data from the USDA, ARS, and the NCDC
**1 patient recovered

Table 2. HUMAN RABIES DEATHS, UNITED STATES 1972

<table>
<thead>
<tr>
<th>Locality</th>
<th>Age</th>
<th>Sex</th>
<th>Nature of Exposure</th>
<th>Post Exposure</th>
<th>Incubation</th>
<th>Duration</th>
<th>Date of</th>
<th>Locality of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipolog City, Philippines</td>
<td>70</td>
<td>M</td>
<td>Biten on hands by dog</td>
<td>None</td>
<td>28 or 30 Days</td>
<td>14 Days</td>
<td>2/22/72</td>
<td>Fresno, California</td>
</tr>
<tr>
<td>Temple, Texas</td>
<td>56</td>
<td>M</td>
<td>Laboratory</td>
<td>None</td>
<td>12 Days</td>
<td>9 Days</td>
<td>3/9/72</td>
<td>Temple, Texas</td>
</tr>
</tbody>
</table>
CASES OF RABIES IN WILD AND DOMESTIC ANIMALS
1953–1972

Fig 2
NUMBER OF RABIES CASES REPORTED BY STATE, 1972

[Map showing cases by state with numbers indicated]
1973 Report of the 
COMMITEE ON IMPORT-EXPORT

Chairman: Dr. C. L. Campbell, Tallahassee, Florida 
Co-Chairman: Dr. C. K. Jewell, Trenton, New Jersey 

Paul D. DeLay, Hyattsville, Md.; John R. Langridge, Greenbelt, Md.; 
Philip D. Cazier, Lanham, Md.; M. G. Hynes, Dublin, Ireland; A. R. 
Miller, Falls Church, Va.; A. F. Kaufmann, Atlanta, Ga.; C. H. Pals, 
Arlington, Va.; W. L. Sulzacher, Beltsville, Md.; G. E. Cottral, 
Greenport, L.I., N.Y.; R. H. Rumler, Brattleboro, Vt.; J. J. Callis, 
Southold, L.I., N.Y.; D. H. Spangler, Lacey, Wash.; L. J. Goss, 
Cleveland, Ohio; Gus Griswald, Philadelphia, Pa.; Claude A. Smith, 
Hyattsville, Md.; H. M. Steinmetz, Washington, D.C.; F. E. 
Henderson, Baton Rouge, La.; G. B. Rea, Salem, Oreg.; Bert 
Hawkins, Ontario, Oreg.; Wilson Powell, Tallahassee, Fla.; Lucian 
Welty, Griffin, Ga.; Clint Booth, Dallas, Tex., Ross W. Gerding, 
Denver, Colo.; John H. Richardson, Atlanta, Ga.; Frank Harding, 
Geneva, Ill.

The Committee on Import-Export is highly gratified in the interest 
shown in the activities of the committee at this year's meeting, the fifth 
since its organization. In re-evaluating its functions, since there had been 
voiced some suggestion that it be combined with the committee on 
Foreign Diseases, the following points were made in rebuttal of this pro-
posal:

1. First and foremost, as was contained in the original charge to the 
committee, its function in outlining guidelines to prevent the 
introduction of foreign diseases.
2. Its role in furthering breed improvement in the importation of 
domestic animals.
3. Expanded markets, not only in this country but in foreign coun-
tries.
4. The emphasis which the committee can add to the expansion and 
 improvement of facilities at ports of importation and exportation.
5. The input which committee members lend to fortifying procedures 
 involving quarantine and diagnostic tools.
6. Its role of support to the Import-Export, Animals and Products 
 Services of APHIS in obtaining funds and personnel.

With the foregoing factors in mind it was the consensus of the com-
mittee to retain its autonomous identity.

The committee expressed its concern over the limited import facilities 
available for handling the ever increasing numbers of domestic livestock 
eligible for entry into the United States in the two import facilities pres-
ently available to handle this class of livestock. The existing accommo-
dations are simply not adequate.
It was pointed out that Congress on September 12, 1964, had authorized the disposition of the Clifton, New Jersey, import station to be replaced with another facility in the New York-New Jersey port area. However, during the nine intervening years, due to the varied road blocks encountered by the United States Department of Agriculture, little if any progress has taken place to culminate this matter. It was brought out in discussion that recommendations for the selection of a site to accomplish the intent of Congress have, on numerous occasions, been approved by the originating responsible agency, namely Animal and Plant Health Inspection Service, but the urgency of the project loses impact before reaching the highest level of authority in the Department.

Therefore, this Committee recommends that the Secretary of Agriculture be apprised of these problems which have resulted in lack of adequate facilities available to replace the Clifton port. The committee urges the Secretary to give prompt consideration to the acquisition of the necessary facilities, including even the possibility of rebuilding on the present site.

The Committee discussed the fact that some three or four years ago the Congress authorized the establishment of a maximum security import station through which livestock from foreign countries could safely be imported into the United States for the purpose of breed improvement. The membership was quite disappointed to learn that the Senate-House Conference Committee on Appropriations failed to implement this intent of Congress by refusing to provide funds requested in the fiscal 1974 budget for the construction of the Fleming Key, Florida, import station. Your committee recommends that U.S.D.A. re-submit a request for construction funds for this facility in such amount as is adequate in view of the escalation of construction costs and that this Association and national livestock organizations be urged to further endorse this proposal.

In the interest of promoting the foregoing projects, an action subcommittee was appointed for the purpose of coordinating the activities of the various livestock entities, so as to establish the interest of the Congress.

It was noted by the committee that the importation of dry milk from throughout the world has increased significantly and poses a threat of introducing dangerous exotic diseases. The current regulations permit the introduction of such products upon merely a signed statement that the product will not be diverted to livestock feeding. There is under consideration at the moment a proposed amendment to the Code of Federal Regulations which would make this product from foot-and-mouth countries a restricted product and could move under bond to an approved establishment for processing to adequately destroy dangerous viruses. This committee endorses this proposed amendment and requests the Administrator of the U.S.D.A. APHIS to expedite the implementation thereof so as to close this dangerous loophole.

The committee was pleased to hear from the Senior Staff Veterinarian of Import-Export, Animals and Products of APHIS Veterinary Services that his agency is beefing up its border security forces between Mexico
and the United States, and has enlisted the aid of Customs and Immigration Services in its patrol efforts to prevent the introduction of hog cholera and other diseases from Mexico into this country.

Respectfully Submitted

C. L. Campbell, Chairman
Your Committee on Animal Welfare met yesterday as scheduled with five committee members and twenty-two non-members present and submits the following report.

1.) There is a need to enact legislation to prohibit the selling and/or trading of exotic animals by dealers and pet stores to individuals:

ACTION: The Committee voted to support fully the American Veterinary Medical Association position on this subject.

REPORT OF THE COMMITTEE

Wild or Exotic Animals as Pets

RESOLVED, that AVMA strongly opposes the keeping of wild or exotic species of animals as pets and believes that all commercial traffic of these animals for such purpose should be prohibited.

Statement About The Resolution

People acquire skunks, raccoons, monkeys, alligators, and other exotic species as pets because they like to possess unusual pets or regard them as status symbols.

These exotic species create disease, diet, and exercise problems different from those of domesticated pets (dogs and cats).

Disposing of an exotic pet can be a traumatic experience with difficulty in relocating such animals. Frequently, zoos will not accept them and they are "too domesticated" to return to the wild, so euthanasia may be the only alternative. Therefore, veterinarians should exert influence to discourage the keeping of wild or exotic species as pets.

2.) There is a need to provide care for animals being transported by air:

ACTION: The Committee recommends that the United States Department of Agriculture seek legislation to amend the animal export
accommodations act to include air carriers under its provisions. The Committee further recommends that the Animal Welfare Act be amended to include common carriers exclusive of the requirements under the jurisdiction of the Federal Aviation Authority.

3.) There is a need for uniformity in method of rabies vaccination and the adoption of a uniform rabies vaccination certificate:

*ACTION:* The Committee recommends the adoption of rabies vaccination methods and the use of the uniform rabies vaccination certificate developed by the rabies committee of this association.

4.) There is a need for new research in the control of animal population:

*ACTION:* The Committee recommends support of various efforts to obtain adequate funding for research in animal birth control with particular emphasis on companion animals and predators.

The Committee recommends that the U. S. A. H. A. support Senate Bill #1388 the “Animal Research Act,” providing funds for animal health research.

5.) There is a need to amend the Horse Protection Act to improve the authority for more effective preparation of cases where violations are committed:

*ACTION:* The Committee recommends the support of Senate Bill #2093.
REPORT OF THE COMMITTEE ON
PARASITIC DISEASES AND PARASITICIDES

Chairman: J. D. Hourrigan, Hyattsville, Maryland
Co-Chairman: H. B. McGrath, Kansas City, Missouri


This committee met in open session on Wednesday to discuss progress and problems of interest to the committee.

COMMITTEE RECOMMENDATIONS

It was brought to the committee's attention that in studies at Washington State University, laboratory cats (111 cats including controls) exposed to commercial flea collars (2,2-dichlorovinyl dimethyl phosphate (DDVP)) exhibited a 42% incidence of an ataxia-depression syndrome with an 8% mortality. Whole blood cholinesterase was significantly (p=0.01) reduced in all DDVP collared cats. Cervical dermatitis occurred in 74% of DDVP collared cats. This information was presented by J. R. Keith Farrell. The committee, with Dr. Farrell's concurrence, elected to forward the data to the Environmental Protection Agency for their consideration and appropriate action.

The matter of recognizing Co Ral (coumaphos) as a permitted dip for use against psoroptes cattle scabies was discussed.

Dipping trials have been conducted at the Parasite Research laboratory (PRL), Albuquerque, New Mexico, and at Amarillo, Texas, using coumaphos against psoroptic cattle scabies.

Eight trials using 30 head of cattle were conducted at the PRL with concentrations ranging from 0.06% to 0.25% and either a 10-second or 1-minute immersion time in a cage vat. The results of the eight trials were failure. The trial at Amarillo used coumaphos at a concentration from 0.30% to 0.36% and the cattle were immersed twice in a portable vat. This trial was successful.

Research aimed at developing another efficacious pesticide is necessary. It would be desirable to have the pesticide effective against mites, grubs, screwworms, and ticks. A pesticide effective against mites and grubs would be desirable for feedlot use and would do much to prevent scabies in the feedlots.
The committee concluded that additional dipping trials are necessary and that this work should be done as soon as possible.

**PSOROPTIC SHEEP SCABIES**

The United States was declared free of psoroptic sheep scabies by Secretary Butz in an announcement at the annual meeting of the National Wool Growers in January, 1973.

The last reported case was in a flock of eight sheep in Gloucester County, New Jersey, which was confirmed on January 26, 1970.

The sheep scabies eradication program began with a quarantine on all territory west of the eastern border of North and South Dakota, Kansas, Nebraska, Oklahoma, and Texas in 1905.

An accelerated program began in August 1960. A total of 2,280 infected flocks was found during this program during which 230,379,906 sheep were inspected and 4,860,609 were dipped.

A total of 6,297,000 sheep was inspected during FY 1973 and no psoroptic scabies found.

This committee is pleased to have played a role in this program which has been successfully concluded.

**PSOROPTIC CATTLE SCABIES**

Psoroptic cattle scabies has been confirmed in a total of 144 herds from October 1971 through June 1973. These herds were located in Texas (64), New Mexico (32), Oklahoma (15), Kansas (14), Nebraska (8), Colorado (6), Iowa (4) and Idaho (1). In FY 1972, 91 infected herds were found and 53 in FY 1973.

A total of 59 counties was placed under quarantine during the above series of outbreaks beginning with the quarantining of 34 Texas Panhandle counties in December, 1971. The number of quarantined counties has been reduced to six—two in New Mexico and four in Texas.

So far in FY 1974, eight infected herds have been found in Nebraska (4) and one each in Arizona, Texas, Indiana and Iowa.

Inspections for FY 1973 were 39,181,970 as compared to 36,231,843 for the preceding year. A total of 2,692,377 head were dipped during FY 1973 as compared to 1,013,000 for FY 1972.

There was a total of seven herds with sarcoptic cattle scabies during FY 1973; however, there were two collections of sarcoptic mites from llamas and one from deer during the year.

There were no reported outbreaks of psorergatic cattle scabies during the year.

**CATTLE FEVER TICKS**

*Boophilus* spp. ticks were reported from 170 premises—one California and 17 Texas counties—during FY 1973. An area quarantine was estab-
lished by the Texas Animal Health Commission and the United States Department of Agriculture on portions of Jim Wells, Duval, Live Oak, McMullen, Nueces, and Kleberg Counties, Texas, in connection with the outbreak discovered at Alice, Texas, August 22, 1972. A total of 71 infestations was associated with this outbreak and 434 premises were quarantined as infested, exposed, or adjacent.

Infested herds and Texas counties involved in the Alice outbreak included Jim Wells (27), Duval (13), Dimmit (1), Houston (2), Kent (1), Nueces (10), Kleberg (14), Live Oak (1), and De Witt (1).

In the border quarantine area *Boophilus* spp. were found on 35 premises. In addition to the Alice outbreak, 40 infested premises were found. Of 121 stray Mexican horses apprehended 21 were infected as was one of 88 stray Mexican horses.

A total of 1,470,525 cattle and 45,808 horses were inspected during FY 1973; 390,788 cattle and 22,310 horses were dipped or sprayed.

A single outbreak of bovine piroplasmosis was reported from Cameron County, Texas. The involved herd was located in a pasture that was surrounded on three sides by the Rio Grande River. It was determined that it was caused by *Babesia argentina*.

A test animal at Texas A & M was treated and saved using imidocarb. This drug has not been cleared for use and animals treated with it cannot be slaughtered for food.

The interval between dipping for *Boophilus* has been extended to 18 days in Texas. The recommended strength of coumaphos is 0.125% to 0.175% when animals are dipped at 14-day intervals and 0.20% when the interval is greater than 14 days, spraying is used, or it is a single treatment for movement.

The two ranges for Delnav are 0.125% to 0.140% and 0.140% to 0.150% with the restrictions on time and method the same as for coumaphos.

Toxaphene has been added as a permitted tickicide during the past year. It is used at a recommended strength of 0.50% to 0.60%. It has replaced arsenic in the border vats at Del Rio, Eagle Pass, Laredo, and Reynosa.

Research on field tests, methods of delivering pesticides, and disposal of used pesticides would be highly desirable.

**CATTLE FEVER TICK RESEARCH ACTIVITIES**

Dr. O. H. Graham, Agricultural Research Services, Kerrville, Texas, reviewed subject research activities.

Although in South America, Australia, Africa, and other regions serious problems have occurred in regard to *Boophilus* spp. ticks developing resistance to pesticides, Dr. Graham’s work has not demonstrated that *Boophilus* spp. ticks in Texas or Mexico are resistant.

Both *B. annulatus* and *B. annulatus* and *B. microplus* interbreed readily. Although hybrid male sterility exceeded 99%, only about 50%
of the female hybrids were sterile. The purpose of this work is to learn if the phenomenon can be used as a biological method of controlling *Boo-
philus* spp. ticks.

**SOUTHWEST SCREW WORM PROGRAM**

A total of 95,642 cases of screwworm were confirmed in the United States during 1972 which was higher than it had been since the program began in the southwest in 1962. The southeast reported 2 confirmed cases in Florida, 1 in Georgia, 2 in Alabama, 2 in Louisiana and 6 in Arkansas, all of which were prevented from becoming established.

The present year has been much improvement in Texas where the problem was primarily in 13 southern counties. A total of 3,692 cases were confirmed in Texas during the first 9 months of 1973. The number of cases in the Western States increased with 3,713 cases confirmed in Arizona, 163 in California and 981 in New Mexico during the same period.

There were also 5 cases in Nevada, 1 in Iowa, 1 in Colorado and 1 in Utah.

**PUERTO RICO AND THE VIRGIN ISLANDS**

The U. S. Virgin Islands and the British Virgin Islands were declared screwworm free as of November 18, 1972. The Island of Vieques, has been free of screwworms since June 28, 1973.

On July 1, 1973, the U. S. Air Force transferred responsibility for the fly delivery system from Special Operations Forces to the Air Force Reserve. This move provided for the direct transport of flies from Mission, Texas to Puerto Rico.

**MEXICO**

As a result of the joint agreement signed by Secretary Butz and Secretary Aguirre, Secretaries of Agriculture from their respective countries, a Joint United States-Mexico Screwworm Eradication Commission was appointed. The Commission has established offices in Mexico City, Mexico, has selected a site for production facilities near Tuxtla Guti-
errez, Chiapas, in the Isthmus of Tehuantepec, and is currently selecting and recruiting personnel. It is anticipated that sterile flies will be available from the Mexican facility by March, 1975.

**RESEARCH NEEDS**

The Southwestern Screwworm Eradication Program has the following research needs:

1. An attractant to lure screwworm flies into field surveillance traps.
2. Oviposition attractants for use in the laboratory and in the field.
3. It is very important that native larval collections be developed and
colonized for production strains which retain broad-base, natural genetic variability.

4. Nutritional studies for larvae-rearing diets.

5. A more universal pesticide which is effective against screwworms, along with ability to kill and control other parasites, such as ticks, mites, and cattle grubs which are serious animal pests. The pesticide must be effective and yet provide minimal residue for environmental impact.

PESTICIDE USE

Public Law 92-516, "Federal Insecticide, Fungicide, and Rodenticide Act," as amended which was signed into law October 21, 1972, provides for the following with respect to the application of pesticides.

SEC. 2. DEFINITIONS.

(e) Certified Applicator, Etc.

(1) Certified Applicator—The term “certified applicator” means any individual who is certified under section 4 as authorized to use or supervise the use of any pesticide which is classified for restricted use.

(2) Private Applicator—The term “private applicator” means a certified applicator who uses or supervises the use of any pesticide which is classified for restricted use for purposes of producing any agricultural commodity on property owned or rented by him or his employer or (if applied without compensation other than trading of personal services between producers of agricultural commodities) on the property of another person.

(3) Commercial Applicator—The term “commercial applicator” means a certified applicator (whether or not he is a private applicator with respect to some uses) who uses or supervises the use of any pesticide which is classified for restricted use for any purpose or on any property other than as provided by paragraph (2).

(4) Under the Direct Supervision of a Certified Applicator—Unless otherwise prescribed by its labeling, a pesticide shall be considered to be applied under the direct supervision of a certified applicator if it is applied by a competent person acting under the instructions and control of a certified applicator who is available if and when needed, even though such certified applicator is not physically present at the time and place the pesticide is applied.

EFFECTIVE DATES OF PROVISIONS OF ACT

(4) A period of four years from date of enactment shall be provided for certification of applicators.

(A) One year after the enactment of this Act the Administrator shall have prescribed the standards for the certification of applicators.

(B) Within three years after the enactment of this Act each State desiring to certify applicators shall submit a State plan to the Adminis-
trator for the purpose provided by section 4(b).

(C) As promptly as possible but in no event more than one year after submission of a State plan, the Administrator shall approve the State plan or disapprove it and indicate the reasons for disapproval. Consideration of plans resubmitted by States shall be expedited.
1973 REPORT OF THE COMMITTEE ON EVALUATION
AND DEVELOPMENT OF STATE-FEDERAL PROGRAMS.

Chairman: J. L. O'Harra, Reno, Nevada
Co-Chairman: J. G. Milligan, Montgomery, Alabama


Meeting: Tuesday, 1:30, Room 1041

The Committee met with 28 members and visitors in attendance. The Committee discussed in depth many pertinent aspects of the various State-Federal programs and reports as follows:

SCABIES PROGRAM

Dr. Hourrigan reported there were 150 outbreaks in eight states between October 1971, and April, 1973. During this outbreak 59 counties were placed under quarantine. Resistance to the program and increasing lack of industry support has created increased incidence of the infestation. To continue and complete this program, strong support from industry to congress and otherwise is vitally important. Embargos on a collective basis by receiving states would place the program in a much stronger position. This committee agrees with U.S.D.A. policy of instigating a strong quarantine program with meaningful releases when State and Federal Officials are in agreement that the problem no longer exists. The Committee has endorsed a resolution to this effect which will appear in the Resolutions Committee, U.S.A.H.A. report. An increased Public Relations and educational program to the industry and greater cooperation from meat inspection in ante-mortem procedures are needed for detection. U.S.D.A. plans to proceed along this line.

BRUCELLOSIS

We are in agreement the Brucellosis program has regressed during the past two years. It is vitally important that all states be cognizant of the uniform methods and rules and it is vital that each state comply with these directives to the fullest extent to attain success. Reversion of Brucellosis funds and reassignment of Federal personnel to other programs has seriously crippled the eradication efforts aimed at Brucellosis.
This Committee has been assured by U.S.D.A., that funding and manpower will be held constant and increased in certain areas to further the program. The Committee concurs with U.S.D.A. that information on deficiencies in any State be made public on any program.

ACCREDITATION

The Committee recommends that U.S.D.A. develop uniform indoctrination procedures for veterinarians receiving accreditation in all 50 states.
Chairman: O. H. Timm, Dixon, California

The Committee wishes to express appreciation to all Federal personnel who gave freely of their time and efforts at this meeting and we hope that mutual benefits will result for all agencies and associations concerned. The State-Federal Relations Committee presents the following statement for mutual consideration and guidance.

Veterinary Science
The group was appreciative of the increasing and informative discussion with Dr. Paul DeLay, Assistant Administrator, of ARS Livestock and Veterinary Services Division.

Considerable time was spent on the recent reorganization of the Division. The Committee is hopeful that increased man-hours can be spent at the research level as a benefit of the reorganization. Also, we feel that the decline in ratio of technicians to scientist is reducing scientific output. It should also be noted that only by shifting manpower will the Toxicology and Entomology Research facility at College Station, Texas be manned to one-third of the planned capacity.

Again, we wish to emphasize the need for protection against the introduction of exotic diseases. We feel the development of the school at Plum Island and the planned Vaccine Pilot Plant at Plum Island are both important projects in achieving such protection.

The Committee wishes to emphasize the increasing importance becoming evident in the area of human-animal related health problems. In this area the research being accomplished by the Division on Mycotoxins should be continued. Also, the work on the chemical structure of plants at the Logan, Utah station has an impact on knowledge of Animal nutrition. This project should also be continued.

Tho it appears that there will be no funding available for new research, we wish to emphasize that there still remains a need for research in the following areas:

Weak Calf Syndrome
Respiratory Diseases of Cattle
Reproductive Problems in Sheep
Respiratory and Reproductive Problems in Horses

The Committee has noted the decline in the budget and warns that if in the future should the budget remain constant that the inflation in costs which has occurred and probably will continue can only result in a reduction in the services which the Division will be able to offer.

Veterinary Services

Our Committee this year feels there is no need to discuss individual program funding because the budget constraints have rather tightly limited program expansion. We know that the needs and goals of all cooperative Animal Health Programs are realistic and objective. They have all been worked out over the years through the cooperation of USDA and USAHA.

There appears to have been some misunderstanding on the part of Veterinary Biologics in regard to several committee reports presented at the Annual USAHA meeting. This committee strongly supports the efforts of Veterinary Biologics to assure the Veterinary profession and the livestock and poultry industries that biologics being marketed are safe and efficacious.

The committee on Biologics of USAHA is charged with "the responsibility for reviewing the status and results of testing activities of the Veterinary Biologics Disease Laboratory and keeping the industry and the profession apprised of new developments relating to biological products and alerting them to any potential problems. This group should be instrumental in evaluating the efficacy and safety of biologics based on available data and report their findings to the USAHA membership." It was never the intention of USAHA to recommend the lowering of standards for testing Veterinary Biologics.

It has become evident during the past year that present constraints on manpower within the USDA and the difficulties the individual states are experiencing in obtaining funds and manpower have decreased our working field force to a point where we can no longer maintain program standards.

The combined goal of USDA and USAHA has always been the protection of the livestock producer and the consumer against disease losses. Our cooperative regulatory programs have proven their effectiveness over the years. We are approaching a point where these cooperative programs can no longer meet the challenges they are presently facing. Just last year we commended Secretary Butz for advancing the Brucellosis eradication goal from 1978 to 1975. The emergencies during the past year have caused diversion of manpower and funds from Brucellosis has diminished the real thrust of the program so that the 1975 goal is no longer realistic. The same situation has occurred on previous occasions and with other diseases. We are increasingly concerned about the image created for Animal Health regulatory programs when established goals
are changed and extended. The industry and public soon lose faith, not only in the individual program, but in all animal health regulatory programs.

Our committee has been greatly concerned that the industry is questioning the integrity of the cooperative programs when we fail to maintain established program standards and this becomes a factor in subsequent disease outbreaks.

Associated with our lack of sufficient manpower to maintain animal health program standards is the increasing use of manpower to enforce laws such as PL 89-544, PL 95-579 and PL 91-540. While these programs have been mandated by the Congress at the insistence of the special interest groups, it is our feeling that our animal health programs are more important economically as well as from the standpoint of consumer and health protection.

Several months ago our committee was apprised again of the restrictions place on USDA-APHIS by the manpower ceiling and that the situation would not change in the foreseeable future. It was suggested by some of our committee members that a thorough study of manpower utilization needed to be completed. Suggestions were made that a regional concept similar to that employed by other Government agencies be studied, consideration be given for more contractual services with the states similar to Project four and that adjustments be made to increase the working field force and decrease the administrative and supervisory ratio.

We appreciate the efforts on the part of the Veterinary Services Staff in proposing a complete reorganization. This proposal came as a complete surprise to this committee and we have not had sufficient time and discussion to evaluate fairly and completely the possible effect and impact the implementation of this proposal will have on our present and future program needs.

It is the committee's firm conviction that with the impact this proposal has had on our cooperating officials in the individual states that in order for our association to lend support to this reorganization, it is imperative that representatives from Veterinary Services meet with the 50 state veterinarians simultaneously as soon as possible to present all of the details of the proposal including location of district and area offices. To assure maximum attendance by the state veterinarians this meeting should be at the expense of APHIS. This committee without such a meeting feels they are not well enough informed to firmly support the proposal or recommend it to the USAHA membership. At this time many doubt whether this program will in fact increase our field work forces and improve our management-work force ratio. There are misgivings about the proposal to join record keeping clerical help from present V. I. C. structure to the state office. It is the feeling of some that this could create more problems than it would solve unless greater use of contractual services is made so that the state veterinarian and his staff can exercise
more authority supervision. Additional questions regarding the implementation of this program will be presented at the meeting with the state veterinarians.

**APHIS, Meat and Poultry Inspection Program**

The USAHA State-Federal Relations Committee wishes to express our sincere appreciation to Dr. Wise, the new Associate Administrator for ANH and the Meat and Poultry Inspection Service, for making it possible for the several members of the Meat Inspection Service to take the time to present to us the status of the present program. We are indeed grateful to Dr. Fullerton, Dr. Jim Payne, Dr. Vic Berry, Dr. Harry Mussmon, Dr. Jerry Synder, Dr. Spauldin, Mr. Charles Paul, Mr. Ralph Johnson and Mr. Henry Cooke for their presentation of the present program activities.

The status of the Federal-State Cooperative Program and the report of the number of participating Talmadge-Aiken establishments plus the report on the new comptibly federal inspected states was indeed interesting.

The USAHA committee wishes to express with pleasure the noticeable improvement in the climate for discussions of meat and poultry inspection programs. The dialogue was carried on in a much more relaxed and cooperative spirit. We feel that much has been accomplished in the Meat and Poultry Inspection Service by such meetings as we are now discussing.

We would like to recommend to the APHIS Administrator that steps be taken to repeat and perhaps increase the number of meetings of Federal-staff inspection personnel with their state inspection counterparts such as the meeting held at the USAHA meeting in Miami last November. We feel that thought should be given to holding meetings on a regional basis in order that the cooperative relationship may be further enhanced.

The committee is pleased with the combination of the ANH and Meat Inspection Service into one single Associate Administrator relationship. With the improved system of animal identification, as it seems to be developing, and since the meat inspection service is now a responsibility of APHIS, we trust the surveillance programs which rely so much on good animal identification methods will play an even greater role in our disease identification and eradication program nationwide. We recommend that all state inspection programs take full advantage of the disease eradication possibilities presented by this program.

The committee was much interested in Dr. Fullerton's remark that perhaps the meat and poultry V. I. C. office might eventually be merged with the state administration. We are not quite clear as to what might be anticipated along these lines. We do express concern that in the program of decentralization of management of cooperative programs that everyone concerned not lose sight of the necessity of one standard of methods
and rules for meat and poultry inspection with one administrator responsible for the activity.

We would like to request that USDA explore further with the Congress the possibility for the development of a meat inspection program whereby all establishments would have equal rights in commerce.

The committee feels that the present system of residue sampling of meat products still leaves much to be desired. We are pleased with the decrease of lapse time for the collection of samples and the time required for analysis by the USDA laboratories and in the time lapse in reporting back to the state. The individual states are aware of their responsibility in investigating and procurement of evidence in cases of excessive or detectable residue.

The committee feels that many of these chemicals that may possibly be detected are extremely important to Agriculture. Therefore, we were much pleased to have reported to us that the Bureau of Veterinary Medicine of FDA feels the prosecution of violators after proper investigation and study as to prevalence of a residue drug or chemical is much preferred rather than the withdrawal of such chemicals and drugs from the market place. The Committee finds this approach very sound and reasonable and an acceptable program. We recommend that USDA-USAHA and FDA collaborate in further developing a sound workable program for the elimination of possible residues.
REPORT OF THE COMMITTEE ON RESOLUTIONS, NOMINATIONS AND INTERNAL AFFAIRS

Chairman: John C. Shook, Mechanicsburg, Pennsylvania; M. D. Mitchell, Pierre, South Dakota; C. L. Campbell, Tallahassee, Florida; J. L. O’Harra, Reno, Nevada; J. F. Quinn, Lansing, Michigan; T. J. Grennan, Jr., Providence, Rhode Island.

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

PRESIDENT: Mr. Olin H. Timm—of California
PRESIDENT-ELECT: Dr. J. F. Andrews—of Georgia
FIRST VICE PRESIDENT: Dr. H. E. Goldstein—of Ohio
SECOND VICE PRESIDENT: Dr. A. E. Janawicz—of Vermont
TREASURER: Dr. J. C. Shook—of Pennsylvania

REGIONAL INDUSTRY MEMBERS — Francis Buzzell—Maine; E. S. Bryant—Connecticut; J. O. Pearce—Florida; J. Finley—Texas; Bob Laramore—Wyoming; Olin Timm—California; J. R. Bishop—Indiana; Ward Van Horn—South Dakota.

Nominations presented to general membership on Tuesday morning, October 16, 1973. Posted on Association bulletin board until Wednesday morning, October 17, 1973 and acted upon at Wednesday’s general session as provided in the Association’s Constitution.

Slate unanimously elected as presented.
Resolution No. 1

United States Department of Agriculture

Seventy-seventh Annual Meeting

Held at St. Louis, Missouri

Dates October 14 - 19, 1973

Source

Subject Matter Individual Identification of Animals

Background Information

Permanent identification of individual equines (horses) is essential for efficient disease prevention and control and for animal certification. Several such systems are being administered by states, breed registries, etc., and new ones are under development. While these may meet the needs of the individual units using them, they are of limited value because they are not nationally coordinated, and the data generally is not retrievable.

A national computer center is being established by the United States Department of Agriculture at Fort Collins, Colorado, with a capability of supporting a national individual identification system for all livestock, which should be utilized.

Resolution

Therefore, be it resolved that the United States Department of Agriculture is urged, in cooperation with appropriate states and industry organizations, to develop a plan encompassing the foregoing systems and assets with a view to getting as many horses as possible sufficiently identified and recorded in retrievable data form.

The United States Department of Agriculture is urged to accept the responsibility for developing this proposed cooperative program.

The foregoing is in concert with recommendations of the International Livestock Brand Conference covering all classes of livestock, and it is believed that the horse industry, due to its size and the existing organizations therein, could well serve as the model in developing programs for the livestock industry.

Secretary
BACKGROUND INFORMATION

Livestock disease-eradication programs are, and must be, based on surveillance of livestock populations. Existing methods of livestock identification needed to trace individual animals to herd or flock of origin lack the necessary degree of efficiency and effectiveness. Work at the Los Alamos Scientific Laboratory has resulted in the development of an electronic animal identification and data-retrieval system that appears to overcome these deficiencies.

RESOLUTION

Therefore, be it resolved that the United States Animal Health Association urge the Secretary of the United States Department of Agriculture to fund to completion the additional research and development needed to make available at the earliest possible time a practical electronic animal identification and retrieval system that is national in scope, such system to be adaptable to computerization and capable of serving the needs of the livestock industry and assisting allied private and government service groups serving the livestock industry and the public interest.
UNITED STATES ANIMAL HEALTH ASSOCIATION

Resolution No. 3

Seventy-seventh Annual Meeting

Held at St. Louis, Missouri Dates October 14 - 19, 1973

Source

Subject Matter Animal Health Research Act (Senate Bill 1388)

BACKGROUND INFORMATION

The Animal Health Research Act was sponsored by the American Veterinary Medical Association, Associated Deans of Veterinary Colleges, directors of state experiment stations, and numerous breed associations. Its objective is to provide funds for animal-health research in veterinary colleges and experiment stations. It will distribute funds to states upon the basis of livestock value and research capacity.

Companion legislation has been presented to both Houses of the Congress, by Representative Melcher and Senator Talmadge.

RESOLUTION

Therefore, be it resolved that the United States Animal Health Association endorse Senate Bill 1388, The Animal Health Research Act, to enable the Secretary of the United States Department of Agriculture to encourage and assist the several states in carrying out a program of animal health research.

Secretary
Resolution No. 4
Seventy-seventh Annual Meeting
Held at St. Louis, Missouri Dates October 14 - 19, 1973
Source USAHA Committee on Foreign Animal Diseases
Subject Matter Membership in Office of International Epizootics

BACKGROUND INFORMATION

The Committee on Foreign Animal Diseases calls attention to the fact that its resolution presented to the seventy-sixth annual meeting in 1972, which reads in part, "be it resolved that the United States Animal Health Association urges that the Secretary of the Department of State take steps for the United States to become a fully participating active member of the Office of International Epizootics as soon as possible," has not resulted in the desired action by the Department of State. With the increasing amount of international commerce in animals and animal products, there is an even greater urgency for United States participation in the activities of the Office of International Epizootics (OIE). This Committee feels that it is extremely desirable to obtain the widest possible participation of all segments of the United States livestock industry toward achieving this goal.

RESOLUTION

Therefore, be it resolved that the United States Animal Health Association solicit the endorsement of the many individual segments of the United States livestock industry in urging the United States Department of State to cause the United States to become an official member of the Office of International Epizootics at the earliest possible date.

[Signature]
Secretary
BACKGROUND INFORMATION

In view of the fact that both Boophilus annulatus and Boophilus microplus are present in some southern counties of Texas and the annual importation from Mexico of approximately 700,000 cattle, many of which are infected with bovine babesiasis (Texas fever), there is constant danger of an outbreak of this disease in the United States.

RESOLUTION

Therefore, be it resolved that the United States Department of Agriculture is urged to provide more support, not only to eradicate the ticks, but also to tighten control on illegal entry of livestock and to further research into improved methods for the prevention and control of babesiasis. It is further resolved that the United States Animal Health Association send copies of this resolution to the following organizations:

American National Cattlemen's Association
Texas and Southwestern Cattlemen's Association
American Quarterhorse Association
Associated Milk Producers, Inc.
United States Treasury, Customs Bureau
UNITED STATES ANIMAL HEALTH ASSOCIATION

Resolution No. 5 Seventy-seventh Annual Meeting

Held at St. Louis, Missouri Dates October 14 - 19, 1973

Source

Subject Matter Texas Fever

BACKGROUND INFORMATION

In view of the fact that both Boophilus annulatus and Boophilus microplus are present in some southern counties of Texas and the annual importation from Mexico of approximately 700,000 cattle, many of which are infected with bovine babesiosis (Texas fever), there is constant danger of an outbreak of this disease in the United States.

RESOLUTION

Therefore, be it resolved that the United States Department of Agriculture is urged to provide more support, not only to eradicate the ticks, but also to tighten control on illegal entry of livestock and to further research into improved methods for the prevention and control of babesiosis. It is further resolved that the United States Animal Health Association send copies of this resolution to the following organizations:

American National Cattlemen's Association
Texas and Southwestern Cattle Raisers Association
American Quarterhorse Association
Associated Milk Producers, Inc.
United States Treasury Customs Bureau

Secretary
UNITED STATES ANIMAL HEALTH ASSOCIATION

Resolution No. 6. Seventy-seventh Annual Meeting
Held at St. Louis, Missouri Dates October 14 - 19, 1973
Source
Subject Matter Imidocarb (4A65) Therapy Against Babesia Caballi

BACKGROUND INFORMATION

There is a need for a chemical agent that will obliterate the carrier state of Babesia caballi in horses and other equidae.

The availability of a practical therapeutic agent will aid in the interstate and international commerce of horses and other equidae.

RESOLUTION

Therefore, be it resolved that the Secretary of Health, Education, and Welfare be urged to give high priority to the approval of Imidocarb Di-isothiuronate (4A65) as a sterilizing chemotherapeutic agent against Babesia caballi when adequate data are presented.

__________________________
Secretary
UNITED STATES ANIMAL HEALTH ASSOCIATION

Resolution No. 7 Seventy-seventh Annual Meeting
Held at St. Louis, Missouri Dates October 14 - 19, 1973
Source Committee on the Evaluation and Development of State-Federal Programs
Subject Matter Psoroptic Cattle Scabies

BACKGROUND INFORMATION

This committee has been made aware of efforts being made by Veterinary Services, United States Department of Agriculture, to publish in the Federal Register a statement of consideration which in effect would affirm a policy to promptly place a Federal quarantine on counties in which an outbreak of psoroptic cattle scabies is confirmed and not lift such quarantine until State and Federal officials of the state concerned have assured the Department that epidemiological studies of the outbreak have been completed, that the disease has been eradicated from the herd or herds concerned, and that the disease no longer exists in the county.

RESOLUTION

Therefore, be it resolved that the United States Animal Health Association support this effort and, through its Executive Committee, urge the United States Department of Agriculture to implement this policy as soon as possible. Such a policy will assist in providing uniform guidance for quarantine procedures when psoroptic cattle scabies occurs.

Secretary
BACKGROUND INFORMATION

Before a practical program for the control and eradication of anaplasmosis in the northwestern United States can be developed, additional epidemiological studies are necessary. The important vector or vectors must be determined, the source of vector infection and natural transmission to cattle must be clarified, and the practicability of eliminating Anaplasma marginale infection from cattle and returning them to infected ranges must be decided.

RESOLUTION

Therefore, be it resolved that such additional research be encouraged and that emphasis be placed upon identification of both Anaplasma marginale vectors and reservoir hosts, methods of transmission, and practicability of eliminating the latent carrier state from cattle pastured on infected ranges.
BACKGROUND INFORMATION

The USAHA Committee on Infectious Diseases of Cattle, through one of its subcommittees, has previously recommended revisions in the original proposal to regulate the interstate movement of bovine semen in commercial use for artificial insemination. The need for the protection of the livestock industry through such regulations is urgent.

RESOLUTION

Therefore, be it resolved that the United States Animal Health Association urgently recommend that the United States Department of Agriculture implement both interstate and import regulations setting forth specific health standards for sires from which bovine semen is collected for such use.
BACKGROUND INFORMATION

The health and well-being of the American people depend on a wholesome meat supply, and the livestock industry endeavors to supply the American consumer with the highest quality product at the lowest cost possible. The control and eradication of disease is the single most important factor in accomplishing this goal, and when special disease problems occur that are sometimes short of the national emergency category but of sufficient urgency to warrant immediate and expedient attention, these outbreaks and special problems have in the past been funded by robbing budgeted disease-control and eradication projects already in progress, and the gains made in these programs have been lost and have had to be repeated following each interruption.

Reference is made to the policy of fighting fires, which at the moment of their discovery are fought with all the tools and equipment necessary for the earliest confinement and expulsion of the conflagration. When the emergency work has been completed, documented expenses are listed and presented to the Congress for payment. This would allow existing budgeted programs to continue at a regular and calculated pace rather than with the continued disruption and loss of progress.

RESOLUTION

Therefore, be it resolved that the United States Animal Health Association urges that the funding of major disease outbreaks be obtained in a manner which does not deplete the resources set aside for regularly programmed projects.
BACKGROUND INFORMATION

In many southern and southeastern states, bovine brucellosis eradication has become increasingly difficult, and satisfactory progress in eradication is not being accomplished.

RESOLUTION

Therefore, be it resolved that the United States Animal Health Association urge the Animal and Plant Health Inspection Service, United States Department of Agriculture, to classify such areas as critical areas. It is further urged that the Animal and Plant Health Inspection Service aid these areas with personnel, money, and material, without jeopardizing other disease-eradication programs, and that properly trained and supervised lay personnel be employed as much as possible for this purpose.
Infectious diarrhea of newborn calves is a major disease problem in both dairy herds and in beef cow-calf herds. Mebus et al. (1) produced neonatal calf diarrhea in experimental calves with field fecal material and with bacteria-free filtrates prepared from feces of the above experimental diarrheic calves. The incubation period was approximately 12-14 hours. The initial technique used for reproducing calf diarrhea (scours) was by surgical approach through a duodenal cannula. After three passages by way of the duodenal cannula in calves, oral passage of the virus was employed. Hysterectomy-derived calves free of E. coli developed severe diarrhea following inoculation with a bacteria-free filtrate. These calves were kept in a hysterectomy hood, inoculated and fed autoclaved milk. Electron microscopy revealed the presence of viral particles in feces from experimental diarrheic calves. The particles had a diameter of approximately 65 m.

Conjugates were prepared in two white rabbits and a calf with viral antigens prepared from feces of two gnotobiotic diarrheic experimental calves. These conjugates produced bright cytoplasmic fluorescence in villous epithelial cells in sections of upper, middle and lower areas of small intestine from experimental calves. Smears of intestinal contents stained by the same procedure also contained fluorescing cells. Smears stained with Wright's stain revealed these cells to be epithelial type.

The neonatal calf diarrhea virus was adapted and propagated in cell culture (2)(3).

A pathogenesis study (4) of this agent in gnotobiotic calves revealed that virus infected the villous epithelium of the small intestine primarily. These studies also revealed that with progression of the disease (diarrhea), the epithelium on the villi of the small intestine changed from a columnar type to a low cuboidal type. The lamina propria of these altered villi also revealed an increase in reticulum-like cells. Viral titers were $10^6$ and $10^8$ in colonic contents of two calves inoculated with cell culture adapted virus and necropsied 2 and 6 hours after onset of diarrhea. A physiochemical characterization of the virus was made (6) (3). The results suggested that the virus may belong to the reovirus group.

With the development of a fluorescent antibody (FA) diagnostic test
(1) an initial survey (6) was made to determine the incidence and distribution of this viral agent. The virus was detected in 69 Nebraska herds in 1968 and 1969. During this same study positive herds were found in South Dakota, Illinois and California.

Limited experimental studies in newborn calves indicated that calves exposed orally to an attenuated virus propagated in bovine kidney cells were protected when challenged 72 hours postvaccination to virulent reo-like virus. These findings led to field trial studies in spring of 1971 (7). An approval was obtained from the State Veterinarian for such a study. The vaccine was prepared by the department and tested in calves for safety and potency prior to release in the field.

Calves were vaccinated by oral administration of approximately 5 ml of cell culture fluid. Herds in which calves were vaccinated were in two categories:

1) 18 herds in which the reo-like virus had been found in previous years; and
2) 17 herds in which calves were currently having diarrhea. The virus was found before vaccination in diarrheic feces in all the herds in this category except 2. In one of these two herds the virus was found in 1970 but not in diarrheic feces in 1971.

In the first category calves were vaccinated within 24 hours after birth. In category two, when vaccination was started all calves which had not had diarrhea and were less than 5 days old were vaccinated; all subsequent calves were vaccinated within 24 hours after birth. Effectiveness of vaccination was analyzed with the Wilcoxon Matched-Pairs Signed-Ranked Test.

Vaccination of 6,338 calves in category 1 resulted in a reduction in the percentage of the calf crop that developed diarrhea in 15 of the 18 herds. Calf mortality was reduced in 17 of the 18 herds. Statistically, the reduction of diarrhea and deaths was significant (P  0.01).

Vaccination of 3,245 calves in category 2 resulted in the incidence of diarrhea being reduced in 14 of the 17 herds and deaths were reduced in 15 of the 17 herds. Statistically reduction of diarrhea and deaths was significant (P  0.01).

Three herds in category 1 (31, 32, and 30) and 4 herds (21, 29, 33, and 35) in category 2 reveal no reduction in diarrhea or deaths. Further examination of fecal material at a later date revealed a corona-like virus in these herds.

These favorable results in controlling diarrhea in the newborn calf using the oral reo-like virus vaccine prompted expanded field trials for 1972.

Norden Laboratories was granted authorization for field trial testing after meeting requirements of the U.S.D.A. These requirements included the following detailed information on development and production of a neonatal calf vaccine: Research and experimental data to establish the need for a product; source of virus, identification, purification, adaptation, modification and maintenance; biochemical properties, growth,
titers, avirulence, antigenicity, and immunogenicity established for the range of passages used in production; seed lot production, virus action, harvest, test for sterility and virus titer; preparation of the product, labeling, moisture control and amount of virus material per dose throughout dating period. Testing was done according to standard requirements and included purity test for bacteria, molds, fungi, and mycoplasma.

General safety testing consisted of oral inoculation of 2 calves under 10 days of age with a double dose of vaccine; no adverse reaction was observed during the 14 day postinoculation period. Two guinea pigs, 350-400 gms each, were inoculated subcutaneously with one field dose, none developed an adverse reaction to the vaccine. Other tests included intracranial (I.C.) mouse safety test. All 10 young adult mice remained well for the 28 day postinoculation period. The suckling mouse test consisted of inoculation of two litters of mice I.C. with .02 ml of vaccine; no deaths occurred during a 14-day observation period.

In addition, a Seed Lot Principle Potency Test was conducted on 25 calves. Twenty were vaccinated 24 hours after birth and challenged 48 to 72 hours postvaccination. Nineteen of the 20 calves remained normal during a 4-week period. One calf had loose stools for 2 days and then appeared normal throughout the period. Five of five challenge control calves developed typical depression and severe diarrhea within 12-32 hours postchallenge. In addition host specific safety tests were run on a group of 6 gnotobiotic calves and a group of 11 normal born calves that remained with their dam. Both groups were vaccinated orally with one dose of properly restored, undiluted vaccine. These calves remained normal and free of any symptoms of postvaccination reaction for 21 days.

The virus was tested for contamination from extraneous viral agents and demonstrated to be free from IBR, PI-3, BVD, Bluetongue virus and Reo I Virus, using conjugated specific antisera.

Host specific safety tests were also conducted on 2 normal calves by intramuscular and two by intravenous injection of one dose each of undiluted vaccine. Calves were observed daily for 21 days. No clinical reactions were observed.

A back passage evaluation was made from one gnotobiotic calf receiving one dose of vaccine and fecal material collected on 3rd day containing vaccine virus was passaged through a second calf. This was repeated through four additional calves. All remained normal.

Additional safety and potency tests were run in calves on various lots of vaccine initially produced.

Serological data was also collected during these trials and the oral vaccination induced a significant rise in antibody titer.

In addition, a serological survey on 80 human blood samples for antibodies to a bovine reovirus-like agent has been made. Three populations were sampled: (1) 35 individuals who had direct or indirect contact with vaccine manufactured from the agent and some of whom had contact with cattle. (2) 25 individuals who had no contact with vaccine but
had contact with cattle. (3) 20 individuals who were urban dwellers and had no contact with vaccine and little or no contact with cattle. Average serum neutralization titers of the group were 77.7, 75.8 and 92.2, respectively.

Adequate data was obtained on 10,411 calves vaccinated with the reo vaccine in 1972. Data was also collected on 5,816 calves in the same herds that were not vaccinated. The incidence of diarrhea was 50% and a death rate of 9.3% or 539 head. Whereas in the vaccinated calves (10,411) had 16.1% or 1,735 head sick with a death rate of 1.2% or 130 head.

In 1973, a field survey concerned with the effectiveness of Scourvac-Reo vaccine was made. Usable data was obtained from 99 herds. A total of 64 beef herds with 8,026 calves and 35 dairy herds with 806 calves were vaccinated.

The median calf morbidity rate before vaccination in both groups when combined was 66%; morbidity in vaccinated calves dropped to 10%. The median calf mortality rate before vaccination was 10%; this dropped to 1% in vaccinated calves. The mortality comparison is almost identical to previous field trials, while the reduction in morbidity in vaccinated calves was better than the reduction experienced in the earlier field trials.

In view of the extensive testing in the laboratory and three years' of field data with uniformly good performance of the vaccine, we can be confident in recommending Scourvac-Reo as effective vaccine against early viral scours.

REFERENCES

A virus thought to be of etiological significance in field cases of calf scours was first detected in feces from scouring calves by University of Nebraska researchers. The virus has been extensively studied and used as a modified live virus vaccine called Scourvax-Reo (produced by Norden Laboratories) for the prevention of calf scours. The vaccine received federal licensing on March 1, 1973 and was released during the 1973 calving season in 49 of the 50 states, Montana being the exception.

The purpose of this paper is to present the information and the rationale which we used in making the recommendation to the State Veterinarian of Montana that the live virus vaccine Scourvax-Reo not be licensed for sale in the State of Montana. The basic research which preceded the production of the vaccine was excellent and the model developed should be of value in the continued study of the Reovirus-like agent and other viruses of animals and man. We do not, however, agree with some of the fundamental interpretations of the research and field testing data.

An analysis of the published information on the vaccine was made at the request of the State Veterinarian of Montana following the announcement of the availability of the product by Norden Laboratories. After a review of the literature published by researchers at the University of Nebraska and a review of information on the product supplied by Norden Laboratories, a representative from Norden visited the Veterinary Research Laboratory at Montana State University to discuss the data and to answer questions which were presented to him. On March 23, 1973 the following recommendation was unanimously accepted by the State Veterinarian and the Montana Board of Livestock. (1) The attenuated live virus vaccine “Scourvax-Reo” should not be released for sale and use in the State of Montana at the present time. (2) Procedures for continued evaluation of the vaccine should be established. (3) Consideration should be given to the establishment of procedures for the determination of the extent of Reovirus-like agent in Montana.

In this paper information will be presented to show that: (1) the Reovirus-like agent has not been established as the etiological agent of calf scours in the colostrum fed calf, (2) the efficacy of the vaccine has not been established, and (3) the safety of the vaccine for use by humans has not been adequately established.

*Calf Scours.* The term “calf scours” is often used interchangeably with “neonatal calf diarrhea” or simply “diarrhea.” Many newborn calves experience a transient diarrhea which does not progress to the clinical
syndrome of severe calf scours. We define calf scours as a disease of newborn calves characterized by the following clinical signs: diarrhea, dehydration, apathy, prostration, followed by death if the calf is not treated.

*Reovirus-like Agent.* Research on the Reovirus-like agent shows that:
1. the virus can be isolated from homogenates of feces and intestinal mucosa,\(^8\)
2. the virus can be purified by high speed centrifugation followed by density gradient analysis,\(^8\)
3. Reovirus-like agent can be cultivated on various types of cell cultures,\(^2,\,6,\,19\)
4. the diameter of the intact virion is 64 nm,\(^8\)
5. the buoyant density of the particle is 1.359,\(^8\)
6. the nucleic acid of the virus is RNA,\(^16,\,19\)
7. the virus is resistant to lipid solvents,\(^16,\,19\)
8. the Reovirus-like agent is serologically unrelated to Reovirus type 1, Reovirus type 3, dog Reovirus, Bluetongue Virus, Bovine Virus Diarrhea Virus and Infectious Bovine Rhinotracheitis Virus,\(^2,\,20\)
9. the absorption of the agent on primary bovine embryonic kidney is 85% complete in 1 hour,\(^20\)
10. mature virions are detectable by fluorescent antibody techniques four hours postinoculation in cell culture,\(^20\)
11. the maximum titer of the virus on primary bovine embryonic kidney culture is reached at 18 hours postinoculation,\(^20\)
12. the virus is stable at a pH of 3.0,\(^20\)
13. the Reovirus-like agent is relatively heat resistant.\(^20\)

The mechanism by which Scourvax-Reo protects the newborn calf against calf scours has not been determined. It has been proposed that protection may occur by viral interference. It has also been shown that the virus will stimulate the production of interferon by infected cells in culture.\(^20\)

The name Reovirus has been attached to this virus before research to justify its inclusion in this group has been accomplished. The critical characteristic is the determination of whether the RNA is double stranded. Also, the lack of evidence for serological relatedness to known Reoviruses and the failure of the agent to hemagglutinate human type O or type A erythrocytes might be considered as reasons for not classifying the Reovirus-like agent in the Diplornavirus group. The use of the complement-fixation test for estimating relatedness has not been reported.

**Safety of the Vaccine.** Safety of the product for use in the newborn calf has apparently been adequately demonstrated. The question of safety for other animals and for man has been given very little attention.

There have been no reported cases of proven infections which have occurred in laboratory workers, or in consumers who have used the vaccine. No research has, however, been reported in which the "virulent" Reovirus-like agent, or the vaccine has been tested in the commonly used laboratory models. Also, reports of tests of the cell cultures used for the propagation of the Reovirus-like agent and the production of the attenuated vaccine have not been published.

The use of animal cell lines for the propagation of live virus vaccines may be unsatisfactory because of the possible presence of contaminating oncogenic, or contaminating nononcogenic viruses. The contaminating
AN ANALYSIS OF SCOURVAX—REO

viruses may be either present in the original tissue, or be introduced in the course of serial propagation. It is known that viruses can grow parabiotically with no readily demonstrable cytolytic changes. Many of the commonly used cell cultures spontaneously produce type C RNA viruses. The viruses may be oncogenic in laboratory animals and one cannot be sure that they are nonpathogenic for man. The tests for possible oncogenic viruses in cell cultures and the tests for oncogenicity of the virus itself represent important aspects of the fundamental investigations which should be conducted on any live virus product.

Etiologic Significance of Reovirus-like Agent in Calf Scours. Attempts have been made to establish the pathogenicity (and thereby the possible etiologic significance) of the neonatal calf diarrhea virus (NCDV) in newborn colostrum fed and colostrum deprived calves. At least sixty-four colostrum deprived calves have been inoculated with NCDV. The NCDV has been shown to cause depression, transient diarrhea and salivation in colostrum deprived calves in the absence of invasive strains of Escherichia coli. In the presence of invasive strains of E. coli, there is apparently a colonization of the small intestine, often a bacteremia with an elevated temperature, sometimes dehydration and death. These clinical signs of disease following inoculation of colostrum deprived calves with the NCDV in the presence of invasive strains of E. coli are thought to be similar to clinical signs of field cases of calf scours, thus the conclusion that the NCDV causes calf scours.

Preliminary results indicate that the pathogenicity of the NCDV in colostrum fed calves is quite different from that in colostrum deprived calves. Five colostrum fed calves were inoculated with the NCDV. Four of the 5 calves had a transient diarrhea which cleared up spontaneously. One calf remained clinically normal following inoculation. The onset of diarrhea following inoculation was variable ranging from 24 to 110 hours postinoculation. The symptoms of dehydration, apathy, morbidity and death that characterize field cases of calf scours were absent. Also absent were most of the clinical signs of disease that appear following inoculation of colostrum deprived calves with the virus. Researchers in Idaho orally inoculated 10 calves within an hour of birth with the NCDV. The calves had access to their dams and therefore had taken colostrum before or soon after inoculation. All 10 of the calves remained clinically normal following inoculation.

The apparent marked difference in susceptibility of colostrum fed and colostrum deprived calves to infectious agents has been reported previously. It has been well established that colostrum deprived calves are easily killed by invasive strains of E. coli while colostrum fed calves are quite resistant to E. coli septicemia. When BVD virus was given to 13 colostrum deprived calves, all got sick and four died from scours. Eight other calves similarly exposed to BVD virus but fed mothers milk showed much milder symptoms. Only one calf died and its mothers milk did not contain viral antibodies.

Study of the pathogenicity of infectious agents in colostrum deprived gnotobiotic calves may be productive because the results are not compli-
licated by the presence of other infectious agents. Discretion must, how-
ever, be used in determining the etiologic significance of any infectious
agent in colostrum deprived calves because this type of a calf may be far
more susceptible to the agent than colostrum fed calves. Many viruses
are "nonpathogenic" in their normal environment, but may exert a patho-
genic effect under abnormal circumstances. The gnotobiotic calf is in fact
an "abnormal" host for the virus. This difference in susceptibility of co-
lostrum fed and colostrum deprived calves seems to be true for the
NCDV, although more colostrum fed calves should be inoculated with
the agent under various disease conditions.

There are no reports on attempts to isolate the Reovirus-like agent
from normal calves. This is important to the establishment of the etio-
logical significance of the virus.

**Efficacy of Scourvax-Reo.** Field trials with similar experimental de-
signs were conducted by the University of Nebraska during the 1971
calving season and by Norden Laboratories during the 1972 calving sea-
son to establish the efficacy of Scourvax-Reo. Reports are available
concerning 10,411 vaccinated calves on the Norden Trial and 9,583 vac-
cinated calves on the Nebraska trial." Control data were collected on
26,166 nonvaccinated calves in the Norden field trial and several thou-
sand calves in the Nebraska trial (the exact number was not reported).
Vaccination in some herds was initiated at the beginning of the calving
season. In other herds, vaccination was initiated sometime after the be-
ginning of calving. Control calves were those calves born the previous
calving season, or calves born early in the calving season prior to the ini-
tiation of vaccination, or both. Once vaccination was started in a herd,
all new born calves were vaccinated. Control data were obtained by ask-
ing the rancher to estimate the incidence of diarrhea and the number of
calves that died of scours during the previous year and/or during the ini-
tial part of the calving season in which vaccination was started. Re-
results of the field trials indicated a significantly lower incidence of diar-
rhea and a significantly lower death rate due to calf scours in vaccinated
than in control calves.

The experimental design in the field trials is unsatisfactory and the
results cannot be considered meaningful for the following reasons. (1)
The experimental design did not effectively minimize the potential for
biased reporting of the results because personnel recording the results
knew which calves were vaccinated and which calves were not vac-
cinated. (2) The design did not minimize the potential for variability be-
tween control and vaccinated calves since the two groups of calves were
born at different times and were, therefore, in the important neonatal
period under different disease conditions. (3) Control and vaccinal data
were collected in a different way. The rancher was asked to estimate the
occurrence and severity of disease in control calves sometime after the
disease occurred, while the results in vaccinated calves could be recorded
at the time period in which the disease was occurring and would, there-
fore, potentially be more accurate.
A much more satisfactory experimental design for the field trial would have been to prepare a vaccine and a placebo the identity of which could not be determined by the individual recording the individual calf results. Every other calf born would be vaccinated and the other calves given a placebo.

It is not clear why properly designed field trials were not conducted. The following reasons have been given. (1) Vaccinated and nonvaccinated calves were not included in the trials at the same time because if the control calves developed diarrhea, the premises would be seeded and the time between vaccination and exposure might be insufficient for development of resistance. (2) A comparison of vaccinated and nonvaccinated animals within the same herd at the same time was not practical because vaccinated animals would shed the vaccine virus and thus indirectly vaccinate the control calves. One wonders why a somewhat smaller, properly controlled field trial was not conducted initially as a means of establishing the best design for the larger field trials.

Summary. The following points summarize our reasons for recommending that the attenuated virus vaccine Scourvax-Reo not be licensed for sale in Montana during the 1973 calving season. (1) Calf scours as we define the disease has not been caused by oral inoculation of colostrum fed calves with the NCDV. (2) The colostrum deprived calf was used to establish the etiologic importance of the Reovirus-like agent in field cases of calf scours. (3) The incidence of the NCDV in normal calves has not been determined. (4) The efficacy of Scourvax-Reo has not been established because of unsatisfactory design of the field trials. (5) The safety of the vaccine for man has not been adequately established.

It seems clear that current and future problems on live viral vaccines are of significant importance to the Veterinary Medical Profession. Clinicians must have scientific justification for the therapy which they recommend. Consumers must have reasonable assurance that the products which they purchase will do what they are advertised to do and are safe.

REFERENCES

3. Fey, Hans. 1972. Colibacillosis in calves. Published by Hans Huber, Bern, Switzerland.


The Biologics Committee was informed that the Animal Plant Health Inspection Service proposes to issue a notice in the Federal Register pertaining to fractions of licensed biological products which are of questionable potency or efficacy. These fractions of combination products involve certain species of the bacterial genera streptococcus, staphylococcus, corynebacterium, aerobacter, salmonella and bordetella which are used to prepare biological products including toxoids, antiserums and bacterins.

The notice will inform biologics licensees that unless data are provided to demonstrate efficacy of fractions derived from these microorganisms, and a potency test is developed to evaluate each serial, licenses for products containing these fractions shall be terminated. A.P.H.I.S. will give favorable consideration to licensing the other fractions of those products for which licenses are so terminated.

Licensees will be given a limited time to provide A.P.H.I.S. with a statement of intent. If the licensee intends to provide the required information he shall be given a reasonable time to achieve compliance. If acceptable information is not provided, the license for each product involved will be terminated.

The Biologics Committee has had insufficient time to fully review this proposal and is concerned that practicing veterinarians and livestock owners have opportunity to comment prior to termination of licenses for biological products which contain fractions derived from these microorganisms.

The Biologics Committee suggests the principle that licenses should be terminated for ineffective biological products; but to prevent elimination of essential products of possible merit, recommends that the notice not be published in the Federal Register until a statement of intent has been published and called to the attention of veterinarians and livestock owners by APHIS, with ample opportunity provided for comments.

The Committee discussed the process of special licensing of biological products by A.P.H.I.S. Information was presented to the Committee by
Drs. M. J. Twiehaus, F. S. Newman, L. Meyer, P. L. Smith, W. Beckendauer, and R. Price on the entire process of producing and evaluating a product for use under the special licensing procedures. This included a thorough discussion of the states' role in administration and use of all biological products.

As a result of these discussions, the Committee on Biologics makes the following recommendation.

The Committee favors the continuance of the special licensing procedures as they are now written. The Committee further recommends the continued responsibility of the state regulatory official in the administration and use of all biological products within the state, including those under either a special or regular license.

Veterinary Services of A.P.H.I.S. should notify the state veterinarians of the issuance of all licenses for biological products, both regular and special. In addition, it should be the responsibility of the licensee to supply data on a specific biological product upon request of the state regulatory official.

Respectfully submitted by the Committee on Biologics, R. B. Bushnell, chairman.
THE WEAK CALF SYNDROME—EPIDEMIOLOGY, PATHOLOGY, AND MICROORGANISMS RECOVERED


INTRODUCTION

The weak calf syndrome is a disease entity of neonatal calves, presently of unknown etiology which is seen predominantly in Southwestern Montana and Southeastern Idaho. The syndrome has been recognized in Montana since 1964, and was first recognized in the Salmon-Challis area of Idaho in 1968 when approximately 400 cases of the syndrome were diagnosed by local veterinary practitioners. The death loss in these 400 cases was approximately 80%, despite the treatment attempts of the local veterinary practitioners. The number of reported and treated cases of weak calf syndrome reached a thousand in the Salmon-Challis area in 1973. As in any disease, a number of cases undoubtedly were not reported, precluding an accurate estimate of the economic impact of the disease in these isolated ranching communities. However, economists from the University of Idaho have estimated a potential gross income loss of $131,000 which was in the ranches affected by this disease in 1973.

Total losses to the livestock industry are difficult to estimate because unthriftness and poor growth have been reported in recovered calves. Ranches that have been affected by this condition report that up to 30 percent of a calf crop may show clinical signs of illness during the calving season.

The disease has been reported with less frequency in both 1972 and 1973 in other ranching areas of Idaho. If the morbidity increases in these areas in future years as it did in the Salmon-Challis areas from 1968 to 1973, the results would be economically disastrous to many cow-calf operators in Idaho.

The disease has been reported in one northern Colorado ranch with a 600 cow herd. The rancher reported a 15 per cent calf loss during that season, contributing a 10 percent net increase in losses over normal. One affected calf was reported saved, but never has grown normally.

The purpose of this presentation is to describe some of the salient clinical and pathological observations that have been made by practitioners and research workers from the University of Idaho, the National Animal Disease Center, Washington State University, Montana State University, and the University of Montana. Presently the University of Idaho and the National Animal Disease Center are cooperating in a study of the incidence, economic impact, etiology and pathology of this condition. Future plans include cooperative studies with Montana State researchers on epidemiological investigations of the syndrome.

Although polyarthritis is the single most common clinical symptom and post-mortem lesion, the condition has been referred to as the "weak calf syndrome" rather than polyarthritis to distinguish it from the disease in calves caused by chlamydial infections. At a recent symposium on the "weak calf syndrome", the term "idiopathic polyarthritis" was proposed as a more specific name for this condition.
THE WEAK CALF SYNDROME

condition. Although several pathogenic agents have been isolated from calves and from cows giving birth to weak calves, a causal relationship has not yet been established between the isolated organisms and the disease in calves.

CLINICAL SIGNS

Field observations have indicated that most "weak calves" are affected by 10 days of age. A majority of affected calves will show signs of clinical illness between three and seven days of age. Approximately 20 percent of affected calves will show illness at the time of birth. The following signs are commonly seen in "weak calf syndrome" cases.

1. Lassitude, depression and severe weakness are seen often accompanied by an inability to stand and consequently to nurse.
2. The body temperature will be normal in the early cases but as in many other conditions, it may decline as death approaches. Rarely is the body temperature above normal.
3. Early in the condition, diarrhea is not seen. Loose, yellow feces may be seen, however, after two or three days of illness. The fecal material is not the watery, fetid material which is present in cases of "neonatal diarrhea".
4. The muzzle is often reddened and crusty.
5. Petechial and diffuse hemorrhages may be present on the conjunctiva and the third eyelid.
6. Many calves will be lame and reluctant to stand. They may stand with an arched back and in a few cases swelling of periarticular tissue and distention of the carpal and tarsal joint sacs can be detected by palpation.
7. Dehydration is not seen in the early stages of the disease, but it may be noted after diarrhea becomes a part of the syndrome.
8. Severe diarrhea, dehydration and the complications of secondary bacterial infection are often seen prior to death in protracted cases.
9. The morbidity on a ranch may vary from six to fifteen percent and will be dependent on previous exposure to the disease. Practitioners believe "that most cows giving birth to "weak calves" will not have an affected calf in the subsequent gestation". Mortality rates will vary from 60 to 80 percent in the untreated cases.

TREATMENT

Presently treatment includes good nursing care, blood transfusions and general supportive treatments. Blood may be given intravenously, but in case the vein cannot be catheterized, it may be given subcutaneously. The blood should be taken from cows that have had weak calves one to two months previously. A total of one to two pints is usually given intravenously. This treatment has increased the recovery rate from 20 to 40 percent to approximately 80 percent during the 1973 outbreak in the Salmon-Challis area. Supportive treatment for diarrhea, dehydration and secondary bacterial infection including electrolytes, fluids and antibiotics, should be given when these clinical signs present themselves.
Page, et al. have described the clinical signs and gross pathology lesions found in a "weak calf syndrome" case in the Bitterroot Valley of Montana. A strain of mycoplasma that appeared to be antigenically distinct from other mycoplasma serotypes of bovine origin was recovered from the placenta of the dam of this calf. The clinical signs of the condition in this herd included birth of premature or stillborn calves, hyperthermia, lassitude, weakness and death within seven days. The clinical signs of premature birth, stillborn calves and hyperthermia were not noted in the Salmon-Challis area of Idaho.

GROSS AND MICROSCOPIC PATHOLOGY

The most striking feature noted during necropsy examination is the characteristic hemorrhage and edema of the subcutaneous tissues of the extremities. The changes are most obvious around hock and knee joint, and the lesions extend distally along the respective extremities. The edema is often most pronounced around the periarticular tissue of the hock joint and in the supporting tissue of the Achilles tendon. These lesions of hemorrhage and edema are seen in 95-100 percent of "weak calf syndrome" cases. Although these lesions have been described as an occasional lesion resulting from experimentally induced chlamydial infection, they are not commonly seen in either natural chlamydial infection or neonatal diarrhea.

The hemorrhage and edema will vary in severity from small, disseminated hemorrhagic areas only in very early cases to lesions which are more diffuse and include edematous fluid. Chronicity tends to increase the severity of the lesion and the amount of subcutaneous tissue involved.

The second most striking gross lesion seen in the weak calf syndrome is a polyarthritis characterized by a hemorrhagic synovial fluid which may be increased in volume and which may contain variable amounts of fibrin. The affected synovial fluid varies in appearance from a thick very hemorrhagic material containing little or no fibrin to a sero-sanguineous fluid which often contains large plaques of fibrin. The periarticular tissue and synovium that are associated with these joints will be thickened by hemorrhage, edema and fibrin deposition. In a few cases, these joint sacs may be distended by the exudative material and can be detected by palpation. In a series of twenty-five "weak calf syndrome" cases from the Salmon-Challis area of Idaho, polyarthritis was present in 65 percent of all cases. Microscopically, the synovitis that accompanies this inflammatory arthritis is characterized by a thickening of the synovial membrane by edema, hemorrhage and an influx of inflammatory cells which are predominantly neutrophils. A few lymphocytes and macrophages will be seen with the inflammatory cells. The hemorrhage is often present deep in the synovial tissue while the fibrin deposition will be noted mainly on the serous surface of the synovial membrane.

Lesions of the forestomachs will be seen in approximately 30 percent of weak calf syndrome cases. The gross lesions include diffuse congestion and hemorrhage occasionally with vesiculation, erosion and ulceration of the epithelial lining of the forestomachs. The hemorrhage which may extend from the epithelial surface deep into the muscular wall may also be visible through
the serosal surface of these organs. The erosions and ulcerations are often associated with secondary infection by bacterial and fungal agents.

Lesions of the abomasum are seen grossly in approximately 30 percent of the weak calf syndrome cases. These lesions may vary from numerous pinpoint areas of the entire mucosal surface of this viscus to larger more diffuse hemorrhagic areas of the entire mucosal surface of this viscus to larger more diffuse hemorrhages. Small erosions and ulcerations are also present varying in size from pinpoint to approximately 0.1 mm. in diameter. Large ulcerations of the abomasal lining may also be seen, associated with hemorrhage and secondary infection by bacteria and fungal agents.

Microscopically, the lesions of the abomasum are recognized as focal accumulations of red blood cells in the lamina propria often associated with necrosis of the superficial mucosal area. Erosions are seen, as are ulcerations of the mucosal surface, which may extend deeply into the submucosal tissue. Inflammatory cells, predominantly suppurative in nature are found in abundance associated with the erosions and ulcerations. Increased numbers of these cells may also be seen associated with the hemorrhages in the lamina propria.

Other less frequently seen lesions include occasional, mild, enteric infections and colitis in which there may be only hemorrhage in the lamina propria or there may be superficial necrosis of the mucosal surface of the intestinal wall. Occasional congestion or hemorrhage with necrosis of Peyer's patches was noted, associated with depletion of the lymphocytes.

MICROORGANISMS ISOLATED

Attempts to isolate microorganisms from the calves with weak calf syndrome have been relatively unsuccessful in the past. Page, et. al. have isolated a mycoplasma organism from the placenta of a cow giving birth to a "weak calf" in the Bitterroot Valley of Montana in 1969. These authors summarized their work by stating that, "23 percent of the animals in the infected herd had circulating antibodies against the mycoplasma strain." A causal relationship between the organism and the calf disease was not demonstrated. Other isolates of the mycoplasma organism have not been made in calves from this area.

Ushijima and Ward have isolated a virus which has not been characterized completely but which has been described as forming multinucleated giant cells with intranuclear inclusion bodies. This viral agent was isolated from the salivary gland tissue, but no causal relationship to the "weak calf syndrome" has been demonstrated by these workers.

Using a series of "weak calf syndrome" cases collected in the Salmon-Challis area of Idaho during the 1973 calving season, the National Animal Disease Center and the University of Idaho have cooperated in an attempt to isolate the microorganisms that might cause this condition. Various tissues were harvested from approximately 25 typical "weak calf syndrome" cases. From these tissue examinations, which as yet, are not completed, several observations have been made. These observations were reported at a symposium in Salmon, Idaho, to cooperating ranchers, practicing veterinarians and research workers.
1. The continued attempts of Page and co-workers at NADC have not been successful in isolating mycoplasma, chlamydia or bacterial agents from tissues from the "weak calf syndrome" cases.

2. McClurkin and Stauber working independently at the NADC and the University of Idaho, but using the same tissue samples have isolated several viral agents. The most common isolate is the BVD virus isolated from two cases at the University of Idaho and from several cases at the National Animal Disease Center. The isolates were made from buffy coat and joint fluid. Secondly, an adenovirus has been isolated from one case at NADC and two cases at the University of Idaho. This virus is ether resistant and was isolated from the buffy coat.

3. At the University of Idaho, a mixed virus has been isolated. One of the virus agents appears to be BVD and the second which is possibly an enterovirus has enhanced cytopathologic effects in the presence of the BVD antibody.

4. A third viral isolate at the University of Idaho is an unidentified virus which has been isolated from the buffy coat and salivary tissue of two "weak calf syndrome" cases.

   As yet no investigations have been carried out to determine whether a causal relationship exists between these viral agents and the disease entity known as the "weak calf syndrome."

Waldhalm, et al. have isolated *Haemophilus somnus* from several cattle which had given birth to weak calves. In an attempt to associate this organism with the disease, one trial has been conducted in which 27 cows were infected in utero at the time of breeding with a strain of *H. somnus*, isolated from the uterus of a cow which had given birth to a calf that had a disease clinically compatible with the weak calf syndrome. Six calves from this group were observed to have clinical illness resembling the weak calf syndrome at the time of birth or soon after. Organisms, including *H. somnus* could not be isolated from the calves when necropsied three to five days after birth, or from the uterus of the dams a few hours after parturition. The necropsy examination of five of these calves did reveal polyarthritis which was indistinguishable from the polyarthritis seen in the weak calf syndrome. However, lesions of hemorrhage and edema in the subcutaneous tissue of these calves was not present. Microscopically, the polyarthritis was indistinguishable from that seen in the weak calf syndrome cases. Lesions of the forestomachs and abomasum were not noted. A meningitis was noted at the gross necropsy examination in four of the six cases. Microscopically this meningitis was suppurative in nature and extended into the subpial tissue of the neural tissue. Perivascular cuffing with neutrophils was seen in sections from two of these brains. Other lesions were not present either grossly or microscopically in these calves. Five control calves were free of gross and microscopic lesions.

Dr. Richard Bull, a nutritionist at the University of Idaho described a high degree of correlation between the number of cases of "weak calf syndrome" on affected ranches and the level of crude protein in the forage being fed one month prior to the calving season. The lower the crude protein level of the forage,
the greater the incidence of the weak calf syndrome cases in this comparison.

One other etiologic factor, alluded to by both cattlemen and practicing veterinarians is the stress of inclement weather during the calving season in the Salmon-Challis area. This season extends from mid-January to April, a period of time when blizzards, extreme variables in daily temperature range and wetness are all prevalent as contributing factors. These stress factors, which can be tolerated by the healthy calf, contribute to the severity of any clinical illness including the weak calf syndrome. Conversely, during periods of good weather many calves that are harboring infections, including the “weak calf syndrome” may not show signs of clinical illness.

SUMMARY

The clinical signs, postmortem changes, and microscopic lesions of a neonatal disease common to Idaho and Montana have been presented. Best estimates to date would indicate that this disease may be caused by one or more bacterial or viral agents, singly or in combination, and that the clinical condition is enhanced in severity by stress factors such as poor nutrition in the dams and the inclement weather common in these geographic areas during the calving season.

REFERENCES
REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF CATTLE

Chairman: N. B. Swanson, Cheyenne, Who.
Co-Chairman: H. D. Anthony, Manhattan, Kans.

John F. Hudelson, Derfver, Colo.; E. E. Wedman, Corvallis, Oreg.;
R. P. Azelton, St. Joseph, Mo.; D. E. Bartlett, DeForest, Wisc.; W. D.
Bolton, Burlington, Vt.; E. A. Butler, Des Moines, Iowa; G. L.
Crenshaw, Davis, Calif.; Joe Finley, Jr., Encinal, Tex.; Sam Guss,
University Park, Pa.; Gerald Shiner, Canan City, Colo.; F. W.
Hansen, Jr., Hyattsville, Md.; E. L. Henkel, Salem, Oreg.; J. H.
Hopson, Albuquerque, N. Mex.; George Lambert, Ames, Iowa;
Rolland McIclymont, Holdrege, Nebr.; J. G. Milligan, Montgomery,
Ala.; R. C. Searl, Ft. Dodge, Iowa; H. Q. Sibley, Austin, Tex.; D. E.
Williams, Guyman, Okla.

The Infectious Diseases of Cattle Committee met Thursday afternoon
in the Centennial Room with more than 64 people present. Dr. Anthony,
Co-Chairman of the Committee and I would like to take this opportunity
to thank all those in attendance for their interest and participation in our
activities.

This is the one committee where I feel the grass roots level of the cattle in-
dustry has an opportunity for input. Infectious diseases of cattle en-
compass a wide variety of subjects, many not presently included in stand-
ing committees of this organization. Not everyone’s priorities can be dis-
cussed, of course, because of the great variation in the geographical areas
of the nation; it is impossible to scan all the current research for recent
important developments in one short committee session.

The committee report is as follows:

Progress reports were presented on the pink-eye bacterin studies
being conducted at NADL, the sudden death syndrome of feedlot cattle
being conducted at Kansas State University, and the Hemophilus Som-
nus research at Iowa State University. The committee urges continued
research efforts on these various projects.

The committee accepted the proposal and the recommendations of the
summit committee on the A.I. regulations. The following statements and/or
recommendations were offered by the sub-committee:

1. That we urge APHIS to publish a revised proposed regulation in the
   Federal Register.
2. That laboratory procedures can be made available. AAVLD prepared
   last year a set of procedures which will be published this year in their
   proceedings for diagnosing the diseases to be included in the
   proposed regulation. The sub-committee recommends that:
   a. AAVLD revise the section on Leptospirosis to stipulate what con-
      stitutes positive and negative tests. Suggest that AAVLD con-
      sider including the use of the USDA-licensed killed antigen in their
      procedures.
b. The section in AAVLD procedures dealing with Trichomoniasis is to be used in the proposed regulation.

3. That laboratory facilities can be available to anyone who needs diagnostic work.

4. That in the case of bulls in individual or joint ownership the regulation exempt semen in the amount of inseminating units equal to the number of cows owned by the owner or owners of the bull X 1.5. Sale or gift of exempted semen is not to be allowed.

5. That Paratuberculosis be left out of the regulation for the present.

6. That semen shall be from bulls tested negative for Vibriosis and in addition shall be processed so that it contains antibiotics in prescribed amounts. The sub-committee on A. I. of the Committee on Infectious Diseases of cattle of the USAHA will determine which antibiotics and the amounts of each that should be used.

7. That this sub-committee be made permanent for the purpose of making recommendations for updating the regulation in the event it becomes a reality.

A resolution concerning these recommendations has been forwarded to the resolutions committee.

A presentation of certain disease problems involved in livestock transportation and marketing was made to the group by Dr. Abraham of Memphis, Tenn., speaking for the National Livestock Dealers Association. Representatives of other industry groups also spoke to the group on this topic. The committee strongly recommends that more research be initiated on the preparation of cattle to withstand the stress of movement and to help withstand the respiratory disease complex peculiar to the movement of these animals.

The committee recognized the leadership of Senator Bellman of Oklahoma and Representative Melcher of Montana as reflected in passage of Public Law 93-86, Section 809, Agricultural Research Act of 1973.

The committee wishes to commend the Livestock Identification Committee for their progress on animal identification, especially for bringing to this convention the presentation and information on the use of electronic equipment.

The committee welcomes any and all suggestions of the membership of this organization that may be pertinent to the responsibilities of this committee for the coming year.

We respectfully submit this report for your consideration and recommend its approval.

Signed
Dr. N. R. Swanson, Chairman
Dr. H. D. Anthony, Co-Chairman
AN EPIDEMIOLOGICAL STUDY OF ANAPLASMOSIS IN OREGON*

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INTRODUCTION

The epidemiology of anaplasmosis in Oregon is especially interesting because of the many variables capable of influencing Anaplasma marginale transmission. National Geographic states: "Oregon combines enough climate and geographic variety to suit a whole continent". Elevation in the state varies from sea level to over 11,000 feet, annual precipitation from 90 inches to less than 10 inches, vegetation from dense conifer forests along the Pacific coast and in the mountainous areas to sparse sagebrush and other dry land plants in the high desert areas of eastern and central Oregon. Variation also exists throughout the state in numbers and species of wild animals, animal parasites and in animal diseases. As expected, all these variation have influenced the type and management procedures of livestock operations located in various parts of the state.

Controversy exists concerning the date anaplasmosis was first recognized in Oregon. The earliest report states that A. G. Moore, a veterinarian in Ontario, Oregon received laboratory confirmation of the disease in 1920." Anaplasmosis has since that time been recognized as an important bovine disease entity on many ranches in eastern and central Oregon and in localized areas of southwestern Oregon. A survey conducted in 1954-55 on bovine serums collected on ranches in eastern Oregon revealed a within-herd-infection rate of from 0 to 99% with an average rate of 60%. Approximately 3,500 cattle were complement fixation (CF) tested. This survey also revealed that cattle which had always remained in irrigated valleys were usually free of infection while those pastured on sagebrush-covered desert ranges were often infected.

A recent study of a 168 cow herd located in this anaplasmosis endemic area showed 43% CF 4+ reactors, 28% suspects and only 29% negatives. Of 120 young calves that moved to this range in April, 24.2% were CF 4+ reactors, 12.5% 3+ suspects and 1.6% 2+ suspects when removed in early September.

Ticks appear to be the primary vectors of A. marginale in Oregon.

*(1) Received for publication from the Department of Veterinary Medicine, Oregon State University, Technical Paper No. 3717 Oregon Agricultural Experiment Station.

**(2) The author wishes to acknowledge the assistance of Robert Gresbrink, Director, Vector Control Program, Oregon Health Division; the Oregon Game Commission, the Office of the State Veterinarian, Oregon State Department of Agriculture and the participating veterinary practitioners.
Two known important vector species, the Rocky Mountain wood tick-
\textit{Dermacentor andersoni-venustus} and the Pacific Coast tick \textit{D. occidentalis}, both three-host ticks, are found in the state. \textit{Dermacentor andersoni} is indigenous to most of central and eastern Oregon while \textit{D. occidentalis} is indigenous to southwestern Oregon. Survey reports indicate that \textit{D. occidentalis} is slowly moving northward.

The California black-legged tick \textit{Ixodes pacificus}, a three-host tick, is indigenous to all of western Oregon while \textit{D. albipictus} the winter tick, is found throughout the state. It appears unlikely that either of the latter two are important transmitters of \textit{A. marginale} in Oregon.

Mule deer (\textit{Odocoileus hemionus hemionus}) are indigenous to Oregon east of the summit of the Cascade Mountains while Columbian black-tailed deer (\textit{O. h. columbianus}) are indigenous west of the Cascade summit. In areas of the high Cascades, crossing of these two sub-species sometimes occurs.

In California black-tailed deer are known reservoirs of \textit{A. marginale}. The possibility of natural latent \textit{A. marginale} carriers in animals other than cattle has been considered for years by eastern and central Oregon ranchers and veterinarians. Mule deer have been incriminated, perhaps unjustly, because of their abundance on cattle ranges and because experimental \textit{A. marginale} infection with transmission to cattle has been demonstrated.

The purpose of conducting this epidemiologic study was to more accurately define endemic bovine anaplasmosis areas within Oregon, assess the importance of the disease in all areas of the state, determine the relationship of ticks and other vectors to the disease prevalence and, further study the relationship of both mule deer and black-tailed deer to bovine anaplasmosis.

**PROCEDURE**

Fifty-one large animal practitioners throughout Oregon were personally contacted regarding the status of bovine anaplasmosis in their practice area. Information requested concerned: (1) prevalence of anaplasmosis and time of year clinical cases were most often observed, (2) relationship of topography, climate and types of vegetation to the disease prevalence, (3) extent of losses, (4) tick numbers observed on cattle and seasons of greatest tick activity, (5) abundance of horse flies, deer flies and mosquitoes, (6) types of cattle generally infected — native or introduced, and (7) long time trends regarding the disease prevalence. All areas of the state were surveyed. Number of years veterinarians had practiced in their respective areas ranged from 7 to 30 with a mean of 19 years.

Distribution, population density and seasonal prevalence of \textit{D. andersoni}, \textit{D. occidentalis} and \textit{I. pacificus} was determined from annual tick surveys conducted by the Oregon Health Division, Vector Control Program. Information regarding distribution, population density and
migration habits of mule deer and Columbian black-tailed deer was acquired from Oregon Game Commission surveys. CF test results conducted on Oregon cattle were supplied by the Veterinary Division, Oregon State Department of Agriculture. Recent information acquired from anaplasmosis studies on Oregon mule deer, American bison (*Bison bison*) and cattle by researchers from Oregon State University and the Animal Parasitology Institute USDA-ARS is also included.

RESULTS AND DISCUSSION

Information acquired from these sources revealed a number of interesting facts concerning the epidemiology of anaplasmosis in Oregon. The disease is endemic in most of eastern and central Oregon except on the eastern slope of the Cascade Mountains and a strip along the northern state boundary. It is also endemic in parts of southwestern Oregon, but is seldom observed in the northern two-thirds of western Oregon. It is primarily a disease of beef cattle; rarely is it observed in dairy cattle.

Ticks are observed on cattle in most of eastern and central Oregon, and are most abundant on cattle grazing sagebrush range land. Here they are most prevalent in the spring and early summer, but at high elevations may be active well into the fall. Clinical anaplasmosis is most prevalent in these areas during the late spring, summer and early fall. Cattle maintained on irrigated pastures, valley meadows or crop lands are seldom parasitized by ticks. *D. andersoni* is indigenous in anaplasmosis endemic areas. However, this tick is also prevalent in range areas of central and eastern Oregon where anaplasmosis is seldom observed. Endemic anaplasmosis is generally limited to sagebrush areas. Mule deer are indigenous to all central and eastern Oregon areas and are abundant on many cattle ranges. They often migrate long distances between summer and winter feeding grounds. Many in the state’s south central portion migrate into known anaplasmosis endemic areas of California for winter feeding.  

The epidemiology of anaplasmosis in western Oregon is quite different. The disease is rarely observed in the northern two-thirds of western Oregon although cattle from other areas, including anaplasmosis endemic areas, are regularly introduced. During the past 20 years only five clinical herd cases were reported. Native cattle appear to have been involved only once, but the method of transmission from introduced infected cattle was not determined. *D. albilpictus* and *I. pacificus* are indigenous, but veterinarians in this area report they seldom observe ticks on cattle. Neither *D. andersoni* or mule deer are found west of the Cascade Mountains. Columbian black-tailed deer are indigenous to the entire area, but generally are not migratory. Those that summer in the high Cascades do migrate short distances to lower elevations for winter feeding.

In southwestern Oregon on the western slope of the Cascade Mountains anaplasmosis is endemic. It is also occasionally observed in the
southern Pacific Coastal areas where it may be endemic in some herds.-

D. alripictus, I. pacificus and (D. occidentalis) are indigenous. Colum-
bian black-tailed deer are prevalent and those in the extreme southern
portion migrate between Oregon and in anaplasmosis endemic areas in
California.7 Results of epidemiologic studies in this area and the Coast
Range of California are similar. Both D. occidentalis, an excellent A.
marginale vector which parasitizes deer and cattle, and the Columbian
black-tailed deer, a natural host, are present. Anaplasmosis control in
this area as in western California, presents a very challenging problem.

The importance of insect transmission of anaplasmosis in Oregon could
not be accurately determined, but little relationship appears to exist bet-
ween the prevalence of clinical anaplasmosis and the prevalence of horse
flies, deer flies or mosquitoes. Transmission by unsterile surgical and
vaccination techniques is not uncommon in endemic areas but is seldom
observed in other areas. This is not in agreement with Minnesota studies
where the disease in one area was commonly transmitted while bleeding
cattle for brucellosis testing and while dehorning.9

Contrary to popular opinion, latent carriers of A. marginale are
probably uncommon in mule deer. For example, mule deer are abundant
during the summer months in the high Cascades. In the fall they migrate
down the eastern slopes into high desert country where anaplasmosis is
endemic. This annual migration to winter feeding grounds often covers
more than 100 miles and takes deer across numerous cattle ranches on
the lower mountain slopes. The deer winter in large herds which disperse
in the spring as migration back to the mountains begins. Although these
deer winter in anaplasmosis endemic areas and remain until late spring
and early summer when D. andersoni is most active, they have not
carried A. marginale back to cattle ranging in the lower Cascades.
These deer have had ample opportunity to develop infection and become
latent A. marginale carriers while on the desert. Mule deer are also
prevalent in the northern portion of central and eastern Oregon where
bovine anaplasmosis is seldom observed.

Recent research conducted by the author, et al. also indicates mule
deer are probably not important reservoirs of A. marginale. Blood was
collected from 31 mule deer in three Oregon bovine anaplasmosis en-
demic areas and inoculated into nonsplenectomized anaplasmosis-free
calves. Each of five calves received blood from five and one from six deer.
Anaplasmosis did not occur in any of the calves during a 106 to 111 day
post-inoculation observation period. The susceptibility of the calves was
subsequently challenged with blood from an infected bovine carrier. All
proved susceptible. Ten of the deer were collected on an eastern Oregon
ranch where acute bovine anaplasmosis is not uncommon. These findings
substantiate research conducted on Wyoming mule deer.

A trial now being completed indicates that American bison may also be
resistant to natural A. marginale infection. This trial was conducted on
perhaps the largest commercial bison herd in the United States. In 1969
the adult herd numbered 1,576 head. The herd was located in an anaplasmosis endemic area and the animals were pastured on typical eastern Oregon sagebrush range. Blood from 132 adult bison was collected and inoculated into nonsplenectomized anaplasmosis-free calves. Each calf received pooled blood from 12 bison — 24 ml intravenously and 180 ml subcutaneously. None of the calves developed signs of anaplasmosis during the 124 day post-inoculation period and none developed positive reactions to the CF and new card test (CT). The calves were then challenged with blood from a bovine *A. marginale* carrier and all became infected.

The observation that clinical bovine anaplasmosis in central and eastern Oregon is generally restricted to the more arid sagebrush areas is of interest. Over the years the disease has had ample opportunity to invade adjacent areas. Cattle, deer and other game animals harboring *D. andersoni* move from endemic areas into these anaplasmosis free areas. Latent *A. marginale* carrier cattle are also commonly transported or driven into these areas. Yet, random CF testing of native cattle shows a very low incidence of infection and acute anaplasmosis is seldom observed. The observation that in Northwestern United States anaplasmosis is more prevalent in cattle pastured on sagebrush ranges has previously been noted.

The reason the disease has not invaded these anaplasmosis free areas is not presently known. Environmental factors such as temperature and atmospheric conditions may in some manner affect *A. marginale* so natural transmission does not occur. Rosenbusch and Gonzales demonstrated the importance of environmental temperatures in transmission of *Babesia bigemina* by boophilis ticks. They found that relatively minor temperature variations in some manner affected *B. bigemina* so infection in cattle did not occur. More likely, however, unfed adult *D. andersoni* present in these areas may have little opportunity to become infected. Transovarian transmission probably does not naturally occur or *A. marginale* would have become established in the tick population of these areas and the disease would be present in native cattle. This is in agreement with the findings of Anthony and Roby who were unable to demonstrate hereditary transmission.

To transmit anaplasmosis it seems logical to assume that adult *D. andersoni* must become infected with *A. marginale* prior to parasitizing cattle. Since endemic anaplasmosis is confined to dry sagebrush range country, it also seems logical to assume that one or more small animal species indigenous to this area may be *A. marginale* reservoir hosts. Larvae and nymphs parasitizing these reservoir hosts could become infected and by transtadial transmission transfer infection to adult ticks. These ticks could harbor the infective agent and transmit the disease to cattle. The disease could be maintained in the small animal reservoir host population by the feeding habits of *D. andersoni* larvae and nymphs.

Conversely, in anaplasmosis free areas no small animal reservoir hosts
may exist. *Dermacentor andersoni* larvae and nymphs are believed to parasitize only small animal species. They, therefore, would not become infected and would not transmit infection to adult ticks. The disease would be transmitted only by partially fed adult ticks leaving an infected large animal host and later parasitizing a susceptible host. However, female ticks once attached generally feed to repletion, drop to the ground, oviposit and die. Males feed as they move about the host fertilizing females. These males can become infected. Although normally they remain on one host they could move between hosts, especially from cow to calf, or under close confinement from cow to cow. Movement of ticks between adult cows on ranges where cattle are widely dispersed, however, seems unlikely. It is doubtful that movement of ticks between hosts is an important method of disease transmission since the disease has not become established in these anaplasmosis free areas where both latent carrier cattle and susceptible cattle are present.

The possibility of *D. andersoni* larvae and nymphs parasitizing cattle still exists. Larvae and nymphs have experimentally been placed on cattle and have fed. Larvae are dark colored, very small and would be difficult to observe on large animals. Since transtadial transmission is known to occur *A. marginale* could be transmitted from an infected cow through the immature tick stages to the adult tick. This tick could then in turn transmit the disease to a susceptible cow. However, if this method of transmission commonly occurred, anaplasmosis should be prevalent in all *D. andersoni* indigenous areas.

It appears that the existence of small animal *A. marginale* reservoir hosts indigenous to the dry sagebrush anaplasmosis endemic areas of Oregon and other northwestern states is very possible and deserves intensive study. Greater knowledge of the epidemiology of anaplasmosis is essential prior to the development of practical prevention, control and eradication programs.

**SUMMARY**

Data from this study were collected from several sources and include all areas of Oregon. Anaplasmosis is endemic in parts of southwestern and most of central and eastern Oregon. The epidemiology in southwestern Oregon is similar to that reported for western California.

In central and eastern Oregon the endemic area is generally limited to the more arid sagebrush country where *D. andersoni* and mule deer are indigenous. However, *D. andersoni* and mule deer are also indigenous to large areas of higher rainfall where clinical bovine anaplasmosis rarely occurs and when it does, it occurs in stressed cattle recently moved from anaplasmosis endemic areas. Native cattle are generally free of *A. marginale* infection. It is conceivable that one or more small animal species indigenous to the arid sagebrush areas is a natural host of *A. marginale*. Larvae and nymphs parasitizing these infected animals could transmit *A. marginale* by stage to stage transmission to the adult ticks.
who in turn could transmit the disease to susceptible bovine hosts.

No relationship appeared to exist in Oregon between the prevalence of clinical bovine anaplasmosis and the prevalence of the ticks *D. albipictus* and *Ixodes pacificus* or mosquitoes, horse flies, or deer flies. The study suggests Oregon mule deer are not important reservoir hosts of *A. marginale*.

REFERENCES

16. Rosenbusch, F. and Gonzales, R.: Garrapatizacion y tristeza. Inves-
REPORT OF THE COMMITTEE ON ANAPLASMOSIS

Chairman: Bert W. Hawkins, Ontario, Oregon
Co-Chairman: W. E. Brock, Stillwater, Oklahoma
Research: F. C. Neal, Gainesville, Fla.; J. A. Howarth, Davis, Calif.;
J. O. Roby, Clarksville, Md.; M. Ristic, Urbana, Ill.; D. G. Luther,
Baton Rouge, La.; J. Lee Alley, Auburn, Ala.; Floyd Frank, Moscow,
Idaho.

State Federal Regulatory: J. L. O'Harra, Reno, Nev.; G. S. Kaley,
Albany, N. Y.; F. E. Henderson, Baton Rouge, La.; R. M. Thomas, Little
Rock, Ark.; J. W. Safford, Helena, Mont.; B. R. McCallon, Adelphi,
Md.; J. F. McCory, Jackson, Miss.; E. E. Kerr, San Francisco, Calif.

Biologics and Pharmaceuticals: J. C. Trace, Ft. Dodge, Iowa; Dreyfus
Froe, Terra Haute, Ind.; G. I. Roberts, Triangle Park, N. D.
Livestock Industry: Bob Laramore, Gillette, Wyo.; Clayton Paddock,
Harrison, Ark.; Robert Gadd, Highmore, S. Dak.; Bill Gallagher,
Stephen, S. Dak.; Doyle McAdams, Jr., Huntsville, Tex.

The Committee on Anaplasmosis met in open session at 1:30 p.m.,
October 15, 1973. Thirty-seven persons were in attendance. Papers con-
cerning current research were presented as follows:

Dr. G. M. Bruening—“Mediated Immune Responses in Anaplasmosis.”
Dr. Richard F. Hall—“Incidence of Anaplasmosis in Northern Idaho.”

Dr. B. R. McCallon presented a map and data showing the results of
a survey of the incidence of anaplasmosis in each of the states. Generally,
the incidence decreases toward the northern states but is greatest in
those states where there is a prevalence of two or more vectors. Many of
the samples tested from north-central states showed a high proportion
of suspect reactions and may not indicate infection with anaplasmosis.
The study shows a surprisingly low incidence in Florida and an unsus-
pectedly high incidence in Wyoming.

Discussion following Dr. McCallon’s presentation indicates that the
disease is increasing in all parts of the country. It was brought out that a
recent regulation following the committee’s resolution of last year now
permits the interstate shipment of anaplasmosis reactor cattle for
slaughter or, if found to react after shipment to a state requiring a nega-
tive test, can be returned to the point of origin. Training courses on the
use of the card test have been set-up in various regions for instruction of
laboratory personnel and Federal field veterinarians.

Dr. Kermit Peterson made a short report on the incidence of anaplas-
mosis in Oregon. There are certain areas in Oregon where anaplasmosis
does not become established although infected cattle are shipped into the
region. The disease appears to be largely limited to the sage brush areas.
Mule, deer and buffalo do not appear to act as reservoirs in these regions
of Oregon from very limited research work carried on.

Discussion was brought out on the Imedocarb drug and application of
it. Different methods of use are under study at this time.

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J. O. Pearce, Jr., moved and Dr. John O'Hurra seconded a motion to adopt a resolution on the need for research which was unanimously passed. BE IT RESOLVED that additional anaplasmosis research in the Northwestern United States be encouraged and that emphasis be placed upon identification of both Anaplasma marginale vectors an reservoir hosts, methods of transmission and practicability of eliminating the latent carrier state from cattle pastured in infected ranges.

STATEMENT OF JUSTIFICATION

Prior to development of practical anaplasmosis control and eradication programs for Northwestern United States additional epidemiological studies are necessary. The important vector or vectors must be determined, the source of vector infection and natural transmission of cattle must be clarified and the practicability of eliminating Anaplasma marginale infection from cattle and returning them to infected ranges must be determined.
THE UTILIZATION OF *BRUCELLA ABORTUS* CULTURING AND BIOTYPING RESULTS IN THE EPIZOOTIOLOGIC INVESTIGATION OF BOVINE BRUCELLOSIS


The authors express their appreciation to the State and Federal work forces in Minnesota for their support and cooperation.

National Data was made available from Dr. W.C. Ray, Chief Staff Veterinarian, Brucellosis Epidemiology, USDA, APHIS, Veterinary Services, Hyattsville, Maryland.

SUMMARY

During an 8 year period, 730 *Brucella abortus* isolates were obtained from 331 cattle herds in Minnesota. Epizootiologic determinations of sources of infection and modes of transmission were significantly increased when culturing and biotyping information was included as part of the total investigation. Results of isolation attempts were used to monitor serologic diagnoses and increase confidence in correctly evaluating herd status. Program modifications based on epizootiologic findings were recommended following evaluations of data from both infected and non-infected herds. There is an urgent need to include bacteriologic culture procedures as an integral part of the epizootiological investigations used to confirm that brucellosis eradication has been achieved in a state or country.

Three *B. abortus* biotypes were isolated from Minnesota cattle. Biotype 1 was predominant and widely distributed in the state. Biotype 4 was confined to the central area of the state but expanded its distribution by movement of infected and exposed cattle. Biotype 2 was usually associated with interstate cattle importation. Isolates indistinguishable from Strain 19 were geographically scattered in the state.

The distribution of *B. abortus* biotypes isolated from cattle in the United States is reviewed and compared with the data from Minnesota.

INTRODUCTION

Bacteriologic procedures for the culturing and biotyping of *Brucella abortus* were included as an integral part of the Cooperative State-

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Federal Brucellosis Eradication Program in Minnesota. The results of bacteriologic examinations from July 1, 1962, through June 30, 1970, are summarized for 730 isolates from 331 herds. These results established another parameter to evaluate a changing brucellosis program.

During this period, Minnesota was qualifying for certified brucellosis free status and all cattle in each county were included in at least 2 (two) area tests. Herds not covered by Brucellosis Ring Test (BRT) surveillance were blood tested at 3 year intervals and all herds were blood tested at least every 6 years. Known infected herds and suspicious BRT herds were blood tested as recommended by the then current Uniform Methods and Rules. The primary means of spread of brucellosis were: introduction of exposed cattle, introduction of chronic undetected carriers and contact transmission by various means from adjacent or neighboring infected herds.

Culture and biotype information was used to evaluate and verify sources of infection for reactor herds and to monitor diagnoses based upon serologic results in both infected and non-infected herds.

The host range and world distribution of Brucella spp. has been described in a report on 550 strains isolated from various species, and preferential hosts for each of the Brucella species were delineated. During the early 1960's, development of several new serologic procedures (supplemental tests) assisted in identifying the characteristics of circulating antibody. In general, each test was used to differentiate between IgG and IgM antibody. Culturing was used in conjunction with some supplemental tests to confirm the presence of infected “carrier” cattle which were negative to standard brucellosis tests.

Results of Brucella isolation attempts were also used to evaluate various serologic tests in brucellosis infected herds and to evaluate the significance of IgM (heterospecific) antibody in non-infected cattle populations. Distribution of brucella biotypes 1, 2 and 4 in classified problem herds has been reported. Better definitive typing procedures have confirmed epizootiologic evidence of persistent Strain 19 infections and identified the need to lower calf vaccination ages.

Several modifications of the brucellosis program have been made to take advantage of this information. One example is the development of the Buffered Brucella Antigen (BBA) and integration of the card test into the program.

The purpose of this paper is to identify and stress the need for using culturing and biotyping techniques in evaluations of the brucellosis program. Their use is an addition to, not a replacement for, complete, in-depth, on the farm, epizootiologic investigations.

MATERIALS AND METHODS
1. Herd Selection and Specimen Collection in Minnesota

During 1962, B. abortus isolates were from problem herds which satisfied one of six original classifications. Beginning July, 1963,
culturing was increased and an attempt was made to isolate *Brucella* from all newly infected herds located in certified brucellosis free counties (20 of 87 total). After July, 1965, newly infected herds in all counties were followed with culture attempts whenever possible. Approximately 75% of the reactors from Minnesota were slaughtered in South St. Paul; this reduced the problems connected with specimen collection. Reactors slaughtered in other states required coordination with the Meat and Poultry Inspection Program personnel who would collect the specimens. Cooperation by this group was excellent; and, with confirmation of shipment and slaughter times, specimens were collected and submitted in satisfactory condition.

Specimens collected at South St. Paul were normally delivered to the laboratory for processing the same day. Tissue specimens that required mailing were either shipped in dry ice or were frozen and shipped with wet ice. Milk samples collected aseptically from lactating dairy animals were also used for culturing. A special attempt to collect milk was made if the animal was not to be slaughtered in South St. Paul. Milk samples were chilled and kept cold during shipment to the laboratory.

2. **Culturing and Biotyping Procedures**

Material obtained for culturing included milk, and/or selected tissues. A complete set of tissues collected at slaughter included: two supramammary, two retropharyngeal, two ischiatic and two iliac lymph nodes, plus a section of spleen, uterus and each quarter of the udder. Selective media containing antibiotics was used under 10% CO₂ atmosphere for all culture attempts. Twenty-two petri plates were used for each full set of tissues.

Confirmation of isolates by conventional typing procedures was conducted by the Biologic Reagents Section, Veterinary Services Diagnostic Laboratory (VSDL), Ames, Iowa, until September, 1966, and thereafter by the Veterinary Services Brucellosis Laboratory, Mission, Texas.

3. **Livestock Population Statistics**

Agricultural statistics for the year 1966 represent the midpoint for this period. Therefore, all calculations were based on these statistics. The number of dairy herds gradually declined while the number of beef herds increased during the eight years.

**RESULTS**

In Minnesota from July, 1962, through June, 1970, serologic reactor cattle were disclosed in 1,899 herds. *Brucella* isolations were attempted in 784 reactor herds (41%). *B. abortus* was isolated from 730 cattle in 331 reactor herds (43% of the herds attempted). Isolates were classified as *B. abortus* biotype 1, 2 and 4; some isolates had characteristics indistinguishable from Strain 19.

The distribution of isolates and their biotype is summarized (Table 1). *B. abortus* biotype 1 was isolated from 169 herds in 56 counties. Biotype 4 was isolated from 125 herds in 32 counties. Biotype 2 was isolated from 15 herds in 12 counties. There were 13 herds in 12 counties from which
multiple biotypes were isolated. Strain 19 like organisms were isolated from 10 herds in 10 counties.

There were five counties with 15 or more *B. abortus* herd isolates (Table 2). The total number of reactor herds, herds with isolation attempts and herd isolations, as well as percentage figures, are also summarized (Table 2).

The number of reactor herds, per county, ranged from 0 to 99, four counties had no reactor herds. In general, the lowest concentration of reactor herds was in the north; southern counties had medium numbers, and the highest concentration was in central Minnesota (Figure 1). This parallels the distribution of herds and cattle per square mile and the herd attack rate (Table 3). There are a few deviations from these general observations. An example is Lake of the Woods County in the far north which had a very small herd and cattle population but had the highest herd attack rate in the state. The deviations are discussed.

**EPIZOOTIC EXAMPLES**

Culturing and biotyping *B. abortus* from cattle was an integral part of the epizootiologic investigation of reactor herds in Minnesota, and it proved to be a successful and effective addition to disease eradication procedures. Several examples illustrate the potential value of these procedures and demonstrate that any state, regardless of its present brucellosis status, could benefit from similar information.

1. **One Herd Outbreak.**

A beef herd was identified as infected, and five *B. abortus* isolates were confirmed. The herd had been imported into Minnesota in several lots during a two to three year period. Each lot was accompanied by certificates and negative brucellosis tests. One first investigation, a previously known reactor herd in the same county was considered the most probable source for infection. A second possibility was exposure to brucellosis prior to, or during, importation into Minnesota from a Western state. Each isolate had an atypical pattern of manometric measurements for biotype 1. No other Minnesota isolates, before or since, have given comparable results. In this case, definitive biotype determinations provided evidence that strongly suggested the herd was infected prior to importation.

2. **One County Epizootic.**

A certified brucellosis free county with no known brucellosis in the previous five years had a rapid and severe spread of infection following the introduction of brucellosis. The epizootic was confined to a small geographic area in the county but 23 new herd infections occurred in two years. This was a herd attack rate of 50%. All herds were infected with biotype 1. The rapid spread to herds within the community indicated neighborhood contacts, but it was impossible to identify the source of infection for each herd. Epizootiologic evidence, including biotype results, favored a cattle to cattle contact spread following a single source introduction. It did not implicate deer, milk trucks, seagulls or traders for intra-community spread, although a tendency often develops during
epizootics to look for the unusual and exotic means of transmission or reservoirs for brucellosis and to ignore the obvious and more common means for spreading the disease. Culture and biotyping as part of the epizootiologic investigation can be used to evaluate probable modes of transmission and aid in the eradication of disease from individual herds. In this, and also other epizootics, eradication of brucellosis was dependent upon the ability to properly test and eliminate all the infected cattle. Reinfection into this area has not occurred.

3. Five Certified-Free Counties.

Five counties in west central Minnesota were certified brucellosis free prior to an outbreak in 1966. The index herd was located with BRT screening. Six infected herds were ultimately identified and biotype 4 was isolated from each herd. The outbreak was the result of movement of exposed infected cattle from a modified certified county following a herd sale. Epizootiologic investigation provided evidence of transmission by exchange of cattle between four of these herds. Results of bacteriologic culture in this epizootic indicated that the six herds were related to a common source and that brucellosis spread rapidly from herd to herd. Two additional epizootiologic factors involved in this outbreak were a 120+ day incubation period and a calfhood vaccinated heifer exposed at one year of age. Later, this vaccinated heifer introduced brucellosis into a clean herd. Research results and observations from epizootiologic investigations in field cases, such as these, instigated recommended changes in both the quarantine time for infected herds and the test ages for vaccinated heifers.

4. Extensive Epizootic.

Two adjacent counties had the most extensive local epizootic during the eight year period. Wadena in early 1963 was a certified brucellosis free county with no known infection, and Cass County had only two known infected herds. During the next seven years, more than 100 reactor herds were identified in the two counties. One hundred and fifty-two isolates from 52 reactor herds were confirmed as biotype 4.

Many hypotheses were developed to explain possible modes of transmission and reservoirs of disease. Sources outside the immediate area appeared to be of little significance because of the involvement of the single biotype. Transmission was obviously occurring within the neighborhood, although the mode was not always identified. Application of two basic principles of disease eradication were successful in breaking the chain of disease transmission. An aggressive approach to eradication was maintained by regulatory personnel and, secondly, an education program for producers stressed the ease and rapidity with which brucellosis can spread. Brucellosis was eradicated from this area and has not reappeared. New reservoirs of infection were not established. Culturing and biotype information played an important role in the education program for these producers.

5. Strain 19 Type Organisms.

Only 10 isolates were designated *B. abortus*, biotype 1,
90 THE UTILIZATION OF BRUCELLA ABORTUS

distinguishable from Strain 19. In most cases, the history was typical: A dairy herd with no recent history of infection or abortion was suspicious on the routine BRT. On the herd blood test following the suspicious BRT, one reactor animal was disclosed. The cow, usually a first lactation heifer, had freshened since the previous negative BRT. There was a significant serial dilution BRT milk titer and Brucella organisms were isolated from the milk of the reactor animal.

Variations of this typical history may pose very important epizootiological questions that cannot be answered without an isolate for identification. The following was one case: Serologic tests following a suspicious BRT disclosed a single reactor which was in the first three months of the initial lactation. The animal had been purchased out of state, six months earlier, at an annual statewide, breed association sponsored purebred sale. The herd of origin was certified brucellosis free, and no test for sale or movement was required. At the time of the last herd certification test, this heifer was not old enough to require a test.

Epizootiologic questions posed: Was this a Strain 19 organism from which no further spread would be expected; or was it a field strain of B. abortus? If it was a field strain B. abortus, where was the source and when did exposure occur? How many, and where, were the other animals that might also have been exposed? Fortunately, a Strain 19 isolate was obtained and no further related infection had occurred.

6. Multiple Biotype Herds.

In thirteen herds, more than one biotype of B. abortus was isolated. All but three herds were disclosed early in the study. Considering the last three cases: The first herd had four B. abortus biotype 1 and one biotype 4 isolates confirmed. Concurrent infection from a dual source was confirmed for this herd. The second herd had biotype 4 isolated initially. Infection was considered eradicated from the herd. After an interval of one year, brucellosis was again diagnosed. Chronic undetected infection with an extended incubation period would normally have been considered in this herd; except that now, biotype 1 was isolated, and a new source of infection was designated. In the third herd, biotype 1 was isolated initially and later a Strain 19 like organism was isolated from a young animal that was calfhood vaccinated while on the farm.

It is neither possible nor necessary to attempt culture on each group of reactors in every herd. In most herds, tissues from one group of reactors will suffice. However, when a herd is infected over a long period of time and new introductions become infected; or, if infection reoccurs after apparent eradication, additional culture attempts are indicated. Multiple biotype herds will become fewer as the eradication program progresses and the opportunity for introduction of brucellosis from two or more sources is reduced.


A significant number and pattern of negative culture results, in conjunction with a better understanding of brucellosis, also prompted program adaptations. While accomplishing area tests in Minnesota, many
herds had only one reactor identified and branded. Subsequent herd tests were negative and the disease did not reappear. With the introduction of supplemental tests, it was observed that these "singleton" reactors, usually non-vaccinates, were negative on all supplemental tests. The standard plate or tube test recorded a reactor titer and the cattle were branded and removed. Tissues from this type of animal were almost always negative for *B. abortus*. The data indicated that some non-infected animals did have significant amounts of circulating IgM antibody (heterospecific) but not IgG antibody. To aid in solving this problem and also others, a buffered low pH *Brucella* antigen (BBA) was developed which eliminates most heterospecific reactions. This BBA antigen was combined with the card test and then adopted as a standard test. In this circumstance, culture results were used to monitor the serological diagnosis. An apparent problem was identified, and after thorough investigations in Minnesota and elsewhere, program modifications were made to alleviate the situation.

**DISCUSSION**

**TISSUE PROCESSING**

Techniques for culturing *B. abortus* from bovine tissues and milk as described do not require extensive laboratory facilities,* With few exceptions, tissues could be processed in present state-federal brucellosis laboratories.

Tissues that will be processed within 24 hours can be stored at refrigerator temperature. If tissues require mailing to the laboratory, they should be frozen, preferably at -40°C, and then mail with ice or dry ice, if available. Milk samples must be chilled shortly after collection and processed within 48 hours for satisfactory results. Dry secretions from non-lactating cows should be handled the same as milk. Overgrowth of culture plates with contaminants will frequently result if specimens are not processed as outlined. In Minnesota, attempts were made to collect tissues and isolate *Brucella* from the initial reactors. The prompt isolation of *B. abortus* helps satisfy the owner and it allows confirmation and biotyping procedures to be rapidly completed so the results are available for epizootiological use. Priority in collecting tissues should be given the supramammary lymph nodes. Collection of additional tissues and fluids increase the probability of isolation. In herds with multiple reactors the isolation rate ranged from 62-85% of the herds attempted. In multiple reactor herds, a preferable procedure is to collect a limited number of tissues from each of several reactor animals. Herds with only one or two reactors will have a lower average herd isolation rate. In these herds a complete set of tissues from each reactor should be collected and processed. Isolations from cattle with heterospecific antibody only, or unusual serologic patterns, were essentially zero; however, under predictable circumstances, such as very recent exposure or very chronic infection, some isolates were obtained. Culture results from cattle classified as suspects were also very low. Negative culture results are no guarantee that *B. abortus* is not present, since culture techniques
are not infallable. Tissue selection and preservation are important factors. However, during the last 3 years of the 8 year period, almost 90% of the herds considered to be actually infected with *B. abortus* prior to culturing did subsequently yield the organism. Only one animal classified as a suspect was positive on culture during this period and it was a "singleton" reactor from a 100% calfhood vaccinated herd.

Upon authorization, in those few states where local facilities are not available for bacteriological examination, frozen tissues individually wrapped and identified can be sent to VSDL, Ames, Iowa. Subcultures for biotyping should be sent on a tryptose agar slant in a double-protection type shipping container.

### GEOGRAPHIC DISTRIBUTION

The geographic distribution of each biotype is summarized (Table 1). Biotype 1 has been the predominant *B. abortus* organism isolated in Minnesota and was distributed throughout the state. Biotype 4 was the second most common isolate. It appeared to be concentrated in the state's north central area where 60% of the biotype 4 infected herds were found in 9 adjacent counties. However, extension to a larger portion of Minnesota has occurred. Five percent of the herd isolates were biotype 2 and a majority were identified early in the study. During the last five years, biotype 2 was associated with cattle imported from other states, usually beef cattle, and only once was transmission to a neighboring herd identified. Strain 19 like organisms were isolated from 10 herds, each in a different county in south and central Minnesota. There are persistent strain 19 infections in beef cattle as well as dairy cattle. Fewer beef animals are detected because of the different surveillance system. There were only three multiple biotype herds disclosed in the last four years. Improved laboratory techniques with additional definitive information may have been partly responsible for fewer multiple biotype herds, but the most important factor was decreased exposure potential to herds. The herd attack rate of 9.2 per 1,000 herds during fiscal year 1963 decreased to 0.8 per 1,000 herds for fiscal year 1970. The potential of having two concurrent infections was all but eliminated by 1970.

### INFLUENCES ON REACTOR HERDS

There were two predominant characteristics of counties with large numbers of infected herds. A localized high concentration of cattle and/or an abundance of cattle moving between herds. Five counties with numerous infected herds are listed (Table 3). Cass and Lake-of-the-Woods Counties have a low herd and cattle concentration when considered on a county basis. However, each county had small geographic areas within its borders in which the cattle concentration was high. The same situation was true for Wadena and Pine Counties. Although the cattle concentration for these two counties was about the state average, local high concentrations of herds contributed to a higher attack rate. In each of these four counties, there was a brucellosis epizootic in such an area. Carver County, which had a high density cattle population, did not experience any local epizootics. In this county, extensive cattle movements were reflected by the isolation and identification of all three bio-
types of *B. abortus*, Strain 19 and a multiple biotype herd.

The influence of periodic area tests on the total number of reactor herds in an area is illustrated by the situation in Pine County and Meeker County. Pine County had two local epizootics, one in 1963 and one in 1966, from which 15 isolates were obtained. There were also 50 "singleton" reactor herds identified on a complete county test in 1964. Meeker County had 45 such herds identified on a complete county test in 1964. Animals in these "singleton" reactor herds were serological reactors on the standard plate test or tube test but all supplemental tests were negative.

Vaccination with Strain 19 is another influence which must be considered, since calfhood vaccination has long been a recommended part of the overall eradication program. There were 156 isolates (21%) from cattle that could be identified as officially vaccinated. A few completely vaccinated herds had very high animal attack rates, but this was unusual. Some herds in counties with high levels (over 50%) of vaccination had *B. abortus* isolated, but neighborhood epizootics did not occur. When there was sufficient brucellosis infection and exposure potential to evaluate the benefits of Strain 19 vaccination; it did appear that the brucellosis attack rate for herds, and cattle within completely vaccinated herds, was less than for those herds, and animals, that were not vaccinated. The exposure potential in Minnesota is now negligible and evaluations of the benefits of Strain 19 vaccination cannot be determined.

**UNITED STATES ISOLATION DATA**

A preponderance of *B. abortus* biotype 1 has been isolated in the United States with fewer of biotypes 2, 4 and Strain 19. The totals as of September 28, 1972, are summarized (Table 4). These isolates were confirmed at either the Mission, Texas, or Ames, Iowa, laboratories of Veterinary Services, APHIS, USDA. This group of 3355 isolates represents 42 states. However, additional isolates from cattle have been obtained but were not confirmed at these two laboratories and therefore, they are not included in the national data.

Twenty states have made few or no *B. abortus* isolations as part of their total epizootiologic investigation procedures (Table 5). National data may reflect, in part, local interest and competency in bacteriology. The data does demonstrate the significant fact that many states do not have sufficient information available to (Table 6): 1) identify predominant biotypes, 2) recognize if biotypes are restricted to certain geographic areas within the states, or 3) confirm spread between states.

The culturing and biotyping of *B. abortus* in various investigative and research projects has helped to confirm much of the epizootiology of brucellosis. The present recommended minimum standards for the brucellosis eradication program contain various modification based on the findings of such studies.

The Minnesota and national data have been presented to emphasize the need for integration of culture and biotype information into an official state brucellosis eradication program. Bacteriology was not used as
a replacement for epizootiologic investigations, but it was used to complement them. A total epizootiologic investigation package must be utilized to expedite the eradication of brucellosis. Conventional and definitive biotyping procedures are additional parameters to be used during investigations. The need for bacteriologic culturing as a routine element of a complete epizootiologic investigation in all areas of the United States is more apparent as brucellosis eradication is approached. There is a definite need to more diligently identify sources of infection and means of spread into free states or areas. This will necessitate a greater effort by many states to include culturing and biotyping of *Brucella* in the eradication program.

REFERENCES

11. Ray, W. C. Personal Communication, Chief Staff Veterinarian, Brucellosis Epidemiology, Veterinary Services, APHIS, USDA, Hyattsville, Maryland.


Table 1. Summary of Minnesota E. abortus Herd Isolates and Biotype Distribution, July 1962 - June 1970.

<table>
<thead>
<tr>
<th>County, or Grouping, by Number of Isolates</th>
<th>Number of herds with each biotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Wadena</td>
<td>28</td>
</tr>
<tr>
<td>Cass</td>
<td>27</td>
</tr>
<tr>
<td>Lake of the Woods</td>
<td>22</td>
</tr>
<tr>
<td>Carver</td>
<td>18</td>
</tr>
<tr>
<td>Pine</td>
<td>15</td>
</tr>
<tr>
<td>12 Counties (6-10 isolates each)</td>
<td>88</td>
</tr>
<tr>
<td>55 Counties (1-5 isolates each)</td>
<td>133</td>
</tr>
<tr>
<td>15 Counties (no isolates each)</td>
<td>--</td>
</tr>
<tr>
<td>Total herd isolates</td>
<td>331</td>
</tr>
</tbody>
</table>

% of total 100% 51% 5% 38% 3% 4%

*One Strain 19 isolate included in the multiple biotype herds.
### Table 2. Summary of Minnesota Reactor Herds Having Animals Cultured, July 1962 - June 1970

<table>
<thead>
<tr>
<th>County, or Grouping, by Number of Isolates</th>
<th>Total Number Reactor Herds</th>
<th>Total Number Herds Culture Attempted</th>
<th>Percent Successful of Herds Attempted</th>
<th>Total Number Herd Isolations</th>
<th>Percent of Total Reactor Herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wadena</td>
<td>53</td>
<td>45</td>
<td>62%</td>
<td>28</td>
<td>53%</td>
</tr>
<tr>
<td>Cass</td>
<td>76</td>
<td>40</td>
<td>68%</td>
<td>27</td>
<td>36%</td>
</tr>
<tr>
<td>Lake of the Woods</td>
<td>28</td>
<td>26</td>
<td>85%</td>
<td>22</td>
<td>79%</td>
</tr>
<tr>
<td>Carver</td>
<td>31</td>
<td>31</td>
<td>56%</td>
<td>18</td>
<td>58%</td>
</tr>
<tr>
<td>Pine</td>
<td>75</td>
<td>56</td>
<td>27%</td>
<td>15</td>
<td>20%</td>
</tr>
<tr>
<td>12 Counties (6-10 isolates each)</td>
<td>335</td>
<td>174</td>
<td>51%</td>
<td>88</td>
<td>26%</td>
</tr>
<tr>
<td>53 Counties (1-5 isolates each)</td>
<td>1146</td>
<td>385</td>
<td>35%</td>
<td>133</td>
<td>12%</td>
</tr>
<tr>
<td>15 Counties (no isolates each)</td>
<td>155</td>
<td>27</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Totals</td>
<td>1899</td>
<td>784</td>
<td>43%</td>
<td>331</td>
<td>17%</td>
</tr>
</tbody>
</table>

*Mean number per county.


<table>
<thead>
<tr>
<th>County</th>
<th>Herds per Square Mile</th>
<th>Cattle per Square Mile</th>
<th>Attack Rate per 1000 Herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wadena</td>
<td>1.33</td>
<td>23.9</td>
<td>74.4</td>
</tr>
<tr>
<td>Cass</td>
<td>0.39</td>
<td>6.4</td>
<td>96.0</td>
</tr>
<tr>
<td>Lake of the Woods</td>
<td>0.18</td>
<td>2.7</td>
<td>123.6</td>
</tr>
<tr>
<td>Carver</td>
<td>3.14</td>
<td>96.2</td>
<td>26.6</td>
</tr>
<tr>
<td>Pine</td>
<td>0.98</td>
<td>20.6</td>
<td>54.3</td>
</tr>
<tr>
<td>State Average</td>
<td>1.02</td>
<td>21.2</td>
<td>22.9</td>
</tr>
</tbody>
</table>
Table 4. Summary of \textit{B. abortus} Biotypes Isolated from U.S. Cattle*

<table>
<thead>
<tr>
<th>Animal Vaccination Status</th>
<th>Total Isolates</th>
<th>1 (69%)</th>
<th>2 (9%)</th>
<th>4 (7%)</th>
<th>St. 19 (14%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinate</td>
<td>705 (21%)</td>
<td>489</td>
<td>64</td>
<td>52</td>
<td>100</td>
</tr>
<tr>
<td>Non-Vaccinate</td>
<td>971 (29%)</td>
<td>719</td>
<td>64</td>
<td>178</td>
<td>10**</td>
</tr>
<tr>
<td>Unknown</td>
<td>1679 (50%)</td>
<td>1344</td>
<td>137</td>
<td>146</td>
<td>52</td>
</tr>
<tr>
<td>TOTALS</td>
<td>3355 (100%)</td>
<td>2552</td>
<td>265</td>
<td>376</td>
<td>162</td>
</tr>
</tbody>
</table>

*As of September, 1972. \textsuperscript{11}

**Exposure to Strain 19 through accident, etc.

Table 5. Summary of Total U.S. \textit{B. abortus} Isolates by States*

<table>
<thead>
<tr>
<th>Number of Total Isolates</th>
<th>Number of States with Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥200</td>
<td>3\textsuperscript{a}</td>
</tr>
<tr>
<td>100-199</td>
<td>6</td>
</tr>
<tr>
<td>50-99</td>
<td>5</td>
</tr>
<tr>
<td>25-49</td>
<td>7</td>
</tr>
<tr>
<td>10-24</td>
<td>9</td>
</tr>
<tr>
<td>1-9</td>
<td>12</td>
</tr>
<tr>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Minnesota, Ohio, Wisconsin.

* As of September, 1972. \textsuperscript{11}
Table 6. Summary of Total U.S. *B. abortus* Isolates by Biotype and States*  

<table>
<thead>
<tr>
<th>Number of Total Isolates</th>
<th>Number of States with <em>B. abortus</em> Biotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>≥200</td>
<td>3(a)</td>
</tr>
<tr>
<td>100-199</td>
<td>4</td>
</tr>
<tr>
<td>50-99</td>
<td>6</td>
</tr>
<tr>
<td>25-49</td>
<td>7</td>
</tr>
<tr>
<td>10-24</td>
<td>8</td>
</tr>
<tr>
<td>1-9</td>
<td>13</td>
</tr>
<tr>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

(a) Minnesota, Ohio, Wisconsin.  
(b) Minnesota.  
(c) Minnesota.  
(d) California.  

*As of September, 1972.*
Figure 1. Numerical Range of Minnesota Brucellosis Reactor Herds by County, July 1962 – June 1970.

0-15 Reactor herds (white) 39 counties
16-40 Reactor herds (black) 37 counties
41-99 Reactor herds (check) 11 counties
PROGRESS OF THE STATE-FEDERAL
BRUCELLOSIS ERADICATION PROGRAM

Gerald J. Fichtner, D.V.M.*

In a report to this association in November 1972, attention was directed toward critical program indicators which strongly suggested that progress toward brucellosis eradication was being significantly reduced. Emphasis was placed on the immediate need to increase the efficiency and volume of surveillance, to apply the basic principles of disease eradication as outlined in the Uniform Methods and Rules, to increase manpower, funds and basic program authority, and to develop a commitment to the psychological attitude of eradication rather than control. Total program effort would need to be increased to reduce the constant prevalence of brucellosis which was apparent at that time.

A year has now passed since that initial warning that the prevalence of brucellosis had increased. A review of program progress at this time might indicate whether the needed increase in total program efforts has been implemented and whether 1 year's effort has brought us nearer the goal of brucellosis eradication from this Nation's livestock.

BLOOD TESTING CATTLE (Figure 1)

The upward trend in cattle blood tested was continued in FY 1973 when over 13.6 million animals were sampled. This 15 percent increase indicates 5.15 million tested on farms or ranches and 8.46 million at packing houses and sales yards under the Market Cattle Identification (MCI) Program.

There was a corresponding 26.6% increase in total brucellosis reactors to 158,000. Of this total, 104,000 were disclosed on the farm or ranch and 54,000 on the MCI program. Although a gradual downward trend in the reactor rate had been observed since 1970, this was abruptly reversed in FY 1973, when 1.16 reactors were disclosed per 100 blood tests conducted as compared to 1.05 the previous year. This reversal in the brucellosis reactor rate must be regarded as a warning signal that positive action must be initiated to reverse this unfavorable trend and allow the program to proceed to a successful conclusion.

MARKET CATTLE IDENTIFICATION PROGRAM (Figure 2)

The upward trend in the number of animals tested under the MCI program continued in FY 1973 when 8.46 million tests were conducted. This 1.2 million increase includes an additional 837,000 samples collected at packing plants and a 357,000 increase at livestock markets. A 61% increase in the number of MCI reactors to 54,000 results in an MCI reactor rate of 0.63. This rate is a marked rise over the 1972 rate of 0.46 and the 1971 rate of 0.52. The MCI reactors were traced to 15,083 herds of origin. Followup tests disclosed infection in 5,352 of these herds with an animal infection rate of 14%. Since this is higher than the 13% infection rate.

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reported for 1972, action to increase the effectiveness of the MCI surveillance program is vital.

**MILK RING TEST RESULTS (Figure 3)**

In FY 1973, 2179 herds (0.23%) reacted to the BRT. 2050 herd blood tests were conducted with infection being disclosed in 592 herds (29%). 4.4 percent of the cattle on the initial herd test were reactors in the infected herds as compared to only 3.5% in 1972.

Although the number of infected dairy herds disclosed by BRT surveillance continued a previously established gradual downward trend, an increase in the numbers of infected animals on the initial herd test should warrant constant vigilance of the following:

1. The BRT must be performed no less than three times per year.
2. The sensitivity of the BRT must be adjusted in large dairy herds.
3. Each BRT suspicious herd must be immediately tested.

**BRUCELLOSIS INFECTED HERDS (Figures 4 & 5)**

A total of 12,435 infected herds were disclosed in the 50 States in FY 1973. This is an 8% increase over the previous year and resulted in a reversal of the continuous downward trend since 1967. 435 of these herds were in the 30 certified States compared to 385 the previous year. The remaining 12,000 infected herds were detected in the 20 modified certified States and exceeded by 900 the number found in 23 such States the previous year.

86% of these infected herds were distributed among only 10 States. Texas accounted for 27.1% of the total, while Louisiana, Mississippi, and Oklahoma had 34.2%. Six States including Alabama, Arkansas, Florida, Georgia, Kentucky, and Tennessee collectively totaled 24.8%. Not one of these 10 States has significantly decreased the number of infected herds found annually during the past 2 years.

Twelve States had 11.4% of the infected herds with only Illinois, Missouri, and South Dakota showing a decrease from the previous year.

In summary, 97.5% of the infection was found in 21 States and Puerto Rico, with 19 of the 22 showing no significant decrease in infection.

**CERTIFICATION STATUS—JUNE 30, 1973 (Figure 6)**

All counties in the United States have now met the requirements for initial modified certified area status. This was achieved during FY 1973 when Nueces County, Texas qualified for this status.

Pennsylvania, Montana, and Hawaii became Certified Brucellosis-Free during the year to bring the total to 30 States and the Virgin Islands. 1,946 counties (62%) held Certified Brucellosis-Free area status at the end of FY 1973. This is an increase of 185 over the previous year and is primarily due to increases in Colorado, Wyoming, South Dakota and Florida. However, during the first 3 months of the current fiscal year, only nine additional counties have qualified for free status. 53% of the Nation's cows, including 79% of the Nation's dairy cows and 44% of the
Nation's beef cows, are located in areas with Certified Brucellosis-Free status.

At the end of FY 1973 there were 2,205 modified certified areas (38%) located in 20 States and Puerto Rico. There were two counties in Oklahoma which lost modified certified status because of certain program deficiencies. 47% of the cows in the U.S., including 21% of the Nation's dairy cows and 56% of the Nation's beef cows, are located in those areas which are listed as modified-certified.

The danger of totally associating the eradication of brucellosis with the attainment of area certification status is evident.

- The granting of "free" area status only indicates a zero prevalence of the disease at that point in time; does not guarantee against reintroduction of infection; and is not a license to reduce surveillance or ignore attention to suspicious and infected herds.
- The granting of "modified-certified" area status only indicates a step toward eradication; was only to be a temporary phase; and should be an area where maximum program effort is exerted.

However, the program is lagging even if measured by progress toward achieving certification status. Only nine modified certified counties were able to reduce the prevalence of brucellosis to zero and qualify for certified free status in the past 3 months, while another 37 counties temporarily lost certification status during FY 1973 due to program deficiencies.

CALFHOOD VACCINATION (Figure 7)

The downward trend in the number of calves vaccinated annually continued in FY 1973 with a 10% reduction to 3.8 million. 50% of the total vaccinations occurred in the 30 Certified Brucellosis-Free States, where an estimated 27.3% of the eligible calves were vaccinated. The remaining vaccinations occurred in the 20 modified certified areas where 16.2% of the eligible calves received Strain 19.

It is also recognized that in order to eradicate brucellosis there must be:

1. continuous and maximum pressure to detect and eliminate any remaining foci of infection,
2. the implementation of necessary program change to prevent reintroduction and spread of the disease, and
3. a decrease and eventual elimination of vaccination.

These three conditions must be concurrent. Although it would be expected that vaccination would be utilized more in those areas where the greatest benefit could be realized and less in those areas where the risk of exposure is minimal, the reverse is true.

SWINE BRUCELLOSIS (Figures 8, 9, 10)

Substantial progress was made in the swine brucellosis program during FY 1973 despite a midyear cutback in the program budget.

During the year 1.2 million swine were blood tested. This represents a
158% increase over the preceding year. This total includes 377,000 tested on farms and 877,000 sows, boars, and stags tested at slaughter under the Market Swine Testing Program (MST). Nine slaughter plants in five States were actively engaged in blood sampling eligible swine at the end of the fiscal year.

Six States — Iowa, Nebraska, South Dakota, Wisconsin, Arkansas, and Montana — initiated statewide validation efforts during the year under the alternate method of validating areas and Montana became the first State to qualify for free status by this method.

The number of Validated Brucellosis-Free areas increased from 198 to 275 during the year. This total includes all counties in Arizona, California, Montana, Nevada, Utah, Vermont, and the Virgin Islands. Other States with validated free counties are: Hawaii—2, Maryland—1, Massachusetts—4, Michigan—13, New Mexico—1, South Dakota—8, and Puerto Rico—55.

There was a 50% increase from 2468 to 3668 in the number of Validated Brucellosis-Free herds during 1973 due in part to the required testing of breeding herds in States validating areas by the alternate method.

During FY 1973 much of the emphasis was placed on improving market swine surveillance by expanding collection of blood samples at slaughter plants and in planning for 1974 swine program activities. Fourteen States —Indiana, Illinois, Iowa, Kansas, Minnesota, Missouri, Montana, Nebraska, New Mexico, Ohio, Pennsylvania, Texas, South Dakota, and Wisconsin—expect to initiate or continue their swine brucellosis program in FY 74 and requested Federal funds for this purpose.

Minnesota adopted regulations late in the year which made it the third State —after Wisconsin and Nebraska—to have mandatory identification requirements for swine.

SUMMARY

In spite of an increase in the number of Certified Brucellosis-Free areas in FY 1973, the only conclusion that can be drawn from an increasing MCI reactor rate, increased numbers of infected herds, a constant BRT suspicious rate, and an increase in the overall reactor rate, is that progress was not made toward the goal of brucellosis eradication.

The question that must be considered at this time is Why, —in spite of available technology; in spite of increasing surveillance to detect foci of infection; in spite of recent efforts by many States to strengthen their programs; in spite of increased manpower in the heavily infected States; in spite of adequate minimum standards to conduct the program; in spite of minimum producer opposition to the program—Why are we lagging in attempting to eradicate brucellosis?

The answer to that question is that we haven’t developed an attitude of seriousness about the urgent task of eradication. This sense of urgency must be upon us today.
### Brucellosis Eradication

#### BLOOD TESTING: CATTLE

<table>
<thead>
<tr>
<th>YEAR</th>
<th>THOUS. REACTORS FOUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>1968</td>
<td>149</td>
</tr>
<tr>
<td>1969</td>
<td>130</td>
</tr>
<tr>
<td>1970</td>
<td>119</td>
</tr>
<tr>
<td>1971</td>
<td>116</td>
</tr>
<tr>
<td>1972</td>
<td>124</td>
</tr>
<tr>
<td>1973</td>
<td>158</td>
</tr>
</tbody>
</table>

#### MILLIONS CATTLE TESTED

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

#### MARKET CATTLE TESTING PROGRAM

<table>
<thead>
<tr>
<th>YEAR</th>
<th>MILLION COWS BLOOD TESTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1967</td>
<td>58.0% 42.0%</td>
</tr>
<tr>
<td>1968</td>
<td>59.0% 41.0%</td>
</tr>
<tr>
<td>1969</td>
<td>59.6% 40.4%</td>
</tr>
<tr>
<td>1970</td>
<td>58.2% 41.8%</td>
</tr>
<tr>
<td>1971</td>
<td>58.4% 41.6%</td>
</tr>
<tr>
<td>1972</td>
<td>52.2% 37.8%</td>
</tr>
<tr>
<td>1973</td>
<td>59.3% 36.7%</td>
</tr>
<tr>
<td>1974</td>
<td></td>
</tr>
</tbody>
</table>
Brucellosis Eradication

MILK RING TEST RESULTS (BRT)

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

FISCAL YEAR

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

FISCAL YEAR

NUMBER STATES
Non-Certified Modified Certified Certified Free
1966 12 29 9
1967 11 29 10
1968 8 29 13
1969 6 29 15
1970 4 27 19
1971 1 27 22
1972 1 22 27
1973 0 20 30
1974
DISTRIBUTION OF BRUCELLOSIS REACTOR HERDS

Percent of Total Reactor Herds Found

- 30 STATES, <30 HERDS: 2.5%
- 12 STATES, 30<300 HERDS: 11.4%
- 6 STATES, 300<1,000 HERDS: 24.8%
- 3 STATES, 1,000<3,000 HERDS: 34.2%
- 1 STATE, >3,000 HERDS: 27.1%

FISCAL YEAR 1973

Cooperative State-Federal

BRUCELLOSIS ERADICATION PROGRAM

- CERTIFIED BRUCELLOSIS FREE: Counties 1,946
- MODIFIED CERTIFIED AREAS: Counties 1,205
- COMPLETE AREA TESTING: Counties 2

JUNE 30, 1973

U.S. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE
Brucellosis Eradication

### CALVES VACCINATED

**MILLION CALVES VACCINATED**

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>1953</th>
<th>'55</th>
<th>'57</th>
<th>'59</th>
<th>'61</th>
<th>'63</th>
<th>'65</th>
<th>'67</th>
<th>'69</th>
<th>'71</th>
<th>'73</th>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

#### BLOOD TESTING: SWINE

**ANIMALS TESTED**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>547,088</td>
<td>532,804</td>
<td>546,021</td>
<td>547,130</td>
<td>538,036</td>
<td>480,834</td>
<td>1,254,187</td>
<td></td>
</tr>
</tbody>
</table>

**REACTING ANIMALS**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
<td>4.9%</td>
<td>0.10%</td>
<td>0.34%</td>
<td>0.32%</td>
<td>0.10%</td>
<td>0.16%</td>
<td></td>
</tr>
</tbody>
</table>

U.S. DEPARTMENT OF AGRICULTURE  VETERINARY SERVICES  ANIMAL AND PLANT HEALTH INSPECTION SERVICE
REPORT OF THE COMMITTEE ON BRUCELLOSIS

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

The Brucellosis Committee of the U. S. A. H. A. met in open session October 17, 1973, 1:30 p.m. Papers were presented by Ray Snell, G. J. Fichtner, E. E. Saulman and John O'Hara. Resolutions were introduced by a delegation of Florida dairymen, Wisconsin Department of Agriculture, and the Virginia Department of Agriculture. No comments supporting or opposing any of the resolutions proposed in open session were received.

The Brucellosis Committee reconvened on October 18, 1973, in closed session to consider all resolutions. Favorable consideration was given to the following:

1.) To amend the Uniform Methods and Rules to allow validation under alternate statewide plan #2.
   1.) (as is on Page 19)
   2.) All sows, boars and stags are tested at slaughter with a minimum of 50% of such swine so tested and representative of all swine herds during a two year period originating from the state seeking validation.
   3.) (as is on Page 19)

REVALIDATION:

1.) Continue testing all sows, boars and stags at slaughter with a minimum of 50% of such swine so tested originating from the Validated State.
2.) When reactors are found, the herd of origin is validated or sent to slaughter within 30 days.

The following motion was adopted: The Chairman of the Brucellosis Committee will appoint a panel to:

1.) Make an immediate review of the research literature and field results on the use of the B. R. T. and other tests on dairy cows.
2.) Recommend a plan to be field tested in large dairy herds where
blood testing is unduly burdensome.
If the research and pilot plan prove effective and contribute to the eradication of brucellosis, the Committee has no objection to the use of B. R. T. on individual dairy cows with all suspect animals to be confirmed by a blood agglutination tests.
The following nine point program was adopted:
1.) That the continued official use of Strain 19 vaccine be encouraged and that the maximum allowable vaccination age be continued at 10 months.
2.) When the official test is used and reactors are found the owner may request an additional test on the reactors within 10 days at his own expense.
3.) That surveillance is a vital part of a Brucellosis reduction program and the Market Cattle Testing program is effective as a surveillance tool. We recommend further attention and effort towards the MCT programs with special emphasis on more effective and complete return of samples from all packing plants and better cooperation between the various departments to bring about more efficient use of MCT programs.
4.) That the regulations governing control of brucellosis in cattle be applied to bison and other known carriers, regardless of federal, state or individual ownership of such carriers.
5.) That officially vaccinated beef females, 24 months of age or older, continue to be allowed a titer tolerance.
6.) That high priority be given to renewal and increased efforts to develop a testing procedure that could determine the difference between infection and vaccinal titre provided to develop alternative types of vaccine with particular emphasis on diagnosis of the disease and as a prophylactic agent in adult cattle.
7.) That funds appropriated for Brucellosis programs be used for Brucellosis programs only.
8.) That to increase the effectiveness of a Brucellosis eradication program, a cooperative state-federal indemnity program for negative exposed cattle and calves be made available and that disaster type loans be made available to owners of depopulated herds.
9.) That, in order to accelerate the Brucellosis Eradication program, an expanded public information and educational effort must be undertaken to create a better understanding, and acceptance of the Uniform Methods and Rules.
In furtherance of the goal accelerating the program, the USDA will provide periodic information to state and federal regulatory and extension agencies, livestock journals, breed associations, and all other interested persons; including
1.) The number of infected herds in each state.
2.) Area certification status.
3.) Program deficiencies in each state.
4.) Significant changes in program procedures.
The USDA Information service will provide such information through specially prepared state release for media within a state for those states where the program warrents such attention, as well as through national roundups.

4.) It was resolved that clarification of the intent of several sections of the Uniform Methods and Rules be provided and that the format as used previously be reinstated. The following additions will be included:

1.) All samples initially tested at markets, in private offices or on the farm will be promptly submitted and confirmed by the central State—Federal laboratory.

2.) Wherever test requirements refer to dairy cattle 20 months of age and beef cattle 24 months of age, there will also be written "As evidenced by the presence of the first pair of permanent incisors."

3.) Eliminate the Standard Plate test for the diagnosis of Brucellosis in swine.

4.) Card positive swine disclosed in a herd which is otherwise serologically, bacteriologically, and epidemiologically negative may be held under herd quarantine until subjected to subsequent tests to determine the status of the animals.

5.) Herd quarantines on swine be clarified with the duration and exemptions now specified for cattle also apply to swine.

6.) The herd quarantine requirements which apply to infected herds will also apply to herds of origin of reactors disclosed at livestock markets.

5.) The following recommendation was adopted pertaining to dealer laws in certified free states.

1.) Any state designated as Brucellosis Free shall develop laws or regulations which require that all dealers in the state maintain adequate records of purchase and sales.

2.) All animals received by the dealer for resale must be brucellosis tested at the time of purchase and at the time of resale.

3.) The premises of all dealers in a Brucellosis Free state should be required to undergo a periodic cleaning and disinfection and there should be periodically a complete depopulation of the dealer premises at the time of cleaning and disinfection so that any chain of infection would be broken.

6.) The following resolution was adopted:

Be it resolved that the funding of major disease outbreaks be obtained in a manner that does not deplete the resources set aside for regularly programmed projects.

STATEMENT OF JUSTIFICATION

The health and well-being of the American people depends on a wholesome meat supply and the livestock industry endeavors to supply the American consumer with the highest quality product at the lowest cost.
possible. The control and eradication of disease is the single important factor in accomplishing this goal, and when special disease programs, sometimes short of the national emergency category, but of sufficient urgency to warrant immediate and expedient attention occur, these outbreaks and special problems have in the past been funded by robbing existing budgeted disease control and eradication projects, and the gains made in these programs are often lost and must be repeated with each interruption.

Reference is made to the policy of fighting fires, which at the moment of their discovery, are fought with all of the tools and equipment necessary for the earliest confinement and expulsion of the conflagration. When the emergency work has been completed, documented expenses would be listed and presented to Congress for payment. This would allow the existing budgeted programs to continue at a regular and calculated pace rather than with the continued disruption and loss of progress.

7.) A recommendation requiring that any future proposals, recommendations or resolutions be in the hands of all Committee members 30 days prior to Brucellosis Committee meeting. It was adopted.

Resolutions not approved by the Committee are as follows:

1.) Florida proposal adult vaccinate was defeated by a vote of 11 to 10.

2.) The Virginia amendment to the Uniform Methods and Rules regarding Brucellosis Critical Areas was tabled for further study and consideration because of lack of time to study the plan.

All proposals submitted to the Committee were considered by all members.
GROWTH INHIBITION TEST FOR MEASUREMENT OF IMMUNE RESPONSE OF ANIMALS VACCINATED WITH LEPTOSPIRAL BACTERINS

D. N. Tripathy, L. E. Hanson and M. E. Mansfield

College of Veterinary Medicine
University of Illinois
Urbana, Illinois

Immune responses of cattle and swine to leptospiral bacterins have been difficult to evaluate because a majority of the vaccinated animals develop either low or no MA titer following vaccination. However, results of hamster protection test and challenge studies show that protective antibodies do develop after vaccination (Negi et al., 1971; Huhn et al., 1973; and Taylor et al., 1973). Evaluation of the immune response with the hamster protection test is not only expensive and time-consuming but cannot be used for those serotypes which are not pathogenic for this species. Similarly, challenge of vaccinated animals has not provided a suitable method for evaluation of antibody response as the signs and lesions vary considerably. In our earlier studies (Tripathy et al., 1972), we reported that sera from animals vaccinated with leptospiral bacterins were able to inhibit the growth of leptospires even after MA antibodies could not be detected. This report provides a further evaluation of the growth inhibition test with some modifications of the procedure.

MATERIAL AND METHODS

Experiment 1.
Sera from a total of 24 cattle vaccinated as follows were tested for growth inhibiting activity with hardjo culture. Ten cattle had been vaccinated with a pomona and hardjo bacterin, nine cattle had been vaccinated with a pomona bacterin plus a hardjo and grippotyphosa bacterin and five cattle had been vaccinated with a pomona bacterin only. The group inoculated with pomona bacterin only served as control group. The Leptospira pomona bacterin used was a commercially available bacterin.* L. hardjo bacterin and the hardjo-grippotyphosa bacterins were prepared in this laboratory using 8-12-day-old-cultures grown in bovine albumin polysorbate 80 medium. The culture was inactivated with formalin and 50% O/W3 adjuvant** was added. Two ml. of vaccine was ad-

This work was supported by University of Illinois Agricultural Experiment Station Hatch Project 70-302.
The authors thank Mrs. R. Marlowe and Mr. W. Manuel for technical assistance.

*Affiliated Laboratories, White Hall, Illinois
**Supplied by Fort Dodge Laboratories
ministered to each animal by the subcutaneous route.

In the growth inhibition test, 1 ml. of liquid polysorbate 80 medium (Ellinghausen and McCullough, 1965) was added to each sterile 1 dram vial. To test each serum sample, five vials of medium were used and 0.05 ml. of inactivated test serum was added to each vial. To each vial a quantity of 0.05 ml. of diluted culture of *hardjo* containing approximately $10^7$ organisms per ml. was added. The vials were incubated at 29°C for two weeks and their contents were then examined under darkfield. Five vials of media inoculated with *hardjo* culture were used as control. All sera were also tested for microscopic agglutinating (MA) antibodies. The vials which had less than ten leptospires per microscopic field under darkfield examinations at magnification of 120X were considered to be positive for antibody while those which had more organisms at this magnification were considered negative for the specific antibody.

**Experiment 2**

Ten swine from one litter were vaccinated at 2½ months of age with a multivalent vaccine containing serotypes *pomona*, *hardjo* and *grippotyphosa* antigens prepared from cell wall material of the organisms and were tested for both MA antibody and growth inhibiting response. This vaccine was supplied by Dr. Johnson from the University of Minnesota (Auran et al., 1972). Each pig was inoculated with 1 ml. of unfrozen bacterin intramuscularly. Sera obtained at different time intervals were tested for antibody response. In the growth inhibition test as described earlier, five vials each containing one ml. of liquid polysorbate 80 medium were used for each serum sample. For occasional serum samples, less test vials were used because of lack of enough serum. Separate tests were conducted with *pomona*, *hardjo* and *grippotyphosa* antigens.

**RESULTS**

The results obtained with sera from vaccinated cattle are summarized in Table 1. Only five out of ten animals of Group I had a MA titer of incomplete 1:100 while sera from almost all animals completely or partially inhibited the growth of *hardjo* one month after inoculation. Growth inhibiting activity was detectable in approximately one-half of the sera at 4½ months while all sera were negative in the MA test.

The results on sera from swine vaccinated with multivalent vaccine of cell wall origin are presented in Tables 2, 3 and 4. It is evident that MA antibody was detectable in few animals and for a shorter duration than the growth inhibiting antibody. At six months none of the vaccinated animals had any MA antibody activity while growth inhibiting activity was detectable in the majority of sera tested.

Results of these experiments were based on the examination of individual vials examined under the darkfield microscope. However, with direct gross observations of the test vials, three definite patterns were identifiable. 1. In the inoculated vials which remained clear, organisms were neutralized to the extent that either no growth or very little growth
was detectable even under darkfield microscopy. 2. In some vials where either the turbidity was less dense than the control vials or agglutinated clumps of organisms were present, the growth was partially inhibited. 3. In vials where growth was not inhibited, the growth densities were comparable to the control tubes. (Fig. 1)

DISCUSSION

Although the results were based on the darkfield microscopic examination of each inoculated vial, it appears that the inhibitory effect could easily be detected by direct examination of the inoculated medium by comparing the turbidity with the controls. However, it is desirable to examine each tube microscopically to determine whether the turbidity was actually due to growth of leptospires rather than by a contaminant. In the latter case, the test cannot be evaluated. It is assumed that reduction in growth whether complete or parital as well as the presence of agglutinated clumps is the result of specific antibody in the serum of vaccinated animals. Different classes of antibody have been shown to be responsible for MA and neutralization (Negi et al., 1972). Whether or not growth inhibition effect is due to one or both classes of antibodies was not investigated in this experiment. The advantage with this test is its simplicity and being inexpensive, a large number of samples can be run in a short time.

SUMMARY

Immune responses of cattle and swine vaccinated with leptospiral bacterins were determined by MA and GI (growth inhibition) tests. To economize the reagents in the test procedure, the GI test was run in one dram vials containing one ml of bovine albumin polysorbate 80 medium to which 0.05 ml. of inactivated test serum and 0.05 ml. of live leptospiral culture containing approximately $10^7$ organisms per ml. were added. Although direct examination of the vials showed differences in growth, each vial was examined microscopically at two weeks after inoculation. GI activity was detectable longer than MA antibody.

REFERENCES

4. Negi, S. K., Myers, W. L., and Segre, D. Antibody response of cattle to Leptospira pomona: Responses as measured by hemagglutination,


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**TABLE 1. RESPONSE OF CATTLE VACCINATED WITH LEPTOSPIRAL BACTERINS AND TESTED WITH HARDJO CULTURE**

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccination</th>
<th>No. of Animals</th>
<th>Antibody Response Measured at 1 MONTH</th>
<th>2 1/2 MONTHS</th>
<th>4 1/2 MONTHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td><em>Pomona</em> plus <em>Hardjo</em></td>
<td>10</td>
<td>5 N</td>
<td>6 +</td>
<td>1 I</td>
</tr>
<tr>
<td>II</td>
<td><em>Pomona</em> plus <em>Hardjo</em> and <em>Grippotyphosa</em></td>
<td>9</td>
<td>9 N</td>
<td>5 +</td>
<td>9 N</td>
</tr>
<tr>
<td>III</td>
<td><em>Pomona</em></td>
<td>5</td>
<td>5 N</td>
<td>1 +</td>
<td>5 N</td>
</tr>
</tbody>
</table>

**M.A. Test:** N = Negative at 1:100 dilution. I = Incomplete for M.A. at a titer of 1:100.

**G.I. Test:** + = Positive for growth inhibition; ± = Where growth was partially inhibited, i.e., some vials showed growth and some did not. N = Negative for growth inhibition.
TABLE 2. RESULTS OF SERA FROM PIGS VACCINATED WITH MULTIVALENT POMONA, GRIPPOTYPHOSA AND HARDJO BACTERINS AND TESTED WITH POMONA CULTURE

<table>
<thead>
<tr>
<th>Test</th>
<th>1 Month</th>
<th>2 Months</th>
<th>4 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.I.</td>
<td>10+</td>
<td>6+</td>
<td>9+</td>
</tr>
<tr>
<td></td>
<td>3±</td>
<td>1±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.A.</td>
<td>1 I</td>
<td>1 1:100</td>
<td>1 I</td>
</tr>
<tr>
<td></td>
<td>9 N</td>
<td>2 I</td>
<td>9 N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 N</td>
<td></td>
</tr>
</tbody>
</table>

G.I. TEST: + = POSITIVE FOR GROWTH INHIBITION; ± = WHERE GROWTH IS PARTIALLY INHIBITED, I.E., SOME VIALS SHOWED GROWTH AND SOME DID NOT; N = NEGATIVE FOR GROWTH INHIBITION.

M.A. TEST: 1:100 REPRESENTS COMPLETE M.A. TITER; I = INCOMPLETE FOR M.A. AT 1:100 TITER; N = NEGATIVE FOR M.A. AT 1:100 DILUTION.

TABLE 3. RESULTS OF SERA FROM PIGS VACCINATED WITH MULTIVALENT POMONA, GRIPPOTYPHOSA AND HARDJO BACTERINS AND TESTED WITH GRIPPOTYPHOSA CULTURE

<table>
<thead>
<tr>
<th>Test</th>
<th>1 Month</th>
<th>2 Months</th>
<th>4 Months</th>
<th>6 Months</th>
<th>8 Months</th>
<th>10 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.I.</td>
<td>6+</td>
<td>7+</td>
<td>8+</td>
<td>5+</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>4±</td>
<td>1±</td>
<td>2±</td>
<td>3±</td>
<td>7±</td>
<td>1±</td>
</tr>
<tr>
<td></td>
<td>1 N</td>
<td>2 N</td>
<td>1 N</td>
<td>3 N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.A.</td>
<td>2 I</td>
<td>2 1:100</td>
<td>4 I</td>
<td>10 N</td>
<td>10 N</td>
<td>7 N</td>
</tr>
<tr>
<td></td>
<td>8 N</td>
<td>3 I</td>
<td>6 N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

G.I. TEST: + = POSITIVE FOR GROWTH INHIBITION; ± = WHERE GROWTH IS PARTIALLY INHIBITED, I.E., SOME VIALS SHOWED GROWTH AND SOME DID NOT; N = NEGATIVE FOR GROWTH INHIBITION; ND = NOT DONE.

M.A. TEST: 1:100 REPRESENTS COMPLETE M.A. TITER; I = INCOMPLETE FOR M.A. AT 1:100 TITER. N = NEGATIVE FOR M.A. AT 1:100 DILUTION.
TABLE 4. RESULTS OF SERA FROM PIGS VACCINATED WITH MULTIVALENT POMONA, GRIFFOTYPHOSA AND HARDJO BACTERIUM AND TESTED WITH HARDJO CULTURE

<table>
<thead>
<tr>
<th>Test</th>
<th>Antibody response at 1 month</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>8 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.I.</td>
<td>2+</td>
<td>4+</td>
<td>4+</td>
<td>3+</td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>4±</td>
<td>6±</td>
<td>6±</td>
<td>5±</td>
</tr>
<tr>
<td></td>
<td>3 N</td>
<td>1 N</td>
<td>1 N</td>
<td>4 N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>M.A.</td>
<td>3 I</td>
<td>4 I:100</td>
<td>I I</td>
<td>10 N</td>
<td>10 N</td>
</tr>
<tr>
<td></td>
<td>.7 N</td>
<td>2 I</td>
<td>9 N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 N</td>
</tr>
</tbody>
</table>

G.I. TEST: + = Positive for growth inhibition; ± = Where growth is partially inhibited, i.e., some vials showed growth and some did not; N = negative for growth inhibition; ND = not done.

M.A. TEST: 1:100 represents complete M.A. titer; I = incomplete for M.A. at 1:100 titer; N = negative for M.A. at 1:100 dilution.

Fig. 1. Leptospiral growth inhibition test showing variation in growth.
1. No growth—complete inhibition
2. Some growth—agglutinated clumps—partial inhibition
3. Marked growth—no inhibition

Fig. 1. Leptospiral growth inhibition test showing variation in growth.
1. No growth—complete inhibition
2. Some growth—agglutinated clumps—partial inhibition
3. Marked growth—no inhibition
REPORT OF THE COMMITTEE ON LEPTOSPIROSIS 1973

Chairman: L. E. Hanson, Urbana, Illinois
Co-Chairman: H. D. Stoenner


The Committee reviewed reports of serologic surveillance, control procedures and leptospiral research. The reports included serologic rates, isolation of a new serotype from cattle, vaccine evaluation, new leptospiral bacterins and public health aspects of leptosperosis.

Leptospirosis continues to be one of the major diseases affecting U.S. livestock. Losses result from interruption of pregnancies, deaths in calves and baby pigs, retardation of growth and reduction in milk production. Serologic studies continue to indicate Pomona, hardjo and grippotyphosa as to the most prevalent serotypes affecting cattle and pomona and grippotyphosa the most common in swine. Reports from several states indicate hardjo which is as common as pomona in cattle is responsible for continuing reproductive problems such as metritis and repeat breedings. A need exists to further evaluate the role of hardjo in sterility problems in cattle.

A new serotype, szwajizak, was isolated from dairy cows in Oregon and identified by the Center for Disease Control in Atlanta, Georgia. The new serotype is a member of the hebdomadis serogroup and antigenically related to the hardjo serotype. Laboratories utilizing hardjo antigens in the serologic testing programs will probably detect the new serotype. Clinical observations of the affected herd indicate the szwajizak serotype was associated with abortions, still-births, reproductive problems and mastitis in the affected cows and deaths in calves up to 4 weeks of age. The identification of this new serotype in clinical leptospirosis in cattle indicates the importance of confirming serologic results by isolation attempts in the diagnosis of leptospirosis.

Reports from some areas of the U. S. indicate satisfactory serologic diagnostic competence has not been achieved by all diagnostic laboratories. The Committee encourages all diagnostic laboratories not using the microscopic agglutination test to utilize the currently available licensed inactivated antigens of pomona, hardjo, grippotyphosa, canicola and icterohaemorrhagice in their leptospiral testing program for livestock.

A serologic surveillance for these serotypes in livestock is needed to evaluate the importance of leptospirosis in domestic animals in all areas of the United States.

The Committee gratefully acknowledges the recent availability of special licensed hardjo and grippotyphosa bacterins as a major effort in providing a more complete spectrum of biologics for the control of
the major serotypes in livestock. Attention now should be directed to the development of multivalent bacterins for the control of *pomona*, *hardjo* and *grippotyphosa* serotypes.

The Committee reviewed research in progress and the importance of continuing research to develop tests for the evaluation of bacterin efficacy. Only through the establishment of reasonable and adequate standards for evaluation of leptospiral bacterin potency by the USDA can the availability of adequate bacterins be assured for the protection of the livestock industry.

The Committee discussed the public health aspects of leptospirosis associated with the disease in livestock and other animals.

A review of a 10 year summary of deaths due to notifiable diseases of man in the United States covering the period from 1960 to 1969 published in the 1972 Annual Supplement of the Morbidity and Mortality Report from the Center for Disease Control indicates leptospirosis was responsible for more deaths in man than any other zoonotic disease except salmonellosis. The significance of leptospirosis as a zoonotic disease demonstrates the need for both the U. S. Department of Health, Education and Welfare and the U. S. Department of Agriculture to provide greater research efforts for the development of control programs for this disease. The veterinarian has a responsibility to alert the owner of infected livestock and pets of the potential health hazards of the disease and should indicate precautions to prevent exposure of the owner.

The Committee again reiterates its position emphasized in previous reports that leptospirosis is not amenable to eradication because of the broad host range. However, it urges that APHIS re-evaluate the position which places priorities on programs for diseases that are considered to be eradicable and include control programs for other major livestock diseases of public health significance. Leptospirosis is a disease of major importance to both man and animals that urgently requires meaningful programs of surveillance and research for development of control programs. The USDA is encouraged to establish a Leptospirosis Reference Center to serotype field isolates.

The Committee recommends that the appropriate information branches of the USDA prepare an updated fact sheet on leptospirosis for the practitioner and the livestock industry. We extend our cooperation in the preparation of such a fact sheet.
DRY COW THERAPY AS A COMPONENT OF MASTITIS CONTROL


Two assumptions are made for the purpose of presenting the topic assigned the speaker, and these are as follows:

1. Mastitis is a problem in dairy herds.
2. Dry cow therapy is a component of mastitis control.

The appropriate choice of the word component, for dry cow therapy in mastitis control, recognizes the complexity of the disease, mastitis, as well as the other factors associated with its control.

It is necessary to mention the other factors recognized as components of mastitis control in order to permit some perspective to be related with dry cow therapy, and these components may be briefly summarized as follows:

a. **Milking system and equipment management.**

   A recent publication (7) provides some information on the effect of physical characteristics of the milking machine on the incidence of new mastitis infections. These findings may be the bases for further improvement in milking equipment design.

   The maintenance of milking equipment operation, in accordance with manufacturers' directions, is essential to udder health.

b. **Environmental hygiene.**

   A clean dry environment is essential for dairy herds to reduce teat contact contamination.

c. **Sanitary hygiene.**

   Sanitary hygiene includes daily cleaning and sanitizing of dairy equipment; the use of a mild disinfectant for washing the udders and teats of cows prior to milking; the use of individual paper towels; the rinsing and dipping of teat cups, first with water and then in a disinfectant between cows; and the use of a post-milking germicidal teat dip.

   There are other measures such as possible immunization and genetic resistance which may become significant factors in mastitis control at some future time.

   In a recently published Special Report (3), the AVMA Mastitis Committee have stated, "Recommended minimal standards of performance for practicing veterinarians who offer mastitis control programs", and illustrated the complexity of the veterinarian's role in mastitis control programs. The dairy farmer requiring effective control of mastitis needs the services of a skilled veterinarian, supported by appropriate Laboratory services, to develop a meaningful mastitis control program for his herd.

   A mastitis control program involves long-term herd prevention of
the disease, where the results of various control measures can only be gauged with time. It is unfortunate that results obtained from dairy herd hygiene and health practices are not reflected in an immediate, obvious, economic response. It is, perhaps, equally unfortunate that many dairy farmers have employed individual components of mastitis control programs, without the consideration, or the advice, essential to overall disease prevention. The results obtained in these cases are frequently less than satisfactory.

Herd health programs must recognize that today's dairy cow is producing more milk than ever before, and accordingly is subjected to increasing metabolic and environmental stresses. Under such circumstances, disease susceptibility might be expected to increase, posing a serious challenge to the herdsman. In the past 20 years, the national milk production has remained fairly stable, while the dairy cow population has decreased by almost 40%. The significance of this information is that the individual dairy cows' productivity has recently shown the greatest increase in the history of milk production.

Loss of milk production due to both clinical and subclinical mastitis, to say nothing of treatment cost, pose an ever-increasing economic burden on the milk producer. Consideration of economics and treatment cost, together with potential market losses, combine to emphasize the need for herd mastitis control programs.

The evaluation of herd mastitis control programs has centered primarily around the measurements of teat dipping and dry cow therapy components. The economic impact of clinical mastitis is apparent, and the production losses due to subclinical mastitis are becoming more apparent.

**Mastitis**

More than 20 different organisms have been associated (8) as the cause of mastitis. 97% of all udder infection has been attributed (4) to 4 gram-positive bacteria (*Str. agalactiae, Str. dysgalactiae, Str. uberis*, and *Staph. aureus*). Accordingly, control programs must be initiated with direct emphasis on the foregoing bacterial organisms. The use of available, effective systems to control major causes of mastitis, which can provide major economic return to the dairy farmer, have yet to be adequately implemented within the dairy industry.

Control of mastitis in a dairy herd is a long-term consideration dependent upon the availability of efficacious and safe teat dips and dry cow therapy products. The efficacy and safety of many commercial teat dips and dry cow treatment products have yet to be adequately substantiated. Until actual published product data is available, the value of many products must remain the subject of conjecture.

English workers published (1) a simple chart to illustrate the herd mastitis control response to teat dipping and dry cow therapy. This material indicates that both of these components play a highly complimentary role in herd health, and further indicate the need for continuing
application of both components in a herd mastitis control program (see Table 1).

**Dry Cow Therapy**

Dry cow therapy consists of the udder infusion of an efficacious and safe antibiotic preparation, at the time of drying off dairy cows, between lactations. The purpose of dry cow therapy is to reduce the frequency of existing infections and to prevent the occurrence of new infections during the dry cow period, prior to the subsequent lactation. Cornell workers found (2) that most new infections were identified within 5 weeks post-calving in each lactation age group. These findings were observed in the quarters of cows previously treated with a dry cow product where approximately 90% of all infections had been reported eliminated by the treatment (see Table 2).

Researchers have measured (1) the proportion of cows, in various herds, infected (with all pathogens) at drying off and calving in the absence of dry cow treatment. A higher percentage of cows were found to be infected at calving than was observed at drying off. It was also observed that the levels of infection at drying off and calving increased for these animals with subsequent lactations (see Table 3).

The value of intramammary antibiotic infusion has been demonstrated (6) in detail. These workers noted a 75% reduction in the number of udder infections at calving, and an 85% reduction in the number of quarters infected, as a result of dry cow therapy (see Table 4 and 5).

In published studies (5), procaine penicillin/dihydrostreptomycin has shown an 80-90% efficacy in reducing the frequency of existing infections at drying off (see Table 6). These results were obtained following the infusion of one million units of procaine penicillin and 1 gram of dihydrostreptomycin in cows’ quarters at drying off. The increase in the infection rate in nonlactating cows, between drying off and calving, was attributed to the persistence of existing infections and the incidence of new infections during the dry period.

A three year study, reported by Cornell Researchers (2), compared two systems of hygiene on 27 commercial dairy farms. In one group of herds, udders were washed with individual paper towels with 600 ppm chlorine in the wash water, milkers wore gloves that were dipped in the wash water before contact with each cow, teats were dipped in a 4% chlorine solution after each milking and all the quarters were treated with one million units of penicillin and one gram dihydrostreptomycin at drying off. Dairymen in the second group continued their existing milk practices but dipped teats and treated quarters at drying off with the same products as the first group. There were no reported differences in response between the two hygiene groups, when the milk yields of these herds were compared with New York Dairy Herd Improvement averages, the two groups on the mastitis control program produced an average of 477 kilograms of milk per cow more per year in each of the 3 years of study (see Table 7).
Mastitis infection within the herds in the preceding study decreased from an initial 28.1% of the quarters infected to 11.5% after one year, 8.1% after two years, and 7.1% at the end of the project. It was also noted from the data obtained in this study that, depending on the age of the animal and the type of infection, each infection reduced milk production by an average of 740 kilograms per cow per year. The herds involved in the foregoing study, carried out over three years, involved slightly less than 2,000 animals for the duration of the work. The dry cow therapy product containing one million units of procaine penicillin and one gram of dihydrostreptomycin, in a slow release base, is not yet available for commercial use. The drug is under current final review by the Food & Drug Administration, Bureau of Veterinary Medicine. The drug will be marketed by Hamilton Pharmacal Company, a division of West Agro-Chemical, Inc. under the name Quartermaster. The product Quartermaster will be available for use in dairy herds by or on the order of a licensed veterinarian and will be available upon prescription from a licensed veterinarian.

REFERENCES

TABLE 1

Diagrammatic representation of trends in the infection levels of cows in
(A) herds not using either teat dip or drying-off therapy and
(B) herds using both techniques. The levels at drying off and calving are based on results from MFE 1 and
MFE 3. Note the lines indicating expected trends in the infection levels in herds that have been using the
control system but cease to teat dip (cd) or fail to treat cows at drying off (ab).


TABLE 2

Change in 305-day lactation milk production per cow infected in one quarter at various
lactation stages as compared to a new infection in one quarter at drying off.

<table>
<thead>
<tr>
<th>Infection started</th>
<th>Lactation 1</th>
<th>Lactation 2</th>
<th>Lactation 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(weeks in lact.)</td>
<td>(kg)</td>
<td>(no.)</td>
<td>(kg)</td>
</tr>
<tr>
<td>0 - 5</td>
<td>-190</td>
<td>4,696</td>
<td>-39</td>
</tr>
<tr>
<td>6 - 10</td>
<td>-116</td>
<td>141</td>
<td>-337</td>
</tr>
<tr>
<td>11 - 20</td>
<td>-168</td>
<td>181</td>
<td>-204</td>
</tr>
<tr>
<td>21 - 30</td>
<td>-104</td>
<td>176</td>
<td>-158</td>
</tr>
<tr>
<td>31 - 40</td>
<td>17</td>
<td>185</td>
<td>-20</td>
</tr>
<tr>
<td>40</td>
<td>125</td>
<td>220</td>
<td>390</td>
</tr>
</tbody>
</table>

TABLE 3

<table>
<thead>
<tr>
<th>Dry period</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>&gt;3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>C</td>
<td>D</td>
<td>C</td>
<td>D</td>
</tr>
</tbody>
</table>

The proportion of cows infected (all pathogens) at drying off and calving. Data from 253 cows no infused at drying off (MFE 1) (36). Note rise in level of infection from drying off to calving for each age group.

D = at drying off;  C = at calving.

### TABLE 4


**The Effect of Intramammary Antibiotic Infusion and Teat Disinfection at Drying-off on the Percentage of Udders Infected at Calving**

<table>
<thead>
<tr>
<th>Experimental Treatment</th>
<th>Control (untreated)</th>
<th>0.2 g. cloxacillin (sodium) in aluminium monostearate base + hypochlorite teat dip</th>
<th>1 g. cloxacillin (benzathine) in aluminium monostearate base + hypochlorite teat dip</th>
<th>S.E. of difference between means 2 and 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of cows: 286, 303, 299

Per cent. of udders infected at drying-off: 53.8, 52.0, 49.9 ± 3.22

Per cent. of udders infected at subsequent calving: 62.0, 26.0, 15.6 ± 3.07

**Note.**—Any two means not underscored by the same line differ significantly at the 5 per cent. level (P<0.05)

### TABLE 5

**The Effect of Intramammary Antibiotic Infusion and Teat Disinfection at Drying-off on the Persistence of Infection Present at Drying-off and on Quarters Becoming Infected in the Dry Period**

<table>
<thead>
<tr>
<th>Experimental Treatment</th>
<th>Control (untreated)</th>
<th>0.2 g. cloxacillin (sodium) in aluminium monostearate base + hypochlorite teat dip</th>
<th>1 g. cloxacillin (benzathine) in aluminium monostearate base + hypochlorite teat dip</th>
<th>S.E. of difference between means 2 and 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of udder quarters: 1,144, 1,212, 1,196

Per cent. of quarters infected at drying-off: 24.2, 27.4, 24.6 ± 2.6

Per cent. of quarters infected at drying-off and persisting with infection at calving: 21.5, 7.9, 3.6 ± 1.82

Per cent. of quarters with a new infection in the dry period: 9.5, 3.3, 1.7 ± 0.9

Per cent. of quarters infected at calving: 30.9, 11.3, 5.4 ± 2.1

Percentage of infections persisting through the dry period: 88.8, 28.8, 14.6 ± 2.1

**Note.**—Any two means not underscored by the same line differ significantly at the 5 per cent. level (P<0.05)

**TABLE 6**

A Comparison of Two Antibiotic Levels of Mastitis Therapy in Nonlactating Cows (a)

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hem. Staph. (Staph. aureus)</td>
<td>1,000,000 Units Procaine Penicillin and 1 Gram Dihydrostreptomycin in 3% Aluminum Monostearate in Peanut Oil*</td>
<td>100,000 Units Procaine Penicillin and 100 mg. Dihydrostreptomycin in 3% Aluminum Monostearate in Peanut Oil (b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>Recovered</td>
<td>Recovery</td>
<td>Treated</td>
<td>Recovered</td>
<td>Recovery</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
<td>---------</td>
<td>---------</td>
<td>-----------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>55</td>
<td>53.9 (c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>136</td>
<td>99</td>
<td>72.8 (d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>135</td>
<td>90.0 (e)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>18</td>
<td>90.0 (e)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,659</td>
<td>3,352</td>
<td>91.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) Treated at the time of drying-off. (b) The authors wish to acknowledge the assistance in this study of Drs. H. Temple, L. Wager, W. Lingquist, L. Field, G. Hayes, and V. Boldt, Field Veterinarians of the New York State Mastitis Control Program. (c) Highly significant difference; P = .005. (d) Highly significant difference; P = .001. (e) No significant difference.

Roberts, S. J. et al.,
XIX WORLD VET. CONG.

**TABLE 7**

Change in mature equivalent production of 23 herds on a mastitis control program (MCP) compared to the New York Dairy Herd Improvement (NYDHI) average.

<table>
<thead>
<tr>
<th>Years on project</th>
<th>Number of lactations</th>
<th>MCP herds</th>
<th>NYDHI herds</th>
<th>MCP - NYDHI</th>
<th>Minus initial difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Milk</td>
<td>Fat</td>
<td>Milk</td>
<td>Fat</td>
</tr>
<tr>
<td></td>
<td>of lactations (kg)</td>
<td>(kg)</td>
<td>(kg)</td>
<td>(kg)</td>
<td>(kg)</td>
</tr>
<tr>
<td>0</td>
<td>1,520</td>
<td>6,124</td>
<td>224</td>
<td>5,992</td>
<td>219</td>
</tr>
<tr>
<td>1</td>
<td>1,637</td>
<td>6,247</td>
<td>239</td>
<td>5,905</td>
<td>219</td>
</tr>
<tr>
<td>2</td>
<td>1,734</td>
<td>6,622</td>
<td>244</td>
<td>5,972</td>
<td>221</td>
</tr>
<tr>
<td>3</td>
<td>1,844</td>
<td>6,757</td>
<td>239</td>
<td>6,122</td>
<td>226</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REPORT OF THE MASTITIS COMMITTEE OF THE UNITED STATES ANIMAL HEALTH ASSOCIATION

Chairman: N. B. Haynes, Ithaca, N. Y.
Co-Chairman: R. B. Bushnell, Davis, Calif.

The annual open meeting of the Committee was held at 1:30 p.m. on October 16, 1973. Twenty-two guests and eight members were present.

Two informal reports were presented. Dr. Robert Bushnell related experience with California dairy herds infected with E. Coli and Mycoplasma spp. His discussion emphasized the fact that mastitis is a disease with a multiple etiology and that correction of management factors is fundamental to adequate control.

Dr. Leslie Wager recounted his experience with herds in New York infected with Streptococcus agalactiae. This organism usually does not cause serious clinical disease but it does impair milk production. Herds which achieve Str. agalactiae free status as a result of quarter culture and intramammary therapy as indicated, consistently experience a production increase in excess of 10%. Herds that become free of Str. agalactiae remain that way unless they subsequently become reinfected. Reinfection is primarily via introduction of infected replacements to the herd. Several case studies were cited as examples.

In the discussion which ensued it was pointed out that aside from the increased production which can be anticipated, there is little incentive for a dairyman to participate in a formal mastitis control program. At the least, a dairyman whose herd is enrolled in such a program should receive quality points on his barn score sheet. The paradox was also cited whereby in most states cattle infected with warts or ringworm are refused admission to fairs and shows while no constraint is placed on the cow infected with udder pathogens, especially Str. agalactiae. The committee reaffirmed its support of the proposals for control of Str. agalactiae infection outlined in prior committee reports dating back to 1967.

INTRAMAMMARY MEDICATION

At present, intramammary preparations are required to be free of potential pathogens but not necessarily sterile. It was the unanimous opinion of the committee that if sterility could be achieved without significant increase in cost, it would be desirable. Greater concern was expressed for, and examples were cited of, on-the-farm contamination of multiple dose vials resulting in transmission of udder infections. The committee was apprised of the imminent availability, on a prescription
MASTITIS

basis only, of a high level antibiotic formula for dry cow therapy.

PUBLIC HEALTH

Reports of isolation of Str. agalactiae from human patients are appearing in the medical literature with increasing frequency. While the true significance of this has not been elucidated as yet it merits investigation.

RESOLUTIONS

As a result of its deliberations, the Mastitis Committee unanimously adopted the following resolutions:

Be it resolved that the USAHA recommends:

1. That the U.S. Public Health Service investigate the relationship between human and animal infections with Str. agalactiae.
2. That the Federal Drug Administration withdraw permission for marketing intramammary medication in multiple dose containers.
3. Initiation, by each state, of a screening survey to determine the prevalence of Str. agalactiae in its dairy cattle population.
4. Adoption, by each state, of a program for the eradication of Str. agalactiae infection in dairy herds, and,
5. That each state prohibit importation of preparturient and lactating dairy cattle unless such cattle are shown to be free from Str. agalactiae infection.

This constitutes the report of the Committee on Mastitis. On behalf of the Mastitis Committee, I respectfully submit the report for approval by the Executive Committee.

R. B. Bushnell C. B. Dearborn
B. R. McCallon E. L. Henkel
R. Weidner K. J. Peterson
D. N. Stern J. S. McDonald
L. F. Williams A. R. Smith
D. A. Kirkbride N. Bruce Haynes
INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS
IN EXTENDED BOVINE SEMEN

Ben E. Sheffy, B.S., M.S., Ph.D. and Mary Krinsky, B.S., M.Ph., Ph.D.
Veterinary Virus Research Institute
New York State Veterinary College
Cornell University, Ithaca, N. Y.

Infectious Bovine Rhinotracheitis Virus (IBRV) was initially isolated in the U.S. in 1956 from cattle with severe upper respiratory tract infection (1). Isolation of a similar virus, Infectious Pustular Vulvovaginitis Virus (IPVV), from genital infections of cattle, had been reported earlier from Europe (2). These viruses have been shown to be serologically identical (3,4), and to be a bovine Herpesvirus (5,6). Other clinical syndromes associated with IBRV infection were abortion (7,8), keratoconjunctivitis (9,10), and meningoencephalitis (11,12). Infection was restricted primarily to tissues derived from embryonic ectoderm. Neonatal calves were highly susceptible and developed more severe IBRV infections than older cattle.

Attenuation of IBRV appeared to be readily effected by serial passage in cultures of embryonic bovine cells (13,14). Safety and efficacy requirements for immunization by intramuscular inoculation were met and products were licensed. As was common in immunization with other modified live virus vaccines, studies suggested production of humoral antibody was followed by virus elimination from the host. Epidemiology was not fully understood, although the possibility of latent infections was recognized (15,16). Initially, vaccines were intended for feedlot cattle. Later, vaccination of calves and breeding cattle was also recommended. Choice of biologicals was extended to include products of attenuated IBRV intended for intranasal inoculation (17) as well as inactivated IBRV to be administered intramuscularly (18). Marked increases in the number of cattle vaccinated with the various IBRV biologicals in the last decade have been recorded. In 1960 (19) a serological survey of New York State dairy cattle showed a 13 percent within herd average incidence of IBRV antibody. In 1971-72 random serological sampling of calves from auction markets in upstate New York showed an average IBRV antibody incidence approaching 50 percent (20). While these increases indicate significant advance in herd health management, serious problems have been created for sire selection committees of artificial insemination (AI) organizations.

ARTIFICIAL INSEMINATION AND IBRV

Processing methods and storage of semen necessary for maintenance of viable sperm are equally favorable for preservation of virus infectivity. Isolations of IBRV from both raw and processed semen (21,22) have been reported. Collections from bulls with genital infections of IBRV can result in contamination of semen with IBRV. To preclude
such a possibility, strict protocols for herd health management of studs have been instituted by AI management. The studs are maintained as isolation units under constant examination and veterinary care, with special attention given to sires during collection periods. Collection and processing of semen are made under near aseptic conditions. Major emphasis, however, has been placed on prevention of IBRV exposure from outside the stud.

In 1965, Snowdon (23) reported sporadic shedding of IBRV from the genital tract of apparently healthy cattle for periods of 578 days after experimental infection. This study served to direct attention to latently infected IBRV serologically positive sires as potential sources of IBRV infection within the stud. Research supported by the industry was initiated directly to study latency and factors affecting IBRV recrudescence.

**IBRV EXCRETION STUDIES**

Pathogenesis studies in adult dairy bulls revealed IBRV shedding in semen beginning 24-48 hours and ending 14-21 days after intravenous infection with virulent IBRV (24). No virus was detected in secretions or tissue explants in these bulls 28 days after infection. However, consistent reexcretion of IBRV from latently infected cattle was demonstrated following 7 daily intravenous inoculations of synthetic corticosteroid (25). Virus excretion was detected 3-4 days after the start of corticosteroid treatment. Virus was shed for 2-6 days. Subsequent studies demonstrated that reactivation and shedding was induced after 3 daily inoculations of steroids. Contact cattle were infected with IBRV (26). Inoculation of ACTH also resulted in recrudescence and reexcretion of IBRV in secretions after treatment followed patterns recorded post primary infection or vaccination. Severity of primary infection influenced both time and duration of post treatment shedding. Virus excretion was often accompanied by very mild or no overt clinical signs of IBRV infection. The majority of cattle studied had been experimentally infected with virulent IBRV (Colo); others had been vaccinated or were exposed to IBRV in the field prior to selection for the AI stud.

**IBRV VACCINATION AND LATENCY**

Vaccination of bull calves with a modified live IBRV vaccine (Colo IBRV-32BK) resulted in shedding of virus from respiratory and genital tracts 2-6 days post inoculation, regardless of whether inoculation was by the intravenous, intranasal, or intrapreputial route. Each method of immunization initiated latent infections of IBRV, as demonstrated by recrudescence and shedding after treatment with synthetic corticosteroids 10 weeks post vaccination (29). These studies suggested that IBRV can be expected to persist in most animals infected with partially attenuated IBRV vaccines.

Vaccination of cattle with inactivated IBRV would not be expected to result in latent infections. IBRV susceptible calves immunized by 2
repeated inoculations of inactivated IBRV vaccine developed high levels of serum neutralizing antibody. However, this level of humoral antibody did not prevent a generalized IBRV infection when these calves were given an intranasal inoculation of virulent IBRV (Colo-2BT). Clinical signs of disease and latent IBRV infections were initiated as a result of virulent IBRV exposure (29) in spite of previous immunization with inactivated virus.

Thus, all cattle with humoral antibody to IBRV must be suspected to be latently infected and potential spontaneous shedders of virus. Cattle in which antibody titers are a result of inactivated virus immunization must be similarly suspect since latent infections can be established in these animals following exposure to virulent IBRV.

**IMPLICATIONS FOR SIRE SELECTION**

The management of AI studs is committed to production of semen free of IBRV. Ideally, studs consisting only of sires serologically negative to IBRV should be retained. Some serologically positive bulls must be recruited to maintain sufficient sires for semen production. This is particularly true for bulls of beef breeds and other breeds where important bloodlines must be retained. Therefore a realistic appraisal of current IBRV immunity and sire selection policy indicates the following possible solutions:

1. Continue to recruit IBRV serologically negative bulls whenever possible.
2. Select young calves with passively acquired antibody and monitor loss of colostral immunity.
3. Segregate serologically positive bulls kept under nearly stress-free management conditions.
4. Maintain careful clinical observation for signs of recrudescence.
5. Test all suspect IBRV contaminated semen for IBRV content.

**THE SEMEN TEST**

A practical laboratory test for presence of IBRV in semen requires utilization of techniques, materials and methods available at any veterinary diagnostic laboratory. Isolation of small quantities of IBRV from nonextended semen is another important consideration. Infection of *in vitro* cell cultures was considered a more sensitive means of demonstrating presence of IBRV than immunofluorescence.

**MATERIALS AND METHODS**

*Tissue Cultures.* Primary testicle cell cultures (CT) from week-old dairy calves were prepared. Culture media consisted of Earle’s balanced salt solution, lactalbumin hydrolysate, and sodium bicarbonate. Growth media contained 10% IBRV antibody-free bovine serum, and maintenance medium contained 2% serum. Growth media contained the following antibiotics: penicillin, 500 units; streptomycin, 0.1 mg; and fungizone, 0.0025 mg, per cc of media. All antibiotics were increased three-fold in
maintenance media.

**Virus.** Standardized SN test IBRV (Colo 5BK-1CT) diluted in tissue culture maintenance medium was used for isolation evaluations.

**Semen.** Fresh ejaculates diluted with either egg yolk-citrate buffer or with pasteurized cows milk (approximately 1:4 dilution) were studied. Diluted semen was held at +4°C prior to initiation of isolation procedures.

**Trypsin Inhibitor.** A 2 percent solution of trypsin inhibitor in 0.25 M phosphate buffer, pH 7.4, was prepared. After 10-15 minutes of gentle stirring, the solution was sterilized by passing through a size 450 Millipore filter. The solution was either used fresh or stored at -70°C prior to use.

**PROCEDURE**

One milliliter of semen was first centrifuged in a refrigerated centrifuge for 10-15 minutes at 600 G. Quantities of IBRV were mixed with the supernatant so the resulting mixture contained virus contents recorded in Table 1. Equal volumes of the IBRV containing seminal fluids and trypsin inhibitor were mixed and then incubated at room temperature (25°C) for 30-45 minutes.

Then 0.2 ml of the semen-inhibitor solution was mixed with 0.5 ml of maintenance medium and overlaid on each of 2 tube cultures of calf testicle cells. The overlaid cultures were incubated at 36°C for 1 hour. The cultures were now washed three times, maintenance medium was added and incubation was continued for 6 days at 36°C. Observation for development of CPE was made daily. If viral effects were not observed on the 7th day, an additional passage was made. Cultures were frozen, thawed and 0.2 ml of the supernatant fluid was inoculated into each of two freshly prepared CT cultures. Second passage cultures not showing CPE 7-10 days after incubation at 36°C were considered negative and discarded.

Toxicity resulting from inoculated semen in the first passage was recorded as follows: T3, cell sheet destroyed; T2, 10-20% of cell sheet destroyed; T1, marginal toxicity, cell sheet intact.

**DISCUSSION OF RESULTS**

Rapid and complete destruction of primary calf testicle cell cultures invariably results after inoculation with undiluted bovine semen or seminal fluids. Pretreatment of seminal fluids with trypsin inhibitor (30) prior to overlay onto cell cultures markedly reduced cytotoxicity. Toxicity recorded in isolations from semen initially diluted 1:4 with egg yolk-citrate buffer and then treated with trypsin inhibitor was 2.5%-T3, 7.5%-T2, and 10%-T1; thus, only 1 sample of 40 could not be assayed. Toxicity recorded as T2 and T1 did not interfere with successful IBRV isolation on second passage. When cows milk was used as the initial diluent, significantly greater toxicity (32%-T3, 32%-T2, and 20%-T1) was recorded than when egg yolk-citrate buffer was used.
When initial dilution was made with egg yolk-citrate buffer, 3-10 TCID\textsubscript{50} of added IBRV per 0.1 ml of the initially diluted semen were reisolated from 76\% of the semen samples tested (Table 1). IBRV from semen were reisolated from 76\% of the semen samples tested (Table 1). IBRV from semen containing 20-30 TCID 50's IBRV/0.1 ml or greater was reisolated from 97\% of the samples tested. These quantities are equivalent to approximately 100-350, and greater than 600-1000 TCID 50's per milliliter of undilute ejaculate. If a final semen extension dilution of 1:25 is taken as an average for the AI industry, the following observations can be made:

1. Fully extended semen containing 27-40 infectious units of IBRV per 1 ml ampoule would have been rejected by this test 97\% of the time.
2. Fully extended semen containing 4-14 infectious units of IBRV per 1 ml ampoule would have been rejected 76\% of the time.

Smaller quantities of virus may have been detected with equal precision if 0.2-0.5 ml of the treated 1:4 diluted semen had been overlaid onto calf testicle cultures instead of 0.1 ml. If greater precision is not required then several ejaculates may be pooled prior to treatment with trypsin inhibitor. Proportionately greater quantities of treated seminal fluids must be overlaid.

**SUMMARY**

A test for isolation of IBRV from semen by direct infection of calf testicle cell cultures was described. Small quantities of virus were consistently detected. The test can be adapted for use in any veterinary diagnostic laboratory.

**REFERENCES**

Table 1

Recovery of IBRV from Bovine Semen

<table>
<thead>
<tr>
<th>IBRV added TCID50/0.1 ml</th>
<th>No. Samples Tested</th>
<th>No. Testing Positive</th>
<th>Percent Recovery</th>
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<tr>
<td></td>
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<td>1st Passage</td>
<td>2nd Passage</td>
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<tr>
<td><strong>Egg Yolk-Citrate Buffer</strong></td>
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<td>40</td>
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<td>0</td>
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<tr>
<td><strong>Cows Milk Buffer</strong></td>
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Mr. Chairman:

Your committee considered proposals submitted from four Regional Meat Hygiene Committee meetings in establishing the agenda for this meeting.

This committee wishes to thank and commend the Regional Committees for the thoroughness of their efforts in developing concrete proposals for consideration. Their proposals represent the thinking of Federal and State Meat Inspection, universities and industry people from the entire nation.

After review and discussion of the Regional Committee proposals, the following topics and recommendations were considered.

TRICHINOSIS ERADICATION

Your committee recommends that the U.S.A.H.A. support the concept of a test and eradication program for trichinosis in the United States. The Scientific and Technical Services Staff of APHIS, USDA has sponsored the development of a serological test for trichina that shows great promise for meeting the requirements for an accurate, rapid and economical means of identifying positive animals. When a practical animal identification system is developed to provide accurate trace back to infected herds it now appears that eradication of trichinosis in the United States will become an attainable goal.

Your committee recommends that the Secretary of the U.S. Department of Agriculture establish a trichinosis eradication program. Such a program to be financed by appropriated funds.

MICROBIOLOGICAL GUIDELINES

Your committee recommends support for a USDA proposal that will establish microbiological guidelines for certain raw and cooked meat and poultry products. We understand there is a proposal which will cover pot pies and several meat and gravy products. The department has future plans to publish guidelines for additional products as good manufacturing practices.

FRESH MEAT NOMENCLATURE

Your committee recommends that the Secretary of Agriculture officially adopt the nomenclature used for identifying retail meat cuts as
In addition, your committee recommends that the state and federal meat and poultry inspection programs continue correlation reviews in plants for the purpose of evaluating and analyzing plant operations and facilities, and official inspection procedures, in order to maintain uniformity and consistency.

**DELAYED POST MORTEM**

Your committee recommends continued use of delayed post mortem procedures as a means of providing inspections at reduced costs for small remote slaughtering operations.

**BRAND AND MARKING DEVICES**

Your committee recommends that strong support be given to implementation of a USDA proposal to permit designated plant employees to account for and control the use of marking devices in processing establishments under appropriate supervision of the inspection service.

**INTERSTATE SHIPPING**

Your committee continues its support for legislation which would authorize interstate shipment of meat & poultry products prepared in state inspected plants.

**NATIONAL FEDERAL-STATE RESIDUE MONITORING PROGRAM**

The National Meat & Poultry Inspection Advisory Committee has recommended State participation in a National Federal-State Residue Monitoring Program. The Committee identified two important benefits in establishing this Program, which pertain to economy and coordination of information between the Federal inspection system and State inspection Programs.

The residues of primary concern are:
1. Chlorinated hydrocarbons
2. Organic Phosphates
3. Carbamates
4. Antibiotics and Sulfa Drugs
5. Herbicides
6. Trace Elements

Slaughtering production data will be collected and computerized so appropriate statistical selection can be made from State & Federal inspected plants. State/Federal or approved private laboratories will be utilized in carrying out analytical tests.

**SEXUAL ODOR OF PORK PRODUCTS**

The problem of sexual odor in pork products was discussed. Several committee members related that they continue to receive consumer complaints. There is indication of a need for further improvement toward elimination of this problem.

**TRAINING PROGRAMS**

*Dr. M. A. Simmons and Dr. Warren Babcock USDA* Training Staff, Denton, Texas reported on a new supervisory performance system being implemented for APHIS Employees. They reported that approxi-
mately 50% of supervisory employees have already been trained.

Dr. Simmons offered the assistance of the USDA for those States interested in learning the system. Several States indicated a strong interest.

*YOUR COMMITTEE* recommended that regional Workshops be continued in 1974 and the Chairman made assignments as follows:

- Dr. James Bell—Southern Region
- Dr. M. D. Mitchell—North Central Region
- Dr. Lawrence Crowell—North East Region
- Drs. Lyle Scott and L. J. Rafoth—Western Region

Respectfully submitted,

E. D. Baker
Committee Chairman
ELECTRONIC ANIMAL IDENTIFICATION
AND TEMPERATURE MONITORING


ABSTRACT

This paper describes a unique system of identifying animals so that a computer-compatible digital code number can be automatically recognized by electronic methods. The transponder attached to the animal is passive and has an unlimited life. It provides a means of measuring body temperature in animals in addition to providing identity. The characteristics of the system and its potential in animal health control are discussed.

INTRODUCTION

Today we plan to present the first public demonstration of a passive electronic identification and temperature monitoring system. This development has been sponsored by the Meat and Poultry Inspection Program and Veterinary Services of the Animal and Plant Health Inspection Service of the United States Department of Agriculture. We are still in an early stage of this development, but we have been invited to present the work at this meeting in order that its impact can be evaluated, particularly by members of the United States Animal Health Association. The equipment on display today is in a prototype form; its purpose is to demonstrate that the basic principles do, in fact, work as predicted and that a portable unit small enough to be useful in the field is possible. Please keep in mind that we have used commercially available components in this design. When this system is manufactured in production quantities, custom-made components will reduce the size and bulk of the system considerably, particularly the transponder.

One of the fundamental ways to rapidly improve the capacity of world food protein resources is to optimize genetic potential, feed selection, the health and stress environment of each individual animal. One of the reasons it has been impossible to realize this optimization, especially in large volume operations, has been the difficulty in identifying individual animals. We will discuss an electronic identity scheme which is intended to provide a computer-compatible digital code using a device attached to or implanted in the body of the animal which, in addition to the identity code, also provides a measure of the animal's body temperature.

Figure 1 is a conceptual view of such a system in operation. Animals here are identified as they pass an interrogation station; at the same time their body temperature is measured and recorded. These data may be transmitted directly to a computer register, read on the spot or recorded

* Sensory Systems Laboratory, Los Alamos, N.M.
** Los Alamos Scientific Laboratory, Los Alamos, N.M.
on a tape cassette for later processing. While the illustration shows the interrogator on a tripod, it will be feasible to use this device (or a simplified version of it) from horseback or vehicle, or even by hand.

Figure 2 diagrams the way in which the system works. Many of you will recognize the interrogator as a Doppler radar transmitter-receiver similar to those used to control vehicular traffic speed. Low-powered radio energy is beamed at a transponder by the transmitter portion of the interrogator. This energy is received and used by the transponder as a power source; that is, the unit on the animal derives its power entirely from the interrogation beam. It has no batteries and hence unlimited lifetime, barring physical damage. With the power derived from the interrogator beam, it generates a digital code and temperature signal. This information is transmitted back to the interrogator at or near the same frequency as that contained in the original radio beam. There is no practical limit on the number of digits which can be generated in the transponder. The demonstration device has a three-digit code. It has been suggested that a national animal identification code contain 14 or 15 decimal digits. Temperature data is given in the demonstration device to about 1/10 of a degree centigrade—this could be read to 1/1000 of a degree if it were needed. The time for an interrogation in the current design is 24/1000 of a second. Two separate interrogations are made and compared. They must be identical before data is displayed. This is to ensure that the accuracy of an identification is compatible with its use by a computer.

The diagram indicates two separate antennas at the interrogator. There is no reason that one antenna cannot serve to beam the transmitted energy out and receive the coded data in, if the two signals are kept separate. This is what we have done in the demonstration unit. One antenna serves the function of the two shown in the diagram.

The transponder shown in the demonstration (Figure 3) is capable of generating any three-digit code desired for test purposes. In production, each transponder would be assigned an unalterable code number, hard-wired or fixed prior to use so that it would be extremely difficult if not impossible, to change an assigned number without complete damage to the transponder. The final approximate size of the electronic circuit part of the transponder will be about one cubic centimeter.

Actually, there are two types of transponders in Figure 3—the first is completely passive; that is, all the operating power is derived from the interrogating signal. This is the type that we expect to find in greatest use in animal identification. The range of this device is limited by the amount of power provided by the interrogator. We are using about five watts for this demonstration (Figure 4) and we expect ranges of about 20 feet. If the transponder is provided with a very small amount of power (sufficient to operate the code and temperature functions only), the transponder can be read by the interrogator at much greater ranges. We have a condition similar to that of a radar set illuminating a target—
in this case the transponder—and the range is limited only by radar range equation and by line-of-sight conditions. We can imagine that animals, such as cattle, could be inventoried by aircraft using a system like this. The power required by the transponder could be provided by a solar cell operated at daytime light levels. One of the demonstration transponders is equipped with a small battery to illustrate this mode of operation.

Figure 5 is a summary of the features of the passive transponder system: almost the entire circuit of the transponder could be fabricated on a single piece of silicon. Using conventional integrated circuit packaging and assembly procedures, we expect reliabilities comparable to those of commercially manufactured integrated circuits. If the identification-temperature scheme is adopted by only the cattle industry (some 35-million cattle per year), or a major part of it, the volume of transponder production would make it one of the most popular integrated circuits ever produced. While the initial development costs are high, such high volume production quantities would ensure a price to the user in an extremely low range. The number of digits is a matter of design choice. Once a code is adopted, however, it would be desirable to standardize it in view of the readout or display capability. The interrogator is simplified in that the transmitter frequency is not critical. No crystal control or modulation is necessary. The batteries in the power supply for the demonstration will operate the prototype continuously for a full day.

Figure 6 is a list of potential or proposed applications. These are offered for consideration and discussion since we have not had the opportunity to support much of this conjecture with experimental data. The balance of this report will be devoted to an outline of the basis for such a projection of applications.

The predominate interest in electronic identification, so far, had been generated by members of the cattle industry, in both beef and dairy production. We believe there is also a need for an application for individual animal recognition and temperature monitoring in swine production; and under certain conditions, sentinel birds in turkey and broiler flocks could be instrumented for detection of infectious diseases. Application to other species would depend on circumstances. Because temperature monitoring is inherent in the identity code, and available to the producer, we believe that one of the most valuable assets will be the recognition of abnormal temperatures. This will allow early segregation of suspect animals and investigation of the cause of the abnormality. Diseased animals in the market process could be detected, identified as to the herd of origin, and isolated rapidly without the tremendous economic disaster of a widespread area quarantine.

In cooperation with the United States Department of Agriculture, Veterinary Services Diagnostic Laboratory, National Animal Disease Laboratory, Ames, Iowa, and the Plum Island Animal Disease Laboratory in New York, members of the Los Alamos Scientific Laboratory
are beginning a series of investigations of the initial temperature profiles of various diseases, in the hope that such information will make rapid diagnosis possible, not only for the important livestock diseases, but perhaps for individual strains or variations of these diseases.

For range cattle the electronic identity code should enable an owner to separate his cattle from those of his neighbors. In this case a portable, simplified, interrogator would be desirable.

At the present time there are some eight-million cattle bred by artificial insemination annually. It has been estimated that this figure might be 30-million if ovulation could be determined more reliably than is possible at present. We are planning a study with a commercial cattle breeding organization to determine whether continuous body temperature can be of use in determining the optimum time for artificial insemination. This information, together with a cow's identity, would permit rather precise selection of favorable genetic traits by drawing on a complete memory bank where stored data on prior progeny would be available when needed.

Another planned use of the temperature identity system is the dairy, where the use of process control concepts could be applied to optimize production. An electronic identity code for individual animals would allow for computer management of feed and ration supplements, environmental stress relief, and for monitoring of milk quality and quantity by individual animal.

We do not believe that electronic identification by itself will stop the theft of valuable livestock. It is feasible to insert the transponder under the hide of cattle or in the rumen, and widespread use of an electronic tag and frequent and positive inventory of animals either on the range or in the market process should offer some deterrent to theft, however.

Figure 7 is an illustration of a possible national identity code. This has been suggested by a special committee of the International Brand Conference under the chairmanship of Mr. Hans. Van Nes, Chief, Bureau of Livestock Identification for the State of California. There are a total of 15 digits represented in this code, one for species, three for the state or country of origin, six for owner, four for individual animal and one for year of birth. The objectives of this committee are to define and recommend a coding technique of sufficient capacity to serve both the national and state requirements for animal identity and disease traceback. Such an electronic code would not supplant current means of establishing legal ownership unless it were proven and accepted as a superior method.

The following Figures 8 through 12 illustrate a hypothetical disease diagnosis procedure based on temperature profiles. These are idealized temperature responses taken from veterinary references. We are impressed by the lack of such continuous records in the literature, especially those involving the early onset of the common infectious diseases. We expect that telemetry of body temperature will lead to its use in diagnosis or recognition of patterns such as these. Currently, experiments
are underway to document the Brucellosis temperature profile. We expect to begin a Foot and Mouth temperature telemetry study next month.

In 1971, the principal author supported a study involving temperature telemetry of dairy cows under summer heat stress in Tucson, Arizona. The work was conducted by the Dairy Science Department at the University of Arizona. We found that elevated brain temperature, as measured in the ear canal at the tympanic membrane, correlated with physiological stress as indicated by adrenal cortical hormones in the blood and by decreased milk production. Cows were instrumented with a small transmitter fixed to the halter (Figure 13) and were free to move to pasture, feed stalls or the milking barn during the measurements. Data from six cows were recorded in the trailer located centrally to the dairy operation. During the course of this experiment, artificial cooling was provided to a herd of about 100 milking cows, while normal environmental temperatures were available to the control group—a similar herd of about 100 cows. Those with artificial cooling increased milk production an average of 20%, over the year and increased their reproduction rate during three months of summer by 100% over the control group. We were impressed by the fact that body stress conditions could be monitored directly by temperature telemetry. A typical record for the six instrumented animals is shown in Figure 14. The horizontal lines represent an arbitrarily assumed normal brain temperature. The period covered here is 24 hours, with increases noted during the day. Those animals whose temperatures did not return to "normal" at least during the night were the ones to lose in milk production.

While we believe brain temperature to be the most valuable measurement for stress and disease detection, general body temperature in the bovine, at least, is quite valuable for determination of abnormal conditions. This is illustrated in a study* with the African buffalo (*Syncerus caffer*) which I conducted with Anthony Sinclair of Oxford University. Because the buffalo is relatively unprotected from solar radiation in equatorial East Africa, we reasoned that a measurement of body temperature would correlate with shade seeking. Buffalo (Figure 15) generally select a habitat, if possible, where both trees and forage are available. We instrumented a bull (Figure 16) for measurement of subdermal temperature by radio telemetry over a period of three days and nights. The data (Figure 17) shows the general elevation of subdermal temperature during the hours of sunlight and the temperature peaks are correlated with shade seeking. The second (dashed) line indicates cooling by rain and resumption of grazing. We concluded this study with the feeling that knowledge of body temperature in this species was fairly predictive for behavior.

Temperature telemetry is a valuable tool for investigation of thermoregulation techniques unique to various species. I have selected two such experiments to illustrate this point. The first deals with a study of the thermoregulation in the zebra (*Equus Burchelli*). These animals inhabit the grasslands of East Africa and adapt to a wide range of temperature environments. Zebra stripes are proposed to serve the function of camouflage from predation. William Hamilton of the University of California at Davis, and I, considered that stripes might better serve as radiation absorbers and reflectors (Figure 18). We found that tanned zebra hide showed $15^\circ C$ differences in temperature under adjacent black and white stripes in the sunlight. A wild yearling was instrumented for black stripe temperature under natural conditions (Figure 19). The record over two days and nights was remarkably stable! The variation of $\pm 1/2^\circ C$ just under the skin occurred with an ambient temperature variation from $8^\circ$ to $32^\circ C$. I was able to investigate skin temperature regulation in a tame zebra in Arizona with probes under adjacent black and white stripes (Figure 20). The data indicates (Figure 21) as much as two-degree differences with rapid readjustment as the animal is led from shade to sunlight. We believe that thermoregulation in the zebra is effected by vascular blood flow shifts under the black or white areas as the zebra's body absorbs or conserves heat from the coat.

The second experiment deals with determining cooling mechanisms in the African elephant. Figure 22 illustrates the large surface area and vascularization of the elephant ear. A. M. Harthoorn, Ian Douglas-Hamilton and I decided to investigate the elephant ear as a convective cooling mechanism by using small temperature transmitters implanted in the body. One transmitter was arranged to measure blood temperature in an artery and a second transmitter measured blood temperature from a vein in the same ear. A third transmitter reported temperature from the collar around the elephant's neck. A receiver mounted on the collar monitored the three channels of data and retransmitted the information over a fourth transmitter which could be monitored at ranges of up to 30-miles. Data recording was done in the vehicle (Figure 23) for a period of about two days. We recorded temperature at the river drinking and when the elephant emerged from cover (Figure 24). The data (Figure 25) shows a dramatic increase in arterial temperature of $9.4^\circ C$ during a 1,000 foot climb with almost perfect blood cooling in the ear. The low point in arterial temperature corresponds to drinking at the river. The momentary increase in venous temperature occurred as the elephant emerged from cover and had his picture taken. He ceased to ventilate his ears during this confrontation.

These experiments, then, illustrate at least some of the potential for understanding the dynamics of physiology with continuous temperature measurements. The availability of low-cost passive identification and temperature transponders will, hopefully, add much more to our understanding of animal health and care.
AND TEMPERATURE MONITORING

Transponder

Interrogator

Figure 1

RF GENERATOR

MIXER

FILTER / AMP

ID DECODER

DISPLAY

VARIABLE LOAD

ID ENCODER

OSCILLATOR

Figure 2
Figure 3

Figure 4
PASSIVE TRANSPONDER FEATURES

UNLIMITED LIFETIME
LOW COST IN VOLUME PRODUCTION
NO PRACTICAL LIMIT ON DIGITS
BODY TEMPERATURE MEASUREMENT
COMPUTER-COMPATIBLE DATA
UNCOMPLICATED INTERROGATOR DESIGN

Figure 5

POTENTIAL APPLICATIONS

ANIMAL REGISTRY - ALL LIVESTOCK SPECIES
EARLY DETECTION OF DISEASE OR STRESS
DISEASE TRACEBACK AND EXPOSURE CONTROL
DISEASE DIAGNOSIS FROM TEMPERATURE PROFILES
OWNERSHIP DETERMINATION IN THE FIELD
DETERMINATION OF OVULATION CYCLES
GENETIC CONTROL IN BREEDING
OPTIMIZATION OF DAIRY MANAGEMENT
DETERRENT TO LIVESTOCK THEFT

Figure 6
SUGGESTED
ANIMAL IDENTIFICATION CODE

Figure 7

FOOT AND MOUTH

°C

Figure 8
AND TEMPERATURE MONITORING

Figure 9

TUBERCULOSIS

Figure 10

BRUCELLOSIS
Figure 11

Figure 12
Figure 13

Figure 14

Figure 15
REPORT OF THE 1973 COMMITTEE ON LIVESTOCK IDENTIFICATION

Chairman: S. H. Flora, Brownsville, Tex.

The Committee heard presentations from Dr. Fred Hanson, Veterinary Services, USDA on their current progress and activities in the field of Livestock Identification, Dr. Alan Morrow of the Minnesota Livestock Sanitary Board presented a formal cattle and swine identification program, Dr. R. C. Knowles of the Infectious Diseases of Horses Committee discussed a resolution calling for equine identification and Dr. Keith Farrell alerted the Committee to the possible interest and need for canine and feline identification. A brief demonstration and discussion of an electronic animal identification and data retrieval system was made by H. A. Baldwin and associates from the Los Alamos Scientific Laboratory.

Information from the Los Alamos group leaves no doubt that an electronic animal identification and data retrieval system is feasible and practical at a price that would appear to be acceptable. Five hundred thousand dollars and three years are needed to develop and prove the hardware. A resolution directed to the Secretary of the United States Department of Agriculture to carry out the further research and development needed was submitted to the appropriate committee for its action.

The Committee makes the following recommendations:

1. To effect an economy of effort and move more rapidly to an effective, efficient animal identification system or systems, useful to all concerned, Veterinary Services of the USDA, in consultation with the States and the livestock industry, develop recommended uniform standards of identification for each or all species of livestock and at all times keep groups and personnel working in research and development of identification methods aware of the recommended standards.

2. To assist in the eradication of tuberculosis, brucellosis, hog cholera, and trichinosis as well as other diseases in swine. Veterinary Services, USDA, require all swine moving in interstate commerce be
uniformly identified as to herd of origin at or before the time they reach the first concentration point. "Trash," "Junk," or cull swine that might be moved in commerce to further feeding shall be so marked that they are clearly identified and moved to immediate slaughter or destroyed. Each state is urged to take companion action to identify swine in interstate commerce.

3. State and Federal Animal Health Officials continue efforts to improve the quality and yield from existing livestock identification systems now in use as well as increasing the number of animals now being identified.

The Committee will notify organized livestock and other interested groups of the actions of this committee and association so that efforts to bring into being an electronic identification and data retrieval system will be in the same general direction.

In keeping with the above recommendations, the Committee specifically endorses the resolution regarding equine identification and a supporting data bank as proposed by the Committee on Infectious Diseases of Horses.

The Committee wished to express its appreciation for the presentations made and the participation of interested persons present.
SWINE VESICULAR DISEASE

J. H. Graves and P. D. McKercher

INTRODUCTION

An outbreak of vesicular disease occurred in a pig-fattening establish-
ment in the Po Valley in Italy in 1966.1 It was diagnosed in the field as
foot-and-mouth disease (FMD) but this was not confirmed by laboratory
tests as identity with any of the types of FMD viruses could not be
shown. Later, through cooperative work by Italian and British workers' it
was shown that the virus that caused the disease, while similar in
structure to FMD virus was stable at low pH whereas FMD virus is
rapidly destroyed below pH 6.5. This virus was classified as a porcine en-
terovirus of the picornavirus group.

In 1970, an outbreak of vesicular disease occurred in a group of pigs
during an experimental FMD vaccine trial in Hong Kong.2 This was also
thought to be FMD but again this was not confirmed by laboratory tests.
The causative agent of the Hong Kong disease was identified as an acid
stable enterovirus serologically closely related to the 1966 Italian virus.

In December of 1972, eight simultaneous outbreaks of a vesicular
disease in pigs occurred in England.3 These were diagnosed in the field as
FMD and slaughter of diseased swine and swine and cattle in contact
with infected pigs was commenced. Five days after the first outbreaks
the viral agent causing the disease was identified as an acid stable en-
terovirus serologically the same as the 1966 Italian and 1970 Hong Kong
viruses.

At the time of the English outbreak, the disease was also reported as
having occurred in Poland, Austria, France and again in Italy. This new
disease was given the name of swine vesicular disease (SVD). There is
reason to believe that SVD is enzootic in Italy whereas the status of the
disease in the other countries in Europe is not known. Considerable con-
cern has been expressed by those responsible for import regulations in
the United States as to the safety of pork products from Europe. Ex-
periments to resolve this concern should such products have been
prepared from SVD-infected swine are proceeding now at a vigorous
pace.

DISEASE DESCRIPTION

Swine vesicular disease can occur in an acute, subacute or subclinical
form. The acute form is of particular concern because the vesicular lesions are indistinguishable from those of FMD. The subacute and subclinical forms, while of low morbidity, permit wide dissemination of the virus due to lack of obvious clinical signs in infected animals.

The incubation period of the disease is from 2 to 14 days. It is readily transmitted by contact of infected with susceptible swine, fecal contamination of premises by infected swine or feeding of virus-contaminated garbage or swill to susceptible swine. The disease can also be produced by inoculating the virus intravenously, subcutaneously, intramuscularly or in the epithelium of the feet, snout or tongue. It is of interest to note that the disease was spread over a wide area of England by trucks carrying susceptible pigs that had previously carried SVD-infected pigs even though these vehicles had been decontaminated between the shipments by standard procedures used for destruction of FMD virus.

The signs of acute SVD are severe lameness with fever of 104-106°F. Involvement of several animals in the herd would be expected. The lesions consist of well-developed vesicles of the nostrils, mouth, tongue, coronary bands, and interdigital spaces of the feet. While foot lesions are invariably present those of the head may not be found. The acute phase of the disease lasts for about 7 days. Pigs of all ages are equally susceptible with essentially zero mortality and good prognosis for recovery. Clinical signs in animals in a herd with acute disease have been reported as low as 20% in the first Italian outbreak to over 80% in the Hong Kong outbreak.

The subacute form of SVD is usually found by examination of apparently normal animals in herds where the acute disease is present or those that have been in contact with acutely-ill pigs. The subacute form was a common occurrence in the English outbreak of 1972 and was only revealed by careful examination of apparently normal animals in the quarantine areas.

There is evidence from field observations that subclinical infection can occur in that animals housed on formerly-infected premises that have had no clinical evidence of the disease have developed significant serum-neutralizing antibody. The resistance of the virus to destruction has led to the conclusion that eradication of the agent from an infected premise may be difficult with the possibility that susceptible swine put in such establishments may develop subclinical infections.

The pathology of SVD is limited to lesions of the skin and central nervous system. The skin lesions are typical of those described for other vesicular diseases such as FMD. The microscopic lesions of the central nervous system involve diffuse encephalomyelitis with perivascular cuffing with lymphocytes and the formation of neuroglia cell foci. The encephalomyelitis is of mild to moderate intensity and is most severe on the 6th day after intravenous inoculation of the virus. Occasionally, clinical
signs of central nervous system involvement consisting of stilted gait and mild flaccid paralysis have been seen in experimentally-infected pigs but such signs have not been reported from the field.

The differential diagnosis of SVD from FMD can only be done in the laboratory. There are some clinical aspects of SVD which favor the decision that it may be the cause of a vesicular outbreak but extreme caution must be exercised in relying on these. One is the fact that SVD only infects swine. Several attempts to infect cattle have been unsuccessful; however, it should be noted that strains of FMD virus have occurred in the field that also do not readily infect cattle. The observation that only a portion of pigs in the herd are involved and some have subacute disease may indicate SVD but again strains of FMD can occur with similar clinical features. Until determined to be otherwise, the vesicular disease in a swine herd must be considered to be FMD. It is imperative that the appropriate samples be submitted through proper channels for laboratory analysis as soon as possible.

Differential diagnosis consists of the complement-fixation test using lesion material as antigen. Swine vesicular disease virus will grow in tissue cultures of swine kidney cells but not those of bovine kidney. Newborn mice less than 24 hours old are susceptible to infection with SVD virus and will die within 3 to 7 days after intracranial inoculation.

**CAUSATIVE AGENT**

Swine vesicular disease virus is a representative member of the enterovirus group of the picornaviruses. All of the known strains have a close serological relationship to each other; however, minor antigenic differences can be shown among the Italy 1966, Hong Kong 1971 and the strains currently in Europe and England.

An interesting relationship of SVD virus has been found which gives a clue as to the possible origin of this disease. The virus kills newborn baby mice and the only viruses in the enterovirus group that routinely do this are the Coxsackie group of human viruses. In light of this observation we tested the ability of 42 antisera against human enteroviruses to neutralize SVD virus. The antisera included the ECHO, Coxsackie and polio serotypes. It was very clear from these experiments that the virus causing SVD was readily neutralized by Coxsackie B-5 antiserum. In addition, it was shown that serum from pigs recovered from SVD neutralized the human Coxsackie B-5 virus. It was further found that of about 50 human sera tested about half significantly neutralized SVD virus.

In support of this close relationship between a human and swine virus are two significant observations. Three pigs were inoculated with Coxsackie B-5 virus grown in pig kidney tissue culture. One was inoculated intravenously, the second intranasally and the third received the virus in its food. All received 10⁴ tissue culture plaque-forming units of virus and while none of these pigs developed any signs of clinical disease, they all developed significant levels of virus-neutralizing serum antibody against
SVD virus by the 14th day. We accepted this as evidence of infection by the Coxsackie B-5 virus.

The second observation has been reported from England. A laboratory worker that had contact with pigs infected with the British field strain of SVD became ill. Comparison of this 5-day postinfection serum-neutralizing antibody titer against SVD virus with that at 14 days showed a significant rise. Subsequent studies based on strain antigenic differences showed that the virus that had caused the disease in this worker was the British strain of SVD virus and not Coxsackie B-5 virus. Thus, the reciprocal infection of the human by the swine virus has been shown. Based on these observations it has been hypothesized that this is a relatively new disease of swine caused by a virus whose origin was man.

A small survey of U.S. swine was done to determine if any evidence of SVD could be found. Serum samples from swine in California, Massachusetts, New Jersey, Kansas, North Carolina and Virginia were tested for their ability to neutralize SVD virus and in no case did any serum neutralize the virus. Also, antiserums to Teschen disease virus, vesicular exanthema of swine virus and common porcine enteroviruses failed to neutralize SVD virus. Based on the accumulated data and absence of a clinical disease like SVD in the field it is concluded that this disease is foreign to this country and that U.S. swine are probably highly susceptible to infection by the virus of SVD.

In summary, we have described a relatively new vesicular disease of swine, evidently exotic to the United States, called swine vesicular disease. The clinical manifestation of the disease cannot be distinguished from those of FMD and would undoubtedly generate considerable diagnostic confusion should it gain entry into this country. The causative agent is an enterovirus of the picornavirus group closely related to the human virus, Coxsackie B-5. Because of its establishment in Europe and Hong Kong, this disease must be considered as a threat to the U.S. swine industry and continued vigilence against its entry is warranted.

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THE EPIZOOTIOLOGY, PREVALENCE AND ECONOMIC ASPECTS
OF BOVINE TRYPANOSOMIASIS IN NIGERIA

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BOVINE TRYPANOSOMIASIS IN NIGERIA, G.O. ESURUOSO

INTRODUCTION

African trypanosomiases are the diseases of animals and man caused
by trypanosomes and transmitted by tsetse flies (Glossina species). The
climatic limits of these flies coincide approximately with the distribution
of trypanosomiases on the continent of Africa. The role of the flies is so
inseparable from the occurrence of the disease that many research
workers consider this group of diseases to be an entomological problem.
But since it has not been possible to control the diseases entirely by en-
tomological methods and it is known that lasting immunity cannot be
achieved through vaccination or following recovery from natural in-
fec tion, these diseases constitute a challenge to immunological research;
it will be right to say that trypanosomiasis is a biological problem, a
definition that will cover both the entomological and immunological
aspects.

A geographical definition of Nigeria, situated between latitudes 4 and
14 north of the equator and longitudes 3 and 14 east of the Greenwich
Meridian shows that the country lies entirely within the climatic limits
of the tsetse fly vectors of trypanosomes. The vegetation and rainfall
provide suitable habitat for the flies for the greater part of the year. The
flies are adaptable enough to avoid inclement weather conditions during
the difficult periods. They are thereby able to maintain the disease in
man and other animals. But by far the most affected animal is the
domestic cattle.

Bovine trypanosomiasis occurs in Nigeria throughout the year,
although more cases are seen at the beginning of the dry and the wet
seasons. Next only to malnutrition, with which it may coexist,
trypanosomiasis is the most damaging and therefore the most important disease of cattle in Nigeria. Even the relatively resistant cattle sometimes succumb to infection with many fatalities (Esusuoso, 1973 in press). The disease occurs in various forms, from hyperacute to chronic, with variations in the virulence of the known pathogens in various localities.

EPIZOOTIOLOGY

HOSTS: There are over ten million cattle in Nigeria; most of them belong to the humped Zebu breeds which are fully susceptible to trypanosomiasis (figures 4, 5, 6, & 7). But there are also the humpless indigenous Muturu cattle and their crosses with the Zebu which are of intermediate resistance to trypanosomiasis. A third group, the N'dama (fig. 11) which are also smallish and humpless, were originally imported from Gunea and the Congo. These are the cattle that show the greatest resistance to the disease. But even these animals sometimes succumb to infection (fig. 12) and die in large numbers (Esuruoso, 1973 in press). The exotic animals in Nigeria include Holsteins, German Brown, South Devon, and Jersey breeds, all of which are fully susceptible to the disease. When not protected by drugs, Holstein cattle die of acute trypanosomiasis (fig 13); those protected with Samorin may still suffer the chronic form of the disease, if the prophylactic treatment is not maintained (fig. 14).

Among the feral group, bushbuck, and warthogs are suitable hosts for trypanosomes. In general, mammalian hosts of trypanosomes can be divided into sufferers and carriers. Bushbucks, warthogs and domestic pigs are carriers which may be the sources of infection for cattle. The domestic pig succumbs readily to *T. simiae* infection but will harbour *T. congolense* without showing any signs of the disease and it is considered the reservoir of infection for cattle with this parasite in western Nigeria.

Parasites: *T. vivax*, *T. congolense*, and *T. brucei* are the main pathogens of cattle in decreasing order of importance. *T. theileri* is found occasionally in combination with *T. vivax* or *T. congolense* infections. The role of *T. theileri* is unknown and *T. brucei* is of low pathogenicity to cattle.

It is generally accepted that both *T. vivax* and *T. congolense* may vary in their virulence according to locality or for unknown reasons. In the southern states of Nigeria, 90% of trypanosomiasis of domestic cattle are caused by *T. vivax*. In the northern states there are great variations. For example, in the North Western State, *T. congolense* is more important in the southern part while *T. vivax* is the major pathogen in the northern part of this state. Nevertheless, it is now obvious that the relative importance of these two pathogens has changed over the last two decades. In the 1950's, most of the cases of trypanosomiasis seen in the northern parts were *T. congolense*, but now there are usually more *T. vivax* than

* Samorin—May & Baker isometamidium chloride
Blood samples taken from Ilorin (Kwara State), Kaduna (North Central State), and Ibadan (Western State) showed that *T. vivax* parasitaemia was encountered three times more frequently than *T. congoense*. But outbreaks of trypanosomiasis in Ekiti and Lalate, two hundred miles apart in the Western State, showed that there were as many cases of *T. vivax* infection as there were of *T. congoense* infection. *T. congoense* is probably as common as *T. vivax* in the sylvatic cycle.

One thousand blood samples taken from Ibadan abattoir earlier this year showed 65% infection rate, seventy-five percent of which were caused by *T. vivax* while *T. congoense* and *T. brucei* were of nearly equal occurrence, and double infections are not uncommon.

**VECTORS AND TRANSMISSION**

It is believed that most of the trypanosomal infections of cattle in Nigeria are transmitted cyclically (by tsetse flies), but the possibility of occasional mechanical transmission by other bloodsucking flies, especially *tabanids* and *Stomoxys* (Baldry and Riordan, 1967), is usually admitted. Very often, an outbreak of trypanosomiasis is the first indication that the tsetse flies are around. In low density areas, a search for the flies may prove negative on first attempt, but eventually the fly was usually found on persistent and more careful searching.

*Glossina morsitans submorsitans*, *G. longipalpis*, *G. fusca*, *G. Medicorum* and *G. palpalis* are the more important tsetse flies responsible for the transmission of bovine trypanosomiasis in Nigeria. The morsitans group or Savanna flies (*G. morsitans submorsitans* and *G. longipalpis*) are important because of their wide dispersal and their generally high infection rate. Up to 76.6% of all *G. morsitans submorsitans* caught along trade cattle routes were infected (Baldry, 1969); whereas the infection rate in *G. palpalis* is usually 2% or less.

Although newly emerged, young adult tenereal tsetse fly shows little discrimination and will therefore obtain its first blood meal (for survival) from any available host (Ford, 1970), the high-infection rate flies in Nigeria (*G. morsitans submorsitans*) often prefer to feed on bovidae, both wild and domesticated (Maclennan, 1973). This is no doubt an important factor in the prevalence of bovine trypanosomiasis, especially in view of the wide dispersal of these flies.

The high plateau area (3,000 ft. above sea level) around Jos (fig. 1) are free from tsetse flies. Most of the plateau areas just below this level are also free. Other free areas include the semi-arid region in the furthest north of the country, and areas cleared for human settlement and for agriculture. Nevertheless, trypanosomiasis may still occur in tsetse free areas. This could be due to transient presence of the flies which had followed migrating cattle, or due to mechanical transmission by *tabanids* and *stomoxys*. In this respect it is necessary to recognize two types of cattle routes; (1) the regular trade cattle route from north to south and (2) the random cattle routes followed by trade cattle awaiting
slaughter. The latter was the source of the trypanosomiasis outbreak at Agege dairy in 1966 in an open area known to be normally free from tsetse flies.

But by far the most important factor for the maintenance of domestic trypanosomiasis is the presence of the disease carriers in the sylvatic cycle. Bushbuck and warthogs are the best known examples of these carriers. It is almost impossible to prevent contact between feral animals and migrating cattle, especially as the tsetse flies will feed on both, but it is known that where game animals have been practically eliminated, the incidence of the disease is usually low.

**ENVIRONMENT**

Environmental factors in trypanosomiasis are numerous and complex. Under the stresses of malnutrition, intercurrent diseases, exposure to extreme weather conditions and overexertion cattle will become more susceptible to trypanosomiasis. In particular the nomadic way of life of the cow Fulani is a response to the shortage of good grazing and the need to avoid tsetse flies in the environment. But as drought sets in, in the Sudan savanna, watering points which also provide the much-needed moisture for the flies, become the meeting point for both fly and host—an event that either can ill afford to avoid.

The stresses of long distant trekking is usually aggravated by increased chances of greater exposure to tsetse flies along the route. These flies are more likely to carry strains of trypanosomes to which the cattle were not exposed at the place from which they had just left. They are also likely to suffer repeated infections along the route.

Infected cattle may be helped to recover naturally if sheltered from the direct mid-day sun and given water and adequate nutrition; these conditions are, however, not readily met during migration. The cattle are therefore more likely to succumb, and die before reaching their destination. As the dry season affects both the cattle and the tsetse flies, the savanna flies which are usually dispersed during the rainy season are known to retreat to the limited river-bed areas where there will be sufficient moisture to maintain them. But in so doing the cattle-fly contact becomes rather close. This remarkable ability to adapt to changing environment is one important factor in the survival of the tsetse fly in extremes of heat; apart from getting the moisture, their source of blood (the cattle) is also brought to them by the waterside.

The internal environment of the host also deserves consideration here. The dynamics of host-parasite interaction forms the biological basis of infection and non-infection of cattle with trypanosomes. The multiplication of parasites inside the mammalian host marks the beginning of infection, to which the host responds by lymphoreticular cell proliferation. The outcome of such interaction may result either in the elimination of the parasites, death of the host or the establishment of an equilibrium in which both parties may from time to time suffer reverses of fortune at varying levels. At any one point during this struggle between host
defence elements and the parasites, it is usually impossible to predict what would happen next, except in the very last stages when the host is dying. Calves often recover spontaneously from trypanosomal infection.

All the breeds of cattle in Nigeria are susceptible to infection with the pathogenic trypanosomes listed above; but the resistant breeds often show few manifestations of the clinical disease. Their reactions are exactly the same in pattern as those of the susceptible breeds; the only difference is in degree and in the ability to limit parasite proliferation and maintain normal blood values. Whatever the mechanism of resistance in the N’dama cattle, it can be overcome and the animal will succumb to infection and even die (figs. 11 & 12). Also among the susceptible Zebu cattle, some breeds are more susceptible than others. The Red Bororo (fig. 9) goes down more readily with trypanosomiasis than the White Fulani (figs. 3-4).

PREVALENCE

When trypanosomiasis occurs in known tests infested territory, there is usually no surprise; but many more cases are now being detected in apparent tsetse free areas of the country, especially in the south. In some cases the explanation lies in the movement of cattle through infested areas from where they are followed by the flies. It is also known that the flies sometimes advance and retreat with changing fauna or adverse environmental conditions. In any one particular case it may be quite difficult to know the actual factors which have produced changes in the Glossina distribution.

Recently at Olokemeji game reserve, the flies were reported to have vanished with the dwindling number of wildbeast in that area (MacLennan, 1973), but a few months later, a major out-break of trypanosomiasis occurred in the N’dama cattle not very far from this area. Also, to the north west of Ogbomosho, near Igbeti, in the old game reserve area near the interstate boundary between the West and Kwara states, a major out-break of trypanosomiasis occurred recently in which over 200 of the 1090 cattle died. It is usually not valid to suppose that certain areas are free from tsetse flies until susceptible hosts have been exposed for some time without incident.

FORMS OF THE DISEASE

Bovine trypanosomiasis may occur sporadically, but usually an out-break in which many animals are involved is encountered. The course of the disease is sometime acute or peracute (as in exotic cattle), but the chronic and subacute forms are more common in the indigenous breeds. The chronic form is usually seen as severe anaemia in which many animals are emaciated, debilitated, with staring coat, sunken eyes, dehydration and marked jugular distension and pulsation. In prolonged cases geophagia and grinding of the teeth are common signs.

The usual sequence of events is shown in figures 4, 5, & 6. An enlarged precrural (fig. 4) or prescapular lymphnode on one side is usually indicative that the animal is being challenged by tsetse flies. After a
variable incubation period of up to three weeks, depending on the rate of challenge, clinical anaemia becomes obvious. The animal becomes progressively weaker and eventually goes into sternal recumbency. The appetite is variable, and animal in sternal recumbency may still attempt to eat grass. After a couple of days in this position, even the head seems too heavy for it to carry, so it adopts the position shown in figure 6. Once recumbent for 48 hours, the prognosis is usually poor, but the animal may remain in this position for up to 5 days before finally falling onto its side to die.

These clinical observations, and the haematological picture, especially the finding of rouleau formation, watery blood, low red blood cell count (may be as low as 0.2-4.8G% (normal-11.5G%) in a weak and deeply depressed animal, even when parasites are not seen immediately, are diagnostic of chronic trypanosomiasis of cattle in Nigeria. At post mortem the heart blood will very often show masses of trypanosomes or a bundle of their flagellae.

ECONOMIC ASPECTS

More than seventy percent of the ten million cattle in Nigeria are exposed to the risk of trypanosomiasis at some period of the year. Economic losses due to the disease can be evaluated in terms of actual deaths, loss of weight, retarded growth, delayed sexual maturity, abortions and reduced milk yield. It will be difficult to investigate all these parameters for all the cattle in the country. Therefore, a few well documented cases will be chosen at random to illustrate the general pattern of losses caused by the disease.

In March 1966, an outbreak of trypanosomiasis occurred in a Fulani herd near Iwo, in which 72 out of the 156 White Fulani cattle of various ages died (Esuruoso, 1972). This represents 46% mortality, and the figure would have been higher if the remaining animals were not treated. Between August and November 1972, a private farmer lost 16/50 (32%) of his N'dama cattle due to the disease. Treatment was necessary to save the rest. In February, 1973, the author witnessed an outbreak of trypanosomiasis in which 206 of the 1090 (19%) Sebu cattle owned by a local chief in Lwara state died of trypanosomiasis. 472 more severely affected survivors were treated by the author as they would have otherwise died also. The death rate among trade cattle trekked 575 miles from north to south unprotected by trypanosidal drug was 11/20 (55%) (Na'isa, 1969). These figures show that mortality in untreated cases of trypanosomiasis is usually high. So, if only 2% of the 7 million cattle at risk are lost this way and each animal is worth 100, the annual loss will be equivalent to 14,000,000 (US $21 million) due to actual deaths.

Weight loss in trypanosomiasis is usually severe (figs 4,5,6, & 7). When trade cattle were exposed to trypanosomiasis on the usual southward trek for slaughter, it was estimated that an animal that survived the journey in spite of clinical trypanosomiasis lost 44 lbs. in body weight. Whereas animals on the same journey, protected by prophylactic treatment against the disease actually gained weight. Since it is estimated
that 200,000 cattle trek southwards every year, most of them un-
protected, the survivor at the end of the journey will usually have lost
weight considerably. In fact the author has found that 65% of cattle
arriving in Ibadan by foot (fig. 8) were suffering from trypanosomiasis.
Therefore if only 100,000 cattle lost 44 lbs. each and the animals are
worth 20K (30c) per lb. live weight, the total loss will be equivalent to
880,000 (US $1.32 million) per annum.

Failure to attain sexual maturity has been associated with early ex-
pposure to trypanosomiasis (Stephen, 1966); the otherwise mature female
showed small ovaries, no sign of ovulation, and the bulls showed no
libido. The economic loss in this case is not easy to evaluate. But since
several cases of abortion are also associated with the disease, losses due
to reproductive disorders generally will be worth about 500,000 annually.

In dairy herds (Moor Plantation, Ibadan) a sudden drop in milk yield is
usually associated with parasitaemia with trypanosomes. The drop is of-
ten up to 50% and it usually takes up to three weeks to return to normal,
even when the animals were treated. In the outbreak that affected 1090
cattle (quoted above), one of the major regrets expressed by the Kwara
Chief was the lack of milk for the calves, resulting from the outbreak.
Losses of this nature all over the country will probably be worth some
500,000 annually. In summary economic losses due to trypanosomiasis in
Nigeria is probably worth more than 15 million (US $23.8 million) an-
nually.

SCOPE

Inspite of the wealth of information that has accumulated over the
years, our knowledge of the biology of tsetse flies remain inadequate for
the purpose of mounting a comprehensive control program against the
flies. We have not been able to arrest or mimic or reproduce the antigenic
lability of the trypanosomes in a manner that we can use this
phenomenon to advantage in the control of the disease. The numerical
and functional exhaustion of body defence elements caused by
trypanosomiasis remains unpreventable. Our knowledge of feral
trypanosomiasis is still inadequate. These are the areas that should
engage our attention in the field of trypanosomiasis research for the next
decade. Alternatively we may seek to promote the biologic equilibrium in
which we would no longer wish to exterminate the fly, the trypanosome
or the wild life reservoir. While this proposition may have much to
recommend, it does not seem to be sufficiently attractive for present con-
sideration.

SUMMARY AND CONCLUSIONS

Bovine trypanosomiasis remains the most important disease of cattle
in Nigeria, since Rinderpest has been controlled by effective vaccination
Economic losses occur annually due to deaths, weight loss, abortions and
infertility. These losses are worth millions of Naira.

The disease is more widespread than can be imagined from the
Glossina distribution maps available from time to time. Many cases of the
disease occur in remote areas—for example the author had to drive a landrover in the bush as there was no access road, for four hours before reaching the Kwara chief who lost 19% of his 1090 cattle due to the disease. Cattle owners are usually anxious to conceal the number of their animals in order to avoid paying taxes on them. Therefore, many cases are usually not reported to the Veterinary authorities. Losses occur in nomadic Zebu herds, in settle Zebu herds, in trade cattle, in newly established N'dama herds and in exotic cattle. The evaluations given above are probably too modest.

The intractable nature of the disease is due largely to the dynamism of a variety of factors both in the epizootiology and in the host-parasite interaction. There are too many variables, such as the adaptive capacity of the tsetse flies, to changing environment and the various hosts, the antigenic lability of the trypanosomes, the interaction between feral and domestic trypanosomiasis, the development of drug-resistant trypanosomes, and the movement of trade cattle across the country by which the disease is spread from tsetse infested to tsetse free areas. If a solution is to be found, a more comprehensive approach to the various aspects of the disease will be necessary, and workers in the various fields of pathogenic trypanosomology will have to collaborate directly at every level. This will be the only way to achieve success in minimum time.

ACKNOWLEDGEMENT

To my technicians who have willingly traveled with me, often at a moments notice, throughout several years of my field study of bovine trypanosomiasis, to the African-American Scholars Council who granted me a traveling fellowship which has enabled me to travel to St. Louis to present this paper, and to the U.S. Animal Health Association Committee who have honoured me by their invitation to attend their 77th annual meeting at which this paper was presented, I am very grateful.

BIBLIOGRAPHY


**Figure 1** — The physical map of Nigeria showing plateau and mountain areas which are largely free from tsetse flies.
Figure 2 — The vegetation map of Nigeria showing actual and potential tsetse infested areas.
Figure 3 — Typical Guinea savanna vegetation zone in which *G. morsitans submorsitans* are widely dispersed.

Figure 4 — Showing various stages of the clinical course of trypanosomiasis in White Fulani (Zebu) cattle.
Trade cattle arriving in Ibadan after a long trek from the northern states are usually emaciated, anaemic and weak; 65% of 1,000 such cattle examined earlier this year were positive for trypanosomiasis.
Keteku x N'dama cattle known to be resistant to trypanosomiasis often succumb to the disease when the fly challenge is high enough.
Figure 11 — Normal N’dama cattle—with good beef conformation in spite of smallish size.

Figure 12 — N’dama cattle in trypanosomiasis
Figure 13 — Holstein cattle in acute trypanosomiasis.

Figure 14 — Holstein cattle in chronic trypanosomiasis when prophylactic treatment was relaxed.
FOOT-AND-MOUTH DISEASE

Major events involving foot-and-mouth disease (FMD) were the return of A22 virus to southeastern Europe, the type C epizootic in central Europe, type 0 virus in central-eastern Europe, the appearance of type A virus in the Iberian peninsula, and Asia I spread into Turkey.

Type A22 FMD virus is considered an exotic strain to continental Europe. Several outbreaks occurred in Greece and Turkish Thrace during April of 1972 in an extensive vaccination campaign sponsored by the European community of nations. By September of 1972, the situation had returned to normal and the A22 was only occurring sporadically in the Asian part of Turkey.

The role of Turkey as the land bridge for passage of exotic viruses of the Middle East and India to the European continent was again emphasized with the spread across Turkey of Asia I virus from Iran. Because of this an emergency meeting was called by FAO of the United Nations in Ankara in the spring of 1973, in order for the European Community of nations to participate in Turkey’s efforts to restrict movement of the disease caused by this exotic virus type.

Near the end of 1972, there were extensive outbreaks of FMD due to type C virus in central Europe with extensive involvement of swine. The first cases were reported in the border area of western Hungary. By
October, the disease was reported from Hungary, U.S.S.R., Yugoslavia and Romania. Czechoslovakia eventually became involved. As the campaigns against type C FMD virus were nearing successful conclusion, cases of type 0 virus infection were reported from Czechoslovakia, Romania and Yugoslavia. The origins and interrelationships of these outbreaks are obscure.

After almost 7 years of freedom from the disease, FMD appeared in Austria in January 1973. This was an extension of the type C outbreaks that had occurred in Hungary. It was possibly introduced by Austrian railway workers that had serviced trains traveling through Hungary. Extensive slaughter and vaccination campaigns were instituted and as of the summer of 1973, 1500 foci had been declared, involving over 65,000 head of livestock, a large proportion of which were pigs. In Austria, also, as the type C infection was about eradicated, type 0 infection appeared about April of 1973.

In October 1972, there was a rapid increase in the number of cases of FMD due to type A infection in the northern part of Spain. Type A5 vaccination was instituted along with other control measures. A new subtype related to A5 and A24 has been encountered possibly due to virus introduction from South America.

Israel has remained free of FMD since February 1971, when their last infection appeared following use of imported vaccine. The peninsular area of Thailand had its first FMD outbreak in 40 years in February of 1973.

RINDERPEST

Sporadic cases of rinderpest occurred in the Near East, usually associated with animals in commerce. Vaccination is being continued on a country-wide basis in several countries of the area, but there is no regional effort beyond that supplied by FAO from the Near East Animal Health Institute in Beirut.

Phase 5 of the Joint Campaign against Rinderpest in Central Africa has emphasized revaccination and surveillance by national veterinary services of the countries which participated in the prior phase of the joint campaign.

NEWCASTLE DISEASE

Newcastle disease continues to be a world-wide scourge of the Poultry industry. The emergency action to eradicate velogenic viscerotropic Newcastle disease from the United States is unique.

AFRICAN SWINE FEVER

African Swine Fever was successfully eradicated from Cuba which was declared free of the disease in April 1972. Since that time there has been no report of the disease in the Western Hemisphere. African Swine Fever continues to be a problem in Portugal and Spain.
HOG CHOLERA

In November 1972, classical swine fever (hog cholera) was declared to be an exotic disease in the United States. However, some foci of infection were still present in 1973 during the last stages of the nationwide eradication program.

SWINE VESICULAR DISEASE (SVD)

This past year saw the spread of a porcine enterovirus first described in Italy in 1966 by Nardelli. The next report of the disease came in 1970 from Hong Kong associated with an FMD vaccine experiment. In December of 1972, this same disease identified as swine vesicular disease was reported in Austria, England, France, and again in Italy.

The outbreak in Austria was characterized by clinical signs indistinguishable from FMD. The animals involved had been imported from Poland and were immediately destroyed. However, laboratory examination showed that FMD virus was not involved but that the disease was due to the porcine enterovirus serologically related to that previously identified in Italy.

Several outbreaks of a vesicular disease in England in December 1972, resulted in slaughter of cattle and swine but again the disease was identified as swine vesicular disease. It soon spread to other swine herds in England, southern Wales and into Scotland.

Swine vesicular disease has been reported in Hong Kong, Italy, France, Austria, Poland and the U.K. The agent is more resistant to heat and pH changes than are the agents of other vesicular diseases and is consequently undergoing intensive investigation at research institutions of the world as to methods of disinfection and safety of pork products. It is serologically and structurally closely related to the human Coxsackie B-5 virus. A preliminary survey of swine sera indicates that SVD is probably exotic to the United States.

VENEZUELAN EQUINE ENCEPHALITIS

Clinical cases of VEE were reported from the northwestern areas of South America. There have been no confirmed cases in horses in the United States for the second consecutive year. The last reported case in Mexico was in September 1972.

CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP)

Shipments of animals across the Red Sea into the Arabian Peninsula have occasionally introduced CBPP into this normally free area. The disease persists across Central Africa.

RESEARCH ON VESICULAR DISEASES

Vesicular Exanthema of Swine. No cases of vesicular exanthema of swine (VES) have occurred in the United States since 1956. In 1972, a picornavirus indistinguishable from VES virus was isolated from an aborting California sea lion on San Miguel Island, California. This isolate
FOREIGN ANIMAL DISEASES

has been designated San Miguel sea lion virus. Inoculation into susceptible swine produces vesicles similar to those of VES. This is the first report of a virus from a marine mammal capable of producing disease in a terrestrial mammal.

Swine Vesicular Disease. On December 11, 1972, an outbreak of vesicular disease in pigs was reported in Staffordshire, England which was diagnosed as FMD by clinical signs, but this diagnosis was not confirmed by serological tests. Within two days following another outbreak nearby, more than one hundred cattle, nearly seven hundred pigs and two hundred sheep had been slaughtered. The virus was described as a porcine enterovirus, similar to the 1966 Italy virus. This disease is now called swine vesicular disease (SVD). Detailed symptoms and lesions are described giving the acute, sub-acute and chronic forms plus the mode of transmission and disinfectants effective. Since February 1973, SVD has spread to Yorkshire and Scotland. The disease was introduced to Austria via thirty pigs imported from Poland, all of which developed clinical signs resembling FMD.

Laboratory confirmation of SVD cannot be made in less than 24-36 hours—following tissue culture passage or overnight complement fixation test.

Vesicular Stomatitis. A bibliography of vesicular stomatitis from 1971-1972 was written by Uskavitch. Antibodies to VS have been demonstrated in a number of Panamanian monkeys. A stomatitis-pneumoenteritis complex termed “Kata” is recognized and described for the first time in sheep in Nigeria.

Replication of VS virus has been demonstrated in lymphoid cells, mouse peritoneal macrophage cultures and an embryo cell line prepared from Marmosa mitis, a small South American pouchless marsupial. Autointerference used as a measure of cross-relationships of VS concludes New Jersey has only distant relationship with Indiana subtypes.

Many studies have been done with transcription and replication of VS virus by using temperature sensitive mutants. The glycoprotein is the only virion protein of VS virus giving rise to and reacting with neutralizing antibody, and intact virus particles for all types and subtypes of VS show cross-reaction in CF and neutralization studies. When uncoated with Tween-ether or Nonidet, the major part of cross-reactivity was found associated with the ribonucleoprotein.

Ascorbic acid has some antiviral activity in tissue culture studies. Distamycin A is an active inhibitor of DNA viruses in cell culture, but does not influence reproduction of RNA viruses such as vesicular stomatitis and Coxsackie B-5.

FOOT-AND-MOUTH DISEASE

Epidemiology and Pathogenesis. In many outbreaks of FMD, it was reported the epidemic decreased when periods of unfavorable weather intervened and was concluded that increase is possible if two periods of
suitable weather are separated by a period of time equal to the incubation period. In the epizootic of Great Britain in 1967/1968, it was concluded that under ideal conditions of droplet size, wind during the night and high relative humidity that infective aerosols could be blown at least sixty miles.

Sellers found that five minutes after the removal of infected pigs from loose boxes, that the air contained 12-16-fold less virus than when the pigs were present. A 400-fold decrease was found after sixty minutes. Heavy spraying (1.2 mm of water in 5 min.) reduced the virus concentration 500-fold compared to 30-fold after light spraying (0.20 mm of water in 5 min.).

The spread of FMD during the epidemic in Hampshire in early 1967 was by an initial infection of pigs eating infected meat scraps. Subsequent spread was by market movement and airborne spread of virus. It was found that the attack rate in the 1967/1968 British epidemic was related to livestock density.

Cottral reviewed the diagnosis of bovine vesicular disease. Type O FMDV was found responsible for a severe outbreak in pigs in India where 66.7% mortality occurred in unweaned pigs only. From experimentally infected buffalo calves, virus was detected in synovial fluid within fourteen hours and persisted until the fifth day after infection.

Three types of FMD virus have been identified from Turkey: Type O, SAT-1 and A22. Three classical types endemic in Brazil are O, A24 and C3. Three other subtypes have been identified and A25 has further been isolated from swine. In two FMD outbreaks caused by Type O in Iran in sheep, 64% of lambs died and among goats, 55% of the kids. Lambs and kids from 1 to 15 days of age were affected, but showed no clinical signs. Lameness and vesicles were sometimes observed in adults and petechial hemorrhages of the organs found at necropsy.

The epidemiology of FMD in Botswana is discussed in both domestic stock and free-living game population. Large scale annual prophylactic vaccination is emphasized and indicates the degree of control which can be achieved in African countries with similar ecological conditions. Brooksby stresses that a study of epizootiology in developing countries is a prelude to any control measures.

Capel-Edwards in reviewing the literature found that FMD in small mammals so far investigated shows susceptibility is not limited to certain taxonomic orders. A classification scheme based on specie's susceptibility is proposed.

Several epizootics of FMD in the USSR have been attributed to the Saiga antelope. During a recent epizootic among game in the Kruger National Park, a group of young adult African buffalo became infected which is the first confirmed clinical FMD case in this species. During an outbreak caused by Type SAT-2 in cattle in Rhodesia, experimental infection was induced in kudu, impala, warthog and brush pigs while mild clinical disease was observed in buffalo. The carrier state was demon-
strated in buffalo, judu and cattle and virus recovered from two wilde-
beest.

A detailed description by Hayes\textsuperscript{44} depicts what would happen to in-
digenous big game animals in the event of FMD in the United States.

In experimental multiple infection of cattle, half of the recovered ani-
mals from one type were completely susceptible to reinfection with any
of the other types tested.\textsuperscript{44} The cumulative effect of resistance from con-
tinued infections were usually less severe with the third and succeeding
FMD types used. The experimentally induced disease in sheep and goats
has also been described.\textsuperscript{47} Evidence indicates the primary site of virus
multiplication in sheep and goats is the pharyngeal area and that the
udder may be infected through the teat canal as well as blood stream.\textsuperscript{48}

The carrier state in cattle was reviewed by Cottral.\textsuperscript{49} In the past three
years oesophageal-pharyngeal (OP) samples from cattle and sheep from
Hamburg Quarantine Station were tested for FMD carrier virus.\textsuperscript{60} Type
A virus isolated from a clinically normal cow showed reduced patho-
genicity in cattle and suckling mice. Serum and OP samples from appar-
etently healthy vaccinated animals were collected in Sao Paulo, Brazil from
an area where no cases of FMD had been reported for six months.\textsuperscript{61}
Virus was isolated from 79 samples in suckling mice and tissue culture
(IB-RS-2) and it was concluded that FMD virus can replicate in the
pharynx of immune animals.

FMD virus Type $A_{22}$ survived on an infected chicken house floor, in
water of an automatic drinking bowl and on the legs of chickens.\textsuperscript{62}

A variety of disinfectants and prophylactic treatment has been re-
ported.\textsuperscript{62} "Experimental evidence showed inactivation of FMD virus by
curing contaminated ox hides with common salt alone, and calcinated
soda mixed with common salt.\textsuperscript{62} Methods of disinfecting the skin of in-
fected cattle and swine have been described.\textsuperscript{66} \textsuperscript{67}

Over an eight year period in France, 1,650,000 susceptible cattle have
been artificially inseminated with semen from FMD vaccinated bulls
without one case of disease developing in females.\textsuperscript{68} During an outbreak
of FMD in bulls in India, many changes were seen in the semen mor-
phology such as immature, micro, tapering or vacuolated heads.\textsuperscript{69} Upon
necropsy, an infected Holstein bull demonstrated a total picture sugges-
tive of severe testicular degeneration and complete absence of spermat-
ogenesis.\textsuperscript{70}

\textit{Vaccine and Prophylaxis.} A report describes immunogenic properties
of experimental strains of FMD virus with inactivation procedures and
adjuvants.\textsuperscript{71} Bachrach\textsuperscript{72} describes problems of vaccination due to high
multiplicity of virus types and hosts, use of modified live virus versus
inactivated virus vaccines as well as application of inactivated vaccines.

Ethyleneimine is a more desirable and faster inactivant than propylenei-
mine or N-acetyleneimine.\textsuperscript{72} Benndorf\textsuperscript{72} proposes the prophylactic
use of adsorbate vaccine in livestock around an outbreak and a live vac-
cine in direct vaccinations in affected stock.

In pigs neonatal thymectomy does not alter their immune response to
It was found that vaccination is adequate for young calves. Labonnardiere describes obtaining viruses for vaccines using both pathogenic and non-pathogenic strains. Promising results were obtained using vaccines prepared from unmodified cattle virus grown in either monolayer or suspended cultures of bovine thyroid cells. Multiple vaccines have been prepared containing brucellosis and FMD and one including rabies. Virus of FMD has been adapted to sheep kidney cell tissue culture; however, following seventeen passages, immunogenicity is reduced by 50%.

Low potency vaccines can be produced by inactivation with acetylthelylenimine or formaldehyde, but Rowlands, et al. showed 0.05% formaldehyde satisfactory probably due to stabilization of 140S virus particles. Inactivation of culture virus with ethylenimine was complete within fourteen hours at 37°C with Types A, O & C. Protection significantly declined at the end of three months in vaccinated piglets with monovalent and trivalent inactivated vaccine containing DEAE-dextran as adjuvant.

Saponified gel vaccine tests in cattle, sheep and goats elicited better antibody response than conventional gel vaccines. The suitability of using adult mice for testing efficacy of three inactivated vaccines has been described. Cross reaction of vaccine viruses and wild viruses has been reported.

Mayr wrote an excellent review of the problems in controlling FMD by vaccination. Since 1962, FAO has spent nearly five million dollars for bi-annual FMD vaccinations for a disease-free buffer in southeast Europe and reports that the area has been re-invaded by A22 introduced by illegal meat movement.

No adverse effect was noticed in FMD vaccinated cattle when dipped in phosphorated, chlorinated or arsenical ascarisides. Beir and Anders indicate vaccination does not stimulate quantitative lymphocyte changes in the blood.

Non-specific neutralizing activity against heterologous virus types was found to be a genetic characteristic of the individual animal involving mainly the IgM serum fraction. The first immunoglobulins to appear in FMD immunized guinea pigs were IgM.

Ginanni and Maglione tested components of FMD vaccine to discover which factor produces early anaphylactic reactions in vaccination of cattle. It was concluded there was constancy in reactions to the complex of cellular lysate, antibiotics and formalin and that virus was not necessary to induce the phenomena.

The interferon inducer, divinyl ethermaleic anyhydride, has been studied with FMD vaccine using mice and swine. Vaccination of pigs continues to be studied.

Intranasal vaccination with a parenterally effective DEAE dextran vaccine was also unsuccessful.
Calves with passive immunity acquired by colostrum, vaccinated at the age of 5-155 days, show little or no response to FMD vaccination. Calves from vaccinated dams only respond to vaccination after four months of age while those from non-vaccinated dams, show a response to vaccine the first few days after birth. There were 982 cases of atypical symptoms and lesions of FMD reported from 3,956 dairy cattle where mass vaccination was practiced with inactivated vaccines, the highest rate among cattle four-twelve months of age. It has been found that vaccinated cattle show a detectable fluctuation in serum antibody titers the first four to seven days following exposure to virus, then a definite rise. The authors postulate the course of infection in immunized cattle depends on the rapidity of new antibody formation as well as the level of neutralizing antibody titer at time of exposure.

Cattle repeatedly vaccinated with Types A&O possessed heterologous neutralizing activity against Types Asia and SAT-1 up to five months in individual animals. Vaccination of heifers have been reported.

Nearly 50% of cattle inoculated one or more times with FMD vaccine containing some carboxymethyl cellulose (CM) gave a positive allergic reaction to an I/D injection CM. Of nearly eight million cattle vaccinated in the Federal Republic of Germany, 576 allergic reactions and 330 abortions were reported with approximately 45% mortality of the allergic reactions. Prior vaccinations predispose the allergic reactions and Frenkel vaccine is more allergenic than tissue culture vaccine.

Of different disinfectants it was found that 1% sodium hydroxide solution inactivated FMD virus completely in 30 seconds, while ten minutes were needed for 0.5 and 0.2% concentrations while 1% potassium permanganate and 3% phenol solution were satisfactory in 10 minutes as was 2% Iosan solution. Disinfection of persons was effective in 3-4 minutes with 2% tartaric acid and 2% acetic acid.

Virus Growth and Serology. Mechanical device for growing virus has been described.

Certain bovine serums inhibited cell susceptibility of IB-RS-2 cell lines to FMD virus with a cumulative effect. Addition of Eagle’s non-essential amino acids to growth medium of primary cultures of bovine and porcine kidney cells increased susceptibility for FMD viruses, whereas Eagle’s essential amino acids had no effect and cystine and ascorbic acid decreased susceptibility.

Classical methods of purification by air-methanol, then butarol-chloroform, then Arcton 113 treatments were inefficient for FMD virus.

Using immunofluorescence techniques, specific FMD virus antigen could be detected in bone marrow, lung, liver and spleen tissue two hours after guinea pigs were infected, with maximum fluorescence after twenty-four hours.

A microculture plaque reduction test was more sensitive than the indirect complement fixation test, but less so than baby mice for titration of FMD virus. Use of tissue culture (BHK-21 cells) resulted in a higher percentage of FMD virus isolations from cattle tongue or foot epithelial
samples than mice.\textsuperscript{107}

The chemical and physical properties of FMD and Maus Elberfeld virus are structurally similar.\textsuperscript{108} All seven serotypes of FMD contain a polypeptide which migrates to the same position in polyacrylamide gel electrophoresis, indicating a group antigen which reacts in complement fixation tests with both hemotypic and heterotypic antisera.\textsuperscript{109}

The virus of FMD was twenty-five times more sensitive to UV radiation than Aujesky's disease virus.\textsuperscript{110} Inositol, sodium glutamate and calcium lactobionate protected FMD virus against inactivation during freeze drying while di-methyl sulphoxide and glycerol were ineffective.\textsuperscript{111} Nearly all FMD virus yield in cell cultures were suppressed with N-phenyl-N-4-hydroxyphenylthiourea.\textsuperscript{112}

Three plaque size variants of FMD Type O were differentiated by serum protection tests and neutralization kinetic studies.\textsuperscript{113} Also, agar gel diffusion tests showed antigenic variation and it was found that all were highly virulent in cattle.\textsuperscript{114,115}

Specific complement fixing and precipitating antibodies equal to blood serum were found in immune ascites fluid from white rats.\textsuperscript{116}

Subtype classification of FMD Types O and C by the passive immunohemolysis test corresponded closely with the complement fixation test results.\textsuperscript{117} The conglutinating-complement adsorption test was as sensitive and less cumbersome and expensive than the complement fixation test for typing FMD viruses.\textsuperscript{118} A system for calculation of bilateral relationships of unknown strains of FMD in relation to reference sera by biomathematics is described.\textsuperscript{119,120}

Only 1 (SAT-2) of the 7 serotypes of FMD showed direct hemagglutination of guinea pig erythrocytes, even after 40-fold concentration of antigen.\textsuperscript{121} No specific hemagglutinin was demonstrated with any FMD antigen using erythrocytes from 38 different vertebrates.\textsuperscript{122} Chernyaer and Sabko\textsuperscript{123} found passive hemagglutination more sensitive than the complement fixation test for identification of FMD virus. Virus coupled to sheep erythrocytes by CRCL3 passively agglutinated with virus-specific antisera and lysed by addition of guinea pig complement was highly specific for differentiation of FMD virus types.\textsuperscript{124}

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DURATION OF IMMUNITY OF HORSES VACCINATED WITH STRAIN TC-83 VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS VACCINE

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SUMMARY

Fifty equines were vaccinated with attenuated VEE vaccine strain TC-83 in July or August, 1971. All horses survived challenge of immunity with an equine virulent VEE strain 9-19 months later. No equine developed clinical signs of VEE infection and only one was viremic. Neutralizing antibody to challenge virus developed to high concentrations in most animals.

INTRODUCTION

During the summer of 1971, an epizootic of Venezuelan equine encephalomyelitis (VEE) entered the United States from Mexico. An attenuated VEE virus vaccine, strain TC-83, developed by the U.S. Army Medical Research and Development Command, Fort Detrick, Maryland, was used with other control methods to stop the outbreak. Although the efficacy of the vaccine had been proved in South and Central America and experimentally used in the USA, only a small amount of serological and VEE virus challenge data existed with regard to duration of vaccine immunity in horses. Spertzel and Kahn reported challenge of immunity studies on horses vaccinated with strain TC-80 and burros vaccinated with TC-81. Twelve and 24 months later, the horses and burros, respectively, were injected with an equine virulent strain of VEE virus. All equines resisted this challenge of immunity. Walton et al. in experimental studies in Panama showed that 6 horses were not susceptible upon challenge with an equine virulent VEE virus 14 months after strain TC-83 vaccination; serum neutralizing (SN) antibody to vaccine and challenge virus was still detectable at the time of challenge. In serological field studies, Walton and Johnson reported that SN antibody to vaccine and a Central American epizootic strain of VEE virus was still detectable in most of 71 Panamanian horses at 20 months and in most of 49 Nicaraguan horses at 30 months after
strain TC-83 vaccination. Duration of immunity was not determined by equine virulent VEE virus challenge. Faced with the inevitable questions about revaccination of horses in high-risk areas of the southwestern United States, the following study was begun to determine duration of immunity in a population of horses that often had evidence of pre-vaccination antibody to the related group A arboviruses, Eastern (EEE) and Western (WEE) equine encephalomyelitis viruses.

MATERIALS AND METHODS

Horses - Fifty mares, stallions and geldings from 1½ to more than 15 years of age were used. Forty-four were ponies, 5 were thoroughbred horses and 1 was a quarterhorse. Six ponies from the VEE susceptible herd kept at Denver were used as control horses for pathogenicity of the virulent virus.

Vaccination - The 44 ponies were vaccinated in California during July, 1971, and the other six horses during August, 1971.* The attenuated VEE virus vaccine, strain TC-83, was administered to all 50 test animals by the recommended method. In addition, 3 ponies received a second vaccination with strain TC-83 in June, 1972 and 17 animals had histories of vaccination with either EEE or WEE killed virus vaccine, or both, before or after the VEE vaccination. The records of the type and producers of the EEE and WEE vaccines were not available.

Challenge of immunity - The immunity of horses was challenged at selected intervals after vaccination. Six ponies were infected with an equine virulent strain of VEE virus at 9 months after vaccination, 6 ponies at 11 months, 6 ponies at 13 months, 6 ponies and 6 horses at 14 months, 12 ponies at 18 months and 8 ponies at 19 months. All work was completed in an insect-secure, viral-safe isolation laboratory at Denver.

The challenge virus strain, 71-180 #4, was isolated on July 12, 1971, in Texas.7 Antigenic characterization by using the kinetic hemagglutination inhibition technique indicated that this strain was closely related to other Central American and Mexican isolants that are all variants IB.8 Horses were inoculated subcutaneously with 1.0 ml of virus from the diluted heparinized blood of the second serial passage in horses. The blood was stored at -70°C, thawed and diluted 1:4 in phosphate buffered saline (PBS), and each horse received approximately 100,000 median embryo intravenous lethal dose units (EIVLDso). When the intravenous technique is used,8 10-day old embryonating hen's eggs are as sensitive as suckling mice for quantitating this virus strain.

Clinical and virologic studies - Temperatures were recorded twice daily. Blood was collected in vacuum tubes with di-sodium EDTA anticoagulant for daily hematologic studies. Leukocyte and differential counts, packed cell volumes, and eosinophil and reticulocyte counts were conducted for most horses. The daily plasma samples were assayed for

*Dr. J. A. Ferguson will report the pre- and post-vaccination serological data on these and other horses in partial fulfillment of the requirements for the Ph.D. degree at the University of California, Berkeley.
the presence of virus by intravenously injecting 0.1 ml of inoculum into 10-day old embryonating hen’s eggs. A 20% suspension was made from dead embryos in PBS. After treatment with aminoethyl-limine, the suspension was tested for VEE viral antigen by using the microtiter complement fixation technique.\(^1\)

**Serologic studies**—The concentration of SN antibody was determined for each horse before challenge and 14 days after VEE virus challenge. Serums were heated at 56°C for 30 minutes. Plaque reduction SN tests were done in Vero (African green monkey kidney) cells by using a tris-oxaloacetic acid buffer system as described by Earley et al.\(^2\) and serial four-fold serum dilutions from 1:8 to 1:131,072.\(^3\) Viral antigens used in the test were the challenge strain of VEE, the LTV-6 strain of WEE and an EEE strain isolated in Panama in 1958.

**RESULTS**

All 50 vaccinated horses survived challenge of immunity. The 6 control horses exhibited typical clinical signs, blood virus concentrations, serological responses, and lesions of VEE infection;\(^6\) all 6 died.

One aged stallion inoculated with challenge virus 9 months after vaccination was febrile from 2-8 days after challenge and psychically depressed on the second day. The plasma specimen taken on the second day yielded 105.5 EiVLD\(_{50}\)/ml. of VEE virus. No other blood samples were taken from this horse for viral isolation attempts. No other horses in the 9 or 11 month post-challenge groups exhibited febrile responses and no change in hematologic values was recorded. Two ponies challenged at 13 months and 2 ponies challenged at 14 months after vaccination exhibited leukopenia 4-6 days after the challenge infection; no other clinical responses were observed for the ponies in these 2 groups. One thoroughbred in the group of 6 horses at 14 months after vaccination had a fever related to a laceration on the leg; hemograms were not done on these horses. At 18 months after vaccination, 3 ponies had elevated temperatures and were depressed and 4 other ponies had leukopenia after challenge; no other clinical responses were observed. One pony had fever and 2 other ponies had leukopenia at 19 months after vaccination; no other clinical responses were observed.

Serum neutralizing antibody responses to VEE, EEE and WEE viruses are presented in Table 1. The serological responses of 6 ponies exposed to virulent VEE virus at 12 months after vaccination were not determined. All horses had detectable SN antibody to VEE virus before challenge and a significant antibody titer rise was noted after challenge.

There was no indication of a heterologous EEE viral SN antibody stimulation. Although there was some change in the WEE SN antibody titers after VEE challenge of some groups of animals, no consistent increase occurred.

In an attempt to provide some data on possible interference by WEE

\(^{*}\)In the plaque-reduction test, 80% neutralization of 50-100 virus plaques at an initial serum dilution of 1:8 was taken as qualitative evidence for presence of specific SN antibody and previous VEE virus infection.
SN antibody on the development of VEE SN antibody after vaccination, we compared the pre-challenge antibody titers of WEE with those of VEE. Although more meaningful data may be derived from the immediately post-vaccination studies to be published by Dr. Ferguson, our data suggest that minimal or nondetectable WEE SN antibody might be correlated with significantly higher concentrations of VEE SN antibody. Comparison of respective VEE geometric mean SN antibody titers for the group of 6 horses at 14 months post-vaccination, however, reveals no difference in response. In all other cases, the VEE SN antibody attained greater concentrations when the WEE antibody titer was low.

History of WEE or EEE vaccination, or both, before VEE vaccination did not correlate with the presence of SN antibody to these two viruses before challenge. We must assume that natural infection occurred in some test animals, especially with WEE virus.

**DISCUSSION**

The evidence is now unequivocal that VEE vaccine strain TC-83 produces solid, long-lasting immunity. Even in horses with pre-existing SN antibody to the related alphaviruses WEE and EEE, resistance to challenge with virulent VEE virus continues for more than 19 months. Although the antibody titers of individual animals against VEE challenge virus were at times only 1:8, except for one horse, there was no detectable difference in their responses as compared with those of horses with greater SN titers. These data strengthen the belief that immunity to VEE infection after vaccination is long lasting. In the report from Nicaragua and Panama, SN antibody was still detectable in most horses 20 and 30 months after vaccination. Although it is only conjecture, this study would support the hypothesis that these field-vaccinated horses would have resisted virulent VEE virus challenge.

An admittedly small amount of data was presented in this paper to suggest that the pre-existence of WEE antibody before vaccination diminishes the magnitude of the VEE antibody response. There is no evidence, however, to suggest that pre-existing WEE antibody in any way affects the horse's ability to withstand virulent VEE virus challenge of vaccine-induced immunity.

The only possible comparison of the serologic responses in these horses after challenge is with the work by Walton et al. who used the plaque reduction SN test in Vero panels. The range of pre-challenge geometric mean SN antibody titers in the present study was great although the values of some horse groups were similar to those of the earlier study. A marked difference is seen in the post-challenge SN antibody titers. Although the titer increase in the earlier study was undramatic, the increases reported here were very great, but not of the magnitude reported for horses that recovered after primary infection with an equine virulent VEE strain. The differences in responses may reflect the status of health of Panamanian versus American horses. The different response may also be a reflection of the influence of the related WEE or EEE viruses on the serological response; the horses in the earlier study
were selected from an area of Panama where there is no serologic evidence of group A arbovirus activity.

Although we have no ready explanation for the viremia detected in one pony, we must consider that this animal may not have been vaccinated successfully. Detection of pre-challenge SN antibody at a titer of 1:8 would refute this suggestion. In addition, in our experience, blood virus titers such as detected in this pony are generally associated with more severe clinical disease; all control horses infected with this same virus source and inoculum have had similar viremia patterns, but died. Other co-existing physiological or pathological conditions may possibly have altered the vaccinal or challenge responses of this 25 year old pony.

Future studies are planned with an ever-decreasing population of available horses with known histories of VEE vaccination. The accumulation of a group of horses for challenge that no longer have detectable SN antibody titers is of particular concern.

REFERENCES


**DURATION OF IMMUNITY OF HORSES**

**TABLE 1**

Neutralizing antibody to Venezuelan (VEE), Eastern (EEE), and Western (WEE) equine encephalomyelitis virus in horses before and 14 days after challenge of vaccination immunity with VEE virus.

<table>
<thead>
<tr>
<th>Months</th>
<th>No.</th>
<th>Neutralizing antibody titer*</th>
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<th>WEE**</th>
<th>EEE**</th>
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<td></td>
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<td>Post-challenge</td>
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<td>Post-challenge</td>
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*Reciprocal of geometric mean neutralizing antibody titer used on 80% plaque reduction.

**Figures in parentheses are percentages of animals with detectable SN antibody (≥ 1:4). Absence of parenthetical data indicates all animals positive. Inclusion of animals without detectable SN antibody in the geometric mean accounts for the absence of definite values (i.e., the use of <).

**TABLE 2**

Comparison of pre-challenge geometric mean serum neutralizing antibody titers to Venezuelan (VLC) and Western (WEL) equine encephalomyelitis virus.

<table>
<thead>
<tr>
<th>Months</th>
<th>Pattern of neutralizing antibody*</th>
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<th>VLE ≤ 102**</th>
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<tr>
<td></td>
<td>No. of horses VLE</td>
<td>WEL</td>
<td>No. of horses VLC</td>
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<td>6</td>
<td>102</td>
<td>2</td>
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*Serum were considered an antibody titer to be indicative when there was a titer increase of at least four-fold.

**Reciprocal of geometric mean serum neutralizing antibody titer used on 80% plaque reduction.
The vaccination program for VEE two years ago and now the EIA control program have vividly confirmed what we have all long known—that there is a very real need for a system or means of permanently identifying individual horses. In fact, accurate health certification and efficient disease prevention programs involving testing, vaccination or isolation are not possible without such a system.

There are several reasons for marking or branding horses. The principle one is proof of ownership as done with the hot iron brand throughout our Western ranch country. It is quite effective for the purpose intended, but in no way identifies the individual horse, so is of little use for identification purposes in a disease control program.

Unquestionably, a system whereby each horse in the country would be identified by his individual brand or mark is ideal—but logistically legally and economically, quite impossible. Consequently, the best we can hope for is a compromise somewhere between utopia and what we now have.

Several effective, unrelated, uncoordinated systems are now in operation—each for a specific purpose. The Preston brand used by the Army is one such system. Using a hot iron brand, an identification number consisting of one letter and three digits was placed on the left side of the neck of each horse. It was a large, legible and convenient. However, the thought of getting modern-day horse owners to extensively use this or any other system involving hot iron brands is out of the question.

The use of acid rather than a hot iron to implace a brand has been tried extensively, especially in cattle. While it seemed to have some advantages, it has never proven to be practical, primarily due to technical problems in application resulting in smudging and inconsistent results.

The other method of external branding that has been extensively tried and used is that of freeze marking. The technique, briefly, is to immerse the branding iron in a mixture of dry ice and alcohol or liquid nitrogen until the iron temperature is lowered to approximately a minus 60 degrees centigrade. The iron is then held firmly against the skin area to be marked for 15 or more seconds, depending upon age, breed and color of the horse. For dark colored horses, a time-temperature ratio is used that will result in a mark of white hair. In grey horses, a little longer exposure time is used which results in a slight balding mark, along with white hair. This technique is painless, effective, rather easily done, and is rapidly gaining in popularity.

Another method and system is the lip tattoo method used extensively in the horse racing industry at Thoroughbred Standardbred and Quarter Horse tracks, for the purpose of accurately identifying horses entered in...
races. It is tightly controlled and quite effective for the purpose intended. The lip tattoo method has not received wide usage elsewhere because of problems in application, also the mark tends to fade after a few years in some horses, especially in those marked while very young.

Another system used very effectively by The Jockey Club is that of photography—that is, photographing the chestnut or "nighteye" on each leg along with a picture of the horse. The system is comparable to the fingerprint system in humans and just as accurate because configuration in chestnuts varies greatly and no two horses are identical. Again, an excellent system for the purpose intended, but hardly adaptable for general usage because of the expertise and equipment required.

Another method, started in New Mexico a few years ago and now used in other Western states is a "Permanent Identification Card" for individual horses issued by the State brand inspection service. The card carries a sketch and detailed description of the horse. It is quite accurate for identification purposes and is valid until the horse changes ownership. Conceivably, it will become popular and widely used in states where brand inspection is required prior to movement or showing. In other areas, there probably will be little incentive for its use even though it is an effective and easy system to operate. Its use should be encouraged.

Interestingly, some eighteen countries are now using and others are considering a similar approach for an international passport for horses. The United States is not one of those countries.

Still other methods have been explored and used. Marking by laser is one. Electronic implants is another. Genetic markers in blood elements are very accurate and have a wide range of usage.

Another system to consider is that used by the individual breed registries—each a little different yet all quite similar. A descriptive certificate is usually stored in a safe place apart from where the horse happens to be. Also, the written and sketched descriptions are made by owners usually when the animal is quite young and are subject to a considerable range of errors. One recent study by a leading horse magazine estimated that up to 30% of existing certificates of registration do not completely or accurately describe the horse upon which issued.

So—it can readily be seen from the foregoing that several methods of marking, branding or otherwise identifying individual horses are available and in use. Also, there are several identification systems, each with different administrative record keeping procedures.

A first step certainly is to create more awareness of the advantages and need for individual horse identification among the horse-owning public, and at the same time, evolve a plan or mechanism whereby all existing systems could be used for the widest and most useful purpose. Such action would require continuous study and central direction and could be accomplished only by some national or federal agency such as U.S.D.A.

This effort in itself should be the prelude to the development and eventual adoption of a national, if not international system for the individual identification of horses. Implementation in this country, at least would
of necessity be voluntary and complete implementation, even if possible, would require many years. However, any partial success would well be worth the effort.

One system that seems to offer much promise is an "angle number symbol system" recently developed by Dr. R. Keith Ferrell at Washington State University. The symbols or brand markers are distinctive, unalterable and designed to incorporate breed registration numbers if desired. The brand may be applied by tattoo, laser or freeze mark. The latter appears to be by far the most practical.

The Arabian Horse Registry of America has been using this system, adapted to specifically fit their administrative system, for two years. The Registry controls the branding irons and Registry employees do all the marking. The program is voluntary and was accepted slowly at first. However, it has gained in popularity, especially among large breeders. To date, 3000 have been marked.

We have a few slides that illustrates some of the methods discussed.

In summary, the need for a system for identification of individual horses is obvious. The purpose of this paper has been to relate existing methods of marking, branding or otherwise identifying and the systems now in operation or available. Hopefully, some universal system will evolve.

It appears that the U. S. Department of Agriculture is the most logical body to develop and coordinate such a system and that there are three actions this organization (USAHA) should take. First, continue and expand efforts to educate horse owners on the value of an individual identification system. Second, encourage more research on methods and systems. Third, urge U.S.D.A. with the consent and cooperation of the horse industry start organizing and implementing a system incorporating presently used methods and programs with a view to getting as many horses as possible effectively identified.

This has intentionally been a brief and sketchy presentation because for those who are familiar with the problem, there is little new to present and for those who are not familiar, it gives a bird's eye view of the picture. One excellent reference book, "Proceedings—Horse Identification Seminar," December 8-9, 1972, Washington State University, is available from the Cooperative Extension Service, College of Agriculture, Washington State University, Pullman, Washington. It covers in detail all methods and systems. Another reference dealing entirely with freeze marking and a proposed international system is "Y-TEX Freeze Marking," available from the Y-TEX Corporation, P. O. Box 1450, Cody, Wyoming 82414.
1973 REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF HORSES

Chairman: Dr. R. C. Knowles, Hyattsville, Maryland
Co-Chairman: Dr. W. O. Kester, Golden, Colorado


The following topics were of major interest to the committee on Infectious Diseases of Horses:

EQUINE ENCEPHALOMYELITIS

The USDA in cooperation with State Agencies has conducted a continuing surveillance for Venezuelan Equine Encephalomyelitis (VEE). During the past year 705 field cases of suspected Encephalomyelitis in horses and other equidae have been investigated and samples were submitted for laboratory confirmation; 116 of these were positive for Western Equine Encephalomyelitis (WEE) 18V for Eastern Equine Encephalomyelitis, and all were negative for VEE.

Along the U. S.-Mexican Border mosquito collections made from Brownsville, Texas to San Ysidro, Calif. during the period April to October 73 revealed only a few positive isolations for WEE, no VEE positive isolations.

The last case of VEE was reported from Mexico in November 1972 near Mexico City; however, a positive isolation of VEE virus was made from mosquitoes in the same area in January 73.

The USDA has accepted an invitation from the animal health authorities in Mexico to help with epidemiological studies of VEE in Mexico. This cooperative effort should help provide an early warning system helpful to both countries.

EQUINE INFECTIOUS ANEMIA (EIA)

During fiscal year 73 a total of 124,837 horses were tested for EIA. Of these, 4,130 or 3.3 were positive to the AGID Test (See FY 73 Map).

The committee recommended that in view of AVMA policy not to officially utilize a man's name on the designation of a diagnostic test, that the agar gel Immuno-diffusion test for EIA (frequently referred to as the Coggins Test) be known officially as the AGID test for EIA.

Several states have initiated EIA control programs.

A subcommittee was appointed to develop a Uniform Methods and rules for EIA. This would supersede the EIA Prospectus with guidelines.

1 virus isolation and 115 serological
58 virus isolation, 122 serological, 8 histopathological.

206
HORSE IDENTIFICATION

The importance of horse identification as an essential to disease control, as well as legal and commercial needs, was reviewed. The committee adopted a resolution urging the USDA to take actions to implement a national individual horse identification system. A sub-committee was appointed to help develop a practical means of identifying horses. (Read resolution).

EQUINE PIROPLASMOSIS (E.P.)

Feasibility of tick eradication in Florida relating to E.P. eradication was reviewed. The project is scheduled when adequate manpower is provided according to state authorities. Chemotherapy against Equine Piroplasmosis was reviewed. Diampron a product found to be effective against Babesia caballi is no longer available for use.

Imidocarb (4A56) has been approved by FDA for experimental use against B. caballi. The committee urged its approval by FDA when adequate data is submitted.

Following a discussion of rules governing importation of horses into the U.S., your committee requests the Secretary of Agriculture to extend present requirements, of freedom from evidence of E.P. in horses to zebras, when they are offered for importation.

OFFICE OF INTERNATIONAL EPIZOOTICS

The committee reemphasized its view expressed last year in a resolution, that every effort be made to secure USDA and U.S. State Department action toward this country joining the Office of International Epizootics.

Respectfully submitted,
R. C. Knowles, Chairman

RESOLUTION ON INDIVIDUAL IDENTIFICATION OF HORSES

It is an established fact that accurate health certification and efficient disease prevention programs involving testing, vaccination, or isolation of horses are not possible without a system for permanent identification of individual horses.

It is also a fact that there are now several such systems being administered independently by various States and Breed Registries and new systems are being developed. These systems are devised to meet the needs and very efficiently serve the purpose intended by the Registries and States administering them. However, they are of limited value in disease prevention and control programs because they are not coordinated on a national basis and data is not retrievable.

A national computer center is being established by the United States Department of Agriculture at Fort Collins, Colorado with a capability of supporting a national individual identification system for all livestock.

It is apparent that existing systems in the horse industry could be coordinated and incorporated into a computerized national system where
by individual identification data could be stored in computer banks ready for retrieval.

Therefore it is urged that USDA in cooperation with appropriate States and Horse Industry Organizations develop a plan encompassing the forgoing systems and assets with a view to getting as many horses as possible effectively identified and recorded in conveniently retrievable data form. Obviously such effort requires continuous study and continuing direction by an administrative office. We are unaware of any office in the horse industry available for this task consequently we urge that USDA accept responsibility for developing this proposed cooperative program.

Further the forgoing is in concert with recommendations of the International Livestock Brand Conference covering all classes of livestock and it is believed that the horse industry due to size and existing organizations therein, could well serve as the model in developing identification programs for the livestock industry.

RESOLUTION ON IMIDOCARB DIPROPIONATE (4A65) THERAPY AGAINST BABESIA CABALLI.

There is a need for a chemical agent that will obliterate the carrier state of *Babesia caballi* in horses and other equidae.

The availability of a practical therapeutically agent will aid in the interstate and international commerce of horses and other equidae.

Therefore it is urged that the Secretary of Health Education and Welfare give high priority to the approval of imidocarb dipropionate (4A65) as a sterilizing chemotherapeutic agent against *Babesia caballi* when adequate data is presented.
### EIA - Acid Tests Reported - Fiscal Year 1973

**Key:**
- **# Positive:** 6,120 (5.35)
- **# Tested:** 114,357

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* = Period January thru June 1973 only.
** = Period January thru Sept. 1973 only.
*** = Period July thru December 1972 only.
**** = Estimated

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**Note:** Preliminary report subject to change.
AGENCY FOR INTERNATIONAL DEVELOPMENT

Dr. Omer J. Kelley

I welcome the opportunity to discuss with you the role of the Agency for International Development (A.I.D), and of its Office of Agriculture in particular, in helping to improve the quality of life in developing countries. Although most of you are no doubt familiar with many of the major problems of these countries, I want to review some of the most monumental ones which we should not forget. First, the developing nations occupy two-thirds of the earth's land surface, and contain 70 percent of its people, most of whom exist on poor diets in mass poverty. Although production of food increased by more than one-third over the past decade, individually there is no more food because of the growing population.

In spite of the fact that the developing countries have doubled the size of their school systems in ten years, they still have more children out of school than ever, and there are no schools at all for more than 300 million children.

Of course, there are other major areas of great concern, as poor health care, short life-expectancy, high unemployment and underemployment and unequal income distribution in all too many localities.

Obviously, to improve or to solve any of the problems of a country requires that the leaders have an understanding of the problems and that they have a sincere desire for betterment. Most have the latter, but some lack the former. Without internal knowledge of the problem, outside assistance can have little real effectiveness. Thus, I believe that donor countries and organizations must do more to educate and to motivate country leaders so that they will want to do everything possible from within.

The Marshall Plan was effective in Europe because of an internal existing education and technological bases as well as country dedication. On the other hand, similar inputs into lesser developed countries have not triggered development in terms of higher living standards. The educational and technological bases were and are just not there to absorb the inputs and transfer them into the necessary improvements.

While much progress has been made in increased production, thus far the richer nations have not been too successful in raising living standards of the poorer nations (too rapid population growth one main factor, poor income distribution in others, etc). I feel strongly that for humanitarian reasons alone we and other donors should not slacken our development efforts. But, beyond these reasons, we must not lose sight of the $10


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billion in fuels and minerals the U.S. obtains annually from certain countries in the developing world, and of the fact that the developing nations now take more than 30 percent of all U.S. exports. Investments of U.S. firms in these countries exceeds $30 billion and is increasing about 10 percent every year.

Obviously, one needs to say no more to establish the linkage of the U.S to these countries. I believe that every passing year will bring closer and growing ties, and that more and more people in the U.S. will see and understand the necessity for a sound U.S. program of technical and educational assistance.

A.I.D.'S TECHNICAL ASSISTANCE PROGRAM

Following this brief statement as to why U.S. involvement is and will continue to be essential, let me turn now to a brief review of how A.I.D. and its Office of Agriculture are going about the complex job of technical assistance.

First, I would like to outline for you the key areas of work we have selected for major agriculture attention.

- food crop production, including high protein crops;
- development of livestock production in relation to land resources and the need for high quality proteins;
- improved management of tropical lands, soil and water resources;
- improving the economic capability of individual nations;
- and development of markets for agricultural products and improving marketing structures and procedures.

In all of these problem areas, wherever possible, we work through the worldwide network of the six international agricultural research centers. In fact, A.I.D. furnishes one-fourth of the core budget for each of these centers. We feel that each center provides the essential mechanism for mobilizing and retaining competent scientific talent in proper number and with the flexibility of operation required to expedite solutions to the problems for which they were chartered to solve.

Since many of you perhaps have had no occasion to deal with these centers, let me take a few moments to tell you about the ones concerned with livestock and how A.I.D. views their very important role.

The International Center for Tropical Agriculture (CIAT) with headquarters in Cali, Colombia has an extensive cattle and swine program aimed at increasing production in the lowlands of Latin America.

Feeding practices have been worked out for swine based on waste bananas, cassava, rice bran and polishings, sugar or molasses and the high-lysine high-protein Opaque-2 maize. The likely relevance of Opaque-2 lines with vitreous kernels makes this avenue of swine development most promising and suggests a real breakthrough in swine production, including the small producer. With high protein costs, the results of this work may well find application in the United States.

Two new international research centers are now being formed for expected initiation of operations early next year in Africa. These centers are: (1) "International Laboratory for Research on Animal Disease"
(ILRAD) to be located at Kebete, Kenya, and (2) "International Livestock Center for Africa" (ILCA) to be located at Addis Ababa, Ethiopia.

ILRAD's creation recognizes that active support of the Governments concerned is necessary in order to attract top scientists and to obtain the highly sophisticated and expensive facilities required for modern-day disease research on animals. Of course, a basic premise of this Center is that it will strengthen and enhance the effectiveness of existing national and regional research programs.

Because much of the information collected at African research stations has never been published, ILCA in Addis Ababa, will attempt the retrieval, storage and classification of this information, in close cooperation with related national, regional and international organizations. Research activities of the Center will concentrate on techniques of rangeland management, livestock production, disease control and marketing. Other areas for investigation include the improvement of reproductive performance, reduction of calfhood mortality, dry season nutrition and parasite control; all of which have international significance.

As mentioned earlier, in addition to supporting the international research centers, our efforts include financial support of centrally funded agricultural projects which have wide application.

One of these projects, "Research on Hemoprotozoal Diseases of Food-Producing Livestock", was started in 1968 under a contract with Texas A&M University. The diseases being studied are those caused by blood parasites transmitted by biting insects or ticks. Purpose of the project is to find effective immunization methods and/or means for controlling the infection process before the onset of debility.

In addition to this research, centered in Colombia, Texas A&M is also training host country scientists in research methodology and helping them to organize problem-oriented research.

Advisory services have been provided also to Peru, Ecuador, Guatemala, and Panama. Exchange of information on research activities has been carried on with technicians in East Africa and Australia. Workshops and seminars are scheduled for several Central American and South American countries.

This project, while directed primarily toward problems of South America, contains many features which are distinctly applicable to U.S. disease problems. Because *Anaplasmosis* is a serious problem in the United States, there is great interest in the development of a therapeutic approach which will eliminate *Anaplasma* infections in carrier animals. The approach of test and treatment currently under consideration will utilize such information as a basis for the U.S. eradication program.

A second aspect of these studies is the development of preimmunization techniques to prevent *Anaplasmosis* and *Babesiosis*. These same procedures would be useful and a necessary prerequisite for cattle being exported from the U.S. to any endemic area of South or Central America.

The importance of this aspect is widely recognized by American cat-
tlemen interested in shipping cattle overseas. In fact, we have had numerous requests for Texas A&M to provide information and assistance in preimmunizing cattle being shipped to infected areas.

Death losses as high as 70-80 percent are common in adult cattle shipped from the U.S. to some zones. Even though a keen demand exists for American breeding stock, a marked reluctance has developed among importers in Latin American countries to the buying of American cattle.

Another centrally funded research project of widespread interest deals with "Research on the Sterility Method of Tsetse Fly Control". Work on this project began in 1963 under an A.I.D. contract with the U.S. Department of Agriculture. Due to the transmission of diseases to man and animals, tsetse fly infestation hampers utilization of over four million square miles of highly productive agricultural land in Africa. The first phase of this project demonstrated that the tsetse fly can be eliminated, or at least controlled, through male sterilization, by adaptation of the technique used so successfully by USDA in control of the screw worm.

The on-going phase of this research envisages the release of sterilized males on about 200 square miles of infested land to determine if such releases will permanently eradicate the natural fly population and to compare the cost with other methods of fly control.

Activities will be tied in with those of the new International Laboratory for Research on Animal Diseases (ILRAD) in Kenya.

A third member of the centrally funded livestock research projects, "Control of Vertebrate Pests—Rats, Vampire Bats and Noxious Birds", was started in 1967 under an A.I.D. contract with the U.S. Department of Interior at its Denver Wildlife Research Center. This project is aimed at finding safe pest control methods which will be effective, economical and adaptable to the social and economic conditions of developing countries.

Control measures developed thus far at Denver and field-tested in Mexico and Brazil, involve a blood anticoagulant (diphenadione), used for human heart patients. A recent breakthrough in vampire bat control has resulted in a high percentage reduction in the pest population and in rabies without danger to desirable species or the environment. As a result, a large-scale bat control program is being initiated in Bolivia and Brazil. Other countries in Latin America, where the vampire bat is indigenous, have requested assistance.

The Denver Wildlife Center has conducted workshops in four countries which have provided a well-trained corps of technicians for control programs.

A four-year project with the University of Florida on "Survey and Analysis of the Problem of Cattle Feeding Systems and Nutrition in the Wet/Dry Tropics of Latin America" is terminating at the end of this year.

The purpose of this project was to identify the nutritive values of a wide range of feed and forage plants in order to establish a data base for improving animal nutrition. Several thousand tropical feed and forage species have been identified and their nutritive values systematically determined and recorded. Sufficient data are now assembled to enable the University of Florida to recommend experimental trials in specific
feeding regimes.

Another example is a cooperative project on foot and mouth disease control in Argentina. Following a March 1962 Argentine-U.S. Joint Conference which led to the formation of the Argentine-U.S. Joint commission of the National Academy of Sciences to handle U.S. participation in the joint program, to be supported by A.I.D., in cooperation with USDA and the Pan American Health Organization. A.I.D. has continued to support this work by periodic renewal of the basic contract.

The original mission of the Argentine-U.S. Joint Commission was not only to aid Argentina in its FMD control program, but to help the Argentines develop methods of processing cooked meat products which could meet U.S. requirements for importation from countries having Foot and Mouth Disease.

An early accomplishment (1962-63) was to recommend development of a cooked meat roll which could be frozen for export without fear of transmitting FMD. This product meets USDA requirements and reports from Argentina indicate that recent exports of this product to the U.S. exceed the volume of salt-cured products formerly exported to the U.S.

Emphasis is now being placed on a continuation of the USDA-NAS-AID Argentina-Pan American Health Organization FMD Center (PAHOOFM-DC) Vaccine Evaluation Project, which was started in 1969. This project in Argentina is concerned with a comparison of vaccines prepared by the USDA's Plum Island Animal Disease Laboratory (PIADL) with those already in use in South America. Two objectives of these trials are: (1) to develop a vaccine which is more immunologically effective than the ones now in use, and (2) to increase the period of immunity in vaccinated animals.

According to an agreement reached in 1970 by the Argentine-U.S. Joint Commission, the Joint Commission will be terminated in July 1974.

Other foreign animal diseases, often referred to as exotic diseases, are a constant danger to the U.S. livestock industry. The increasingly serious threat of introducing these diseases may be attributed to such factors as the increasing volume of modern airborne international trade to satisfy the demand for animal protein; the high degree of susceptibility of U.S. livestock; the unawareness of the average international traveler; the movement of animal products for biological or industrial use; migratory animals and birds; and the international movement and transport of infected insect vectors. The threat is also more serious to the U.S. now than in past years because of the increasing concentrations of susceptible livestock and poultry and the continuous national flow of animals through sales yards, markets, show rings, and race tracks by train, truck, and air such that undetected disease can be widely spread in a matter of hours and prior to suspicion or clinical appearance and diagnosis.

211(d) LIVESTOCK

A.I.D. has a small grant program known as 211 (d) grants. Four grants in the livestock area have been given to the following U.S. Universities:

- Texas A&M to expand its competence in the Design and Execution of Livestock Development Programs in the Tropics Emphasizing
Ruminant Livestock Production Systems through Improved Breeding and Disease Control.

- University of Florida to expand its competence in Ruminant Livestock Development Programs for the Tropics: with special emphasis on production and nutritive evaluation of forages.

- Purdue University to expand its competence in the Design and Execution of Ruminant Livestock Development Programs for the Tropics: with emphasis on the analysis of systems of production and marketing.

- Tuskegee Institute to expand its competence in the Design and execution of Ruminant Livestock Development Programs for the Tropics: with emphasis on design and organization of systems to integrate technological information and disseminate it along with needed physical inputs.

All working together these four Universities provide a great U.S. resource to A.I.D. and the developing countries having serious livestock problems.

It is gratifying that we have professional workers like the members of this Association who will continue to take the lead in designing research and action programs to eliminate the costly animal diseases.

TECHNICAL ASSISTANCE IN LIVESTOCK

Another important category of assistance to developing countries is that of providing direct technical help on livestock problems, upon requests from developing countries, or from a regional or country office of A.I.D. Such requests may vary from asking for help in developing country livestock programs, to making surveys for suggested livestock activities, to helping solve animal disease problems, to assisting in developing range management and livestock production recommendations, as is currently underway for the six drought stricken countries in West Africa.

Livestock activities of A.I.D., however, are only one component of the efforts utilized to increase both the quality and quantity of food available to a hungry world. Many of A.I.D.'s other activities are similar to those just described for animal agriculture, and fall into the same categories of international centers, centrally funded projects and technical assistance.

SUMMARY

The overall direction of U.S. assistance is toward a highly focused program dedicated to doing a better job of applying the scientific and technological resources of the world to the major human problems in the developing countries.

As a great nation, we have no alternative but to continue, in cooperation with other developed nations, a unified effort to help those countries in which the leaders sincerely want to achieve better health and living conditions for their people.

That is what Development Assistance is all about. That is what the Agency for International Development was created to do. Be assured that our livestock and animal health programs are making a most significant contribution toward that end.
Disorders produced by chemicals (poisonings) are an important portion of the total disease problem in farm animals. Since animals are almost entirely dependent upon man for providing the essentials of proper nutrition, housing, and their total environment, diseases produced by chemicals appear to be even more important in veterinary medicine than in human medicine.

Not only is the relative incidence of diseases more variable in domestic animals than in man, but farm animals are different beasts than man. Their anatomical differences are obvious. Coupled with differences in structure are physiological differences in organ size and function, and in capacity and limitations of tissue activity. Differences are also found when the biochemistry of individual tissues and animals are examined. Enzyme capabilities in the digestive tracts, metabolism and biotransformation systems in the liver, and transport and other enzymatic processes throughout the body differ markedly from species to species and between domestic animals and man. Indeed, the occurrence of these variations is the basis for the entire group of comparative sciences.

Of equal or more disease-producing significance than anatomical, physiological, and biochemical differences are the effects of domestication and the resulting characteristics that our animal population have developed through years of close affiliation with man. Domestic animals have essentially become creatures of habit and have maintained the specific feeding, grazing, watering, and daily routine habits that man has imposed. Irregularities in diet and routine produced by mismanagement leave the animals with little alternative. Due to their complete domestication, animals have become entirely dependent upon man for their total care and well-being. They thus have become subject to man's whims, his attempts at efficiency or "cutting corners", and also to his stupidity and errors. The domestic animal is forced to feed and be housed where man wills. Feed, water, and therapeutic or prophylactic application of chemicals make animals susceptible to errors in dosages, feed ingredients, faulty applications of chemicals, and general mismanagement, with the potential for toxicity always present.

With such a wide variety of potential sources of errors due to animals' complete lack of independence and total acceptance of their environment, it is common to find chemical poisonings occurring under wide circumstances. The epidemiological pattern of animal poisonings is closely related to man's ability to properly manage his livestock. The following

Presented at the Seventy-Seventh Annual Meeting of the United States Animal Health Association, October 16, 1973 in St. Louis, Missouri.
are a listing of common farm animal toxicities by species and discussions of several types of livestock poisonings that reflect the variety and importance of this area to the veterinary practitioner.

**TOXICOSES COMMONLY OBSERVED IN FARM ANIMALS**

**Horses.** This species is usually well protected by man from foreign chemicals. Toxicoses in horses usually develop from naturally-occurring toxins, unintentional lapses of judgement, or inappropriate or overzealous drug therapy by trainers or owners. The following is a summary of the more common poisonings observed:

- Poisonous plants—hepatic cirrhosis, gastroenteritis, neurological disorders producing icterus, cyanide-containing plants;
- Insecticides and rodenticides—organophosphorus compounds, strychnine;
- Drug reactions—anthelmintics, tranquilizers, analgesics;
- Snake bite.

**Cattle.** The variable husbandry practices associated with different geographic locations and types of cattle result in variation in the common observed poisonings. In general, dairy cattle are more closely confined and exhibit insecticide or heavy metal intoxication most frequently. Beef or range cattle often encounter poisonous plants or dietary contaminants as the agents responsible for intoxications. Following is a summary of the most common toxicoses observed in cattle:

- Heavy metals—led, arsenic, mercury, fluorine, molybdenum;
- Insecticides and herbicides;
- Nitrate—nitrite, oil, and salt contamination of diet;
- Poisonous plants—braken fern, equisetum, sweet clover, ergot, mycotoxins, fescue, cyanide and selenium-containing plants, oak, algae, thiaminases.

**Sheep and Goats.** Since sheep and goats range widely over land that is otherwise often unusable, poisonous plants are a major part of the factors producing toxicosis in these species. The list below summarizes the commonly-occurring poisonings:

- Poisonous plants—photosensitizers, cyanogenic, selenium-containing, oxalate-containing; lupine, death camas, locoweed, larkspur, cicuta, conium, cocklebur, vetches, and laurels, white snake-root;
- Insecticides and anthelmintics;
- Heavy metals—copper, thallium, arsenic, lead;
- Nitrate, sulfur, fluorine, salt, sodium chlorate.

**Swine.** The close confinement of most pigs and the concentrated feeding schedule results in dietary toxins producing most of the poisonings. As shown in the following summary, heavy metal and insecticide poisonings are also frequent:

- Salt, coal-tar and petroleum products, nitrate-nitrite, wood preservations;
- Heavy metals—mercury, copper, arsenic, lead, zinc, thallium;
- Poisonous plants—mycotoxins, cocklebur, pigweed and nightshade,
conium, cicuta, crotalaria, buttercup, photosensitizers;  
Insecticides and herbicides.

_Poultry._ The short life span of most commercially-raised poultry and 
the wide range of environment for wild birds results in a great variation 
in the poisonings commonly observed in this species. These are listed 
below:

- Insecticides;
- Heavy metals—arsenicals, mercury;
- Poisonous plants—locust, corn cockle, crotalaria, oleander, tobacco, 
nightshade, sprouted potatoes, mycotoxins, algae, botulism;
- Rodenticides;
- Carbon monoxide;
- Drugs in feed and water—salt, sulfonamides, coccidiostats, fungicides.

**FERTILIZERS**

The problem of synthetic materials, such as nitrates and ammonia, and 
also naturally-occurring organic material, such as manure, contributing 
to animal poisonings may seem remote. Unfortunately, synthetic and 
natural fertilizers are an important cause of livestock toxicoses. The 
most obvious situation results from the excessive use of nitrate-
containing fertilizers on crops and the accumulation of high nitrate levels 
in the harvested product. These nitrate concentrations in animal feeds 
can produce acute or chronic nitrate intoxication and widespread 
economic loss. 

Even the application of manure to fields can result in high levels of nitrates developing in plants growing in such areas. Not only do cash crops accumulate toxic levels of nitrate, but certain weeds, such as pigweed (_Amaranthus retroflexus_), are capable of building up high levels of nitrate in their organic matrix when grown on soils containing concentrations of nitrates or nitrate-releasing organic 
matter.

An instance of a vacant feedlot that had grown a lush crop of weeds and 
was used to provide green pasture for cattle illustrates this problem. 
Within a few hours after a large group of feeder cattle were turned into 
the weed-covered feedlot, the owner found 17 cattle dead and numerous 
others in various stages of toxicity. Even though the cattle were prompt-
ly removed from the lot, approximately one-third of the animals died 
from nitrate poisoning. Analysis of the weeds growing in the area 
revealed concentrations of nitrate as high as 4.5%.

A complicating situation is the relationship between adverse growing 
conditions and the accumulation of nitrates in plant materials. Although 
levels of nitrate may be moderate in the soils supporting grain crops (par-
ticularly corn and sorghum) or a variety of weeds, under the influence of 
a drought or the application of plant hormone herbicides, these common-
ly-grown plants may accumulate excessively high and toxic concentra-
tions of nitrate. The wide-scale losses in the middle 1950's due 
to livestock consuming drought-affected corn and sorghum were largely 
due to nitrate toxicity. The application of 2,4-D to weeds frequently per-
mits these plants to develop transient toxic concentrations of nitrate; if 
consumed during this temporary phase, nitrate poisoning may result.
CHEMICAL TOXICITIES IN FARM ANIMALS

NATURAL TOXINS

Toxicoses due to plants are not usual in man, but livestock poisonings due to a variety of poisonous plants are a significant part of veterinary toxicology. The basic factor underlying most plant poisoning problems is mismanagement. Livestock are frequently allowed to graze wide areas of natural range land. When weather conditions are bad, pastures may provide less forage than expected and limited plant material for animal consumption and overgrazing frequently results. Under conditions of repeated overgrazing, naturally-occurring desirable pasture grasses die out and weeds, many of them poisonous, take over the range. While such weeds are normally unpalatable, hungry livestock may be forced to consume them and thereby become poisoned. Although most owners recognize overgrazing and the resulting hunger that results in their animals, mismanagement aggravates the loss of native pasture grasses and speeds the introduction and multiplication of poisonous plants. When owners do not provide supplemental feeding for animals on such poor pasture land, the livestock are forced to consume the noxious weeds.

A variety of poisonous plants are available to livestock and household pets and present hazards to their health. The cyanide-producing forages are the most common cause of animal sicknesses due to plants. Usually the sorghums (Johnson grass, sudan grass, milo) are responsible for losses. In addition, arrowgrass (Triglochin spp.), elderberry (Sambucus spp.), wild cherry (Prunus spp.), and the pits of several common fruits (apple, peach, apricot) contain compounds with the potential of releasing cyanide upon ingestion. Toxicities usually result from ignorance on the part of owners who feed such plant materials to their animals or who throw fence-row clippings into pastures to utilize the material for forage. Adverse weather conditions and wilting frequently increase the toxic potential from this group of poisonous plants.

Halogeton (Halogeton glomeratus) and black greasewood (Sarcobatus vermiculatus) are two plants that contain high levels of oxalic acid in their plant matrix. Although these plants are only commonly found in the ranges of mid-western states, the oxalic acid is an extremely potent toxin and upon ingestion combines with serum calcium and magnesium. The clinical situation resulting from the sudden drop in the blood level of calcium and magnesium causes large-scale acute deaths. The loss of thousands of sheep annually in the Rocky Mountain area is directly due to the ingestion of one or both of these poisonous plants. Only recently a loss of several thousand sheep in one herd resulted in the erroneous claim that the release of experimental nerve gas had caused their death. Upon detailed examination it was found that owner-error had resulted in the sheep consuming the oxalate-containing plants and the large-scale deaths. Since the calcium and magnesium oxalate salts formed are excreted in the urine and become crystalized in the kidney tubules, causing an inability to pass urine, animals that survive the acute syndrome commonly develop renal failure.

Locoweeds (Astragalus ssp. and Oxytropis ssp.) are other common and
characteristic causes of livestock losses in the western states. The plants are not usually consumed by livestock but under adverse growing conditions are inadequate natural forage, cattle, sheep, and horses may be forced to consume them. Once livestock taste the plants they may develop a like for their flavor and will then preferentially consume them, even if other feed is supplied. Although horses and ruminants may consume locoweeds, horses are much more sensitive to the toxic principle's effects. Hence, as in one incident when cattle and horses were grazing the same pasture, horses develop locoweed poisoning well before cattle show even the earliest signs of intoxication. The clinical syndrome is one of weight loss and mental derangement. Animals become easily aggravated and undergo bizarre temperament changes. Deaths frequently result from self-inflicted injuries due to running through fences, falling down wells, or drowning in ponds or streams. The toxin has an affinity for the nervous system and characteristic microscopic lesions are detected in the neurons of the brain.

Selenium is a chemical found in certain types of rock and hence specific types of soils. Plants growing on such soils may accumulate levels of selenium in their plant structure varying from only a few ppm to several-10,000 ppm. While any plant growing on soils containing selenium may build up low levels of this chemical, specific plants, such as poison vetch (Astragalus spp.) and woodyaster (Xylorrhiza spp.), have the ability to selectively take up and accumulate massive amounts of selenium. Some of these plants (goldenweed, Oonopsis spp. and princesplume, Stanleya spp., for example) will only grow on soils high in selenium; hence, they are called "indicator plants", since they indicate the fact that selenium is present in the soils on which they are growing. Toxicity due to selenium will produce various clinical signs, depending upon the concentration of the chemical in consumed plants. Low levels of selenium may result in weight loss, deformed hooves and hair loss, and the birth of deformed young. Larger amounts of selenium cause liver damage. A nervous syndrome, similar to that seen with locoism, commonly develops if large amounts of selenium are ingested.

Several other poisonous plants produce such rapid death that owners may report only that the animals were found dead. Waterhemlock (Cicuta spp.) grows in wet areas and contains the highest toxin concentrations in chambers just above the roots. Animals become exposed by crushing the plant in the stream in which it is growing and then consuming the toxin-containing water, or they may actually consume one or more mouthfuls of the plant material. The toxin is so severe that violent muscle spasms may knock the animal off its feet and cause death within minutes. Cattle poisoned by waterhemlock have been found with walnut-size pieces of the plants still in their mouths.

Common oleander (Nerium oleander) is not only responsible for sudden animal losses, but has also resulted in several human fatalities. Only a small amount of the plant material or toxin-containing sap is required to produce poisoning. Cases have developed from individuals consuming meats cooked using oleander branches as spits. The toxin affects cardiac function and produces rapid death in all species of animals, but is most
Cocklebur (Xanthium spp.) is a weed capable of infesting almost any barren vegetation. It appears in the early spring as one of the first forms of green vegetation. Since the plant is so common, it is fortunate that its most potent state is during the early growth period, but unfortunately this is just when other green vegetation is limited. Cattle and hogs may be browsing pastures when early spring rains produce cocklebur sprouting. The resulting sprouts contain high concentrations of hydroquinone, a potent liver toxin capable of producing massive liver necrosis and death within a few hours. The mature cocklebur plant is not palatable to livestock, but the burrs containing the seeds may sprout in the fall following a warm rain. Hence, poisoning may not only occur in the spring, but also in the fall when sprouting cockleburs again provide lush forage for grazing animals.

The Japanese yew (Taxus cuspidata) is an ornamental plant that recently has been shown to produce acute poisonings in animals consuming clippings. The plant is frequently installed around fences and animals may become exposed to clippings or may browse the plant directly through the fence. The toxic principle has not yet been fully defined, but horses and ruminants have been found dead by amazed owners following access to this plant. Even though oleander and yew are attractive plants to beautify landscapes around homes and other structures, their toxicity provide great hazards not only for livestock, but also for children accustomed to placing foreign objects in their mouths.

Some of the lower members of the plant family, the fungi, are capable of producing a variety of toxins. This particular group of poisons, mycotoxins, has become a focal point for scientific investigation during the past decade, and studies on aflatoxins have been especially productive. This latter group of mold poisons is produced by specific strains of the fungi Aspergillus flavus and Penicillium spp. As with all fungi, a source of nourishment and proper amounts of moisture and heat must be present to support growth. Under suitable conditions, these fungi, and others capable of producing toxins, will grow and in their growth processes will produce their toxins. Hence, the toxins are products of fungal growth; the mere presence of the fungus does not necessarily indicate that its particular toxin is also present. Conversely, the toxin can be present and viable fungi may no longer exist in the sample. Mold toxins commonly develop in stored grains and on certain feedstuffs subjected to unusual weathering or storage conditions. Although moldy feed is usually grossly identifiable, the spoiled feed may be mixed into a ration or otherwise offered for livestock consumption through ignorance or by intention. Animals will usually reject extremely spoiled feed, but well-diluted feeds or rations offered hungry cattle may result in acceptance and toxicity.

Most fungal toxins affect the liver and produce lesions varying from frank necrosis to biochemical interference with enzymes or blood-clotting mechanisms. Digestive track disturbances, photosensitization, poor feed utilization, abortions, and reproductive failures have been also
associated with mycotoxin consumption. Unfortunately, only a few of the toxins of potential fungal origin have presently been identified. The experimental production of tumors by aflatoxins in some laboratory animals has resulted in concern over the concentrations of this toxin in grains destined for human consumption. There is no doubt that this interesting area will continue to attract the attention of toxicologists, chemists, and animal scientists for some time to come.

FEED ADDITIVES

The addition of chemicals to livestock rations for the purpose of increasing feed efficiency and reducing disease is a characteristic unique to animal production. Although this practice has greatly benefited the livestock economy, it is not without danger. Whenever foreign compounds are added to feeds, the possibility of error and resulting animal or human hazard increases. While the presence of chemical residues in human foods and their potential contribution to adverse effects in man is of most concern to public health officials, animals directly consuming feeds containing feed additives are also likely to become grossly toxic. This may be due to improper mixing of the ration, incomplete following of feeding recommendations, faulty husbandry, or mismanagement. In these instances, acute poisonings result from livestock consuming feeds to which they are unaccustomed or because the feed contains unusually high levels of one or more toxic materials. Two such common poisonings are those produced by excessive or improper feeding of urea or salt.

The factors associated with the development of urea poisoning in cattle are listed in Table 1. Urea is offered ruminant animals to provide an economical source of protein to the consuming individuals. The production of ammonia from the urea by the ruminal microorganisms is normally followed by incorporation of the ammonia into bacterial protein;

<table>
<thead>
<tr>
<th>Mismanagement and the Development of Urea Poisoning</th>
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<tbody>
<tr>
<td>Only roughage fed before urea offered</td>
</tr>
<tr>
<td>No previous urea fed</td>
</tr>
<tr>
<td>Switch to high urea ration suddenly</td>
</tr>
<tr>
<td>&quot;Bully cattle&quot; hog feed</td>
</tr>
<tr>
<td>Cattle unusually hungry, overeat</td>
</tr>
<tr>
<td>Feed instructions not followed</td>
</tr>
<tr>
<td>Accidentally fed wrong mixture</td>
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<tr>
<td>Improper or incomplete feed mixing</td>
</tr>
</tbody>
</table>
CHEMICAL TOXICITIES IN FARM ANIMALS

this protein is digested in the intestinal tract of the ruminant and serves as a source of nutrient protein. Under conditions of mismanagement, the production of ammonia becomes excessive and the rumen microorganisms are unable to utilize the ammonia in its entirety. Ammonia (i.e., urea) poisoning then results, with the onset of clinical signs within minutes and death often following in 1-2 hours.¹,²,⁴,¹¹,¹₂,¹₈

Salt is a necessary dietary ingredient, but in excess or in the absence of sufficient fresh water it may become toxic.⁴,¹⁷ Toxicity occurs most commonly in swine, but also occasionally in cattle, due to a variety of management factors leading to the accumulation of sodium ion in the central nervous system. If the owner then discovers that water was unavailable to the animals for a period of time, and then provides unlimited access to water, the osmotic pressure produced by the sodium results in increased central nervous system pressure and an acute neurological and convulsive syndrome.¹⁸ While the condition in cattle is somewhat more chronic and digestive signs are more prominent, the underlying cause of salt poisoning is human error resulting in poor animal husbandry practices.

AGRICULTURAL CHEMICALS

This group of compounds includes insecticides, herbicides, and fungicides; the latter two have the least potential for toxicity.¹⁹ The newer herbicides and fungicides are in general relatively safe, and only the use of some of the older more toxic chemicals or exposure to organic solvents used to carry the chemicals in their application are likely to produce poisoning.

The insecticides are a much more complicated and diverse group of foreign compounds. As causes of animal poisonings, the chlorinated hydrocarbons and the organophosphorus and carbamate materials are hazardous. Fortunately, effective treatments are available for the organophosphorus and carbamate compounds; the chlorinated hydrocarbons are less effectively treated. The widespread use of insecticides around farm animals results in considerable hazard to these species. Poisoning usually occurs from accidental exposure via spray drift or due to the intentional application of the insecticide to control livestock insects. Toxicity may result from improper dilution of the concentrated material or too frequent application of acceptable amounts. Both problems are diminished by users properly reading the labels of the respective products and abiding closely by the specified recommendations.

While direct toxicity to the exposed animals is a foremost concern, many of the agricultural chemicals are capable of accumulating in feed or food supplies following their application to the environment. Hence, the widespread use of insecticides to animals on pasture may result in hay or feedstuffs growing on neighboring fields developing residues of these foreign chemicals. Likewise, if proper precautions in marketing the exposed cattle are not followed, the meat and by-products from such animals may contain high residues of the applied chemicals. Such matters are of vital interest to governmental regulatory agencies and provide a
significant concern in their efforts to protect not only animal, but also human health.\textsuperscript{10}

The government's concern with the accumulation of foreign compounds in not only foods, but also the general environment, has resulted in the consideration of restrictions on the use of many of the insecticides currently in use. Foremost in such action was the recent banning of DDT from routine usage in the United States. This has been followed by other chlorinated hydrocarbons being examined as a preliminary for similar restrictions. Some of the heavy metal materials used as herbicides and fungicides are being re-evaluated by regulatory agencies because of their hazard and adverse environmental impact. Whether they will indeed be banned from use or only restrictions placed on their applications remains to be seen.

**HEAVY METAL TOXICANTS**

Among the variety of heavy metal chemicals potentially toxic to farm animals, lead is one of the most frequent causes of poisoning.\textsuperscript{11} It is the most common toxicant in cattle and is available through the ingestion of paint, lead solder or battery terminals, building materials, and various lead-containing sprays. The poisoning is usually acute and neurological in effect. Blindness, incoordination, and severe convulsive seizures are observed in effected animals. As cattle become older, the lead toxicity is usually expressed as a chronic syndrome with the convulsive pattern less pronounced. Although acute cases are treated successfully with chelating agents (calcium EDTA), the identification of a lead source for the clinical outbreak is a valuable diagnostic aid for the puzzled veterinarian. Adult cattle with lead poisoning respond well to removal from the source, good nutrition, and nursing care.

Arsenic is a less-frequent intoxication, but very dramatic when observed. It results from the topical application or oral absorption of arsenic-containing insecticides or orchard sprays. Severe gastrointestinal irritation results with a bloody diarrhea common. Deaths are acute. Treatment in the early stages with BAL (2 mg/lb.) every 4 hours and the application of digestive tract protectants is useful. An organic form of arsenic, arsenilic acid, is used as a feed additive for swine. Toxicity results if feed levels greater than 100 ppm are fed, and incoordination with posterior ataxia leading to paresis is seen. Interestingly, appetite and alertness of the affected animals is normal. Removal of the swine from the feed source results in progressive recovery.

The addition of copper to feed rations and supplements has also contributed to poisonings in sheep and swine. Because of the intentional inclusion of copper, but the omission of molybdenum, the resulting biological imbalance produces a characteristic toxicity.\textsuperscript{12} While the ingestion of copper (and even the sporadic worming with copper sulfate in sheep) leads to chronic accumulation of copper in the system, the signs of poisoning are acute and lead to death 36-72 hours after onset. Icterus and hemoglobinuria may be observed, but usually the animals are "just found dead." The necropsy examination is characteristic—generalized icterus,
dark metallic-colored kidneys, a swollen friable liver, and a bladder with brown-black urine. The blood is watery and often fails to clot normally. Treatment is to correct the copper-molybdenum dietary ratio by spraying 100 mg. ammonium molybdate and 1 G. sodium sulfate/head/day on the feed, adding 1 lb. sodium molybdate to every 200 lb. of salt, or applying 4 ounces of molybdenum superphosphate/acre to the pastures.

Fortunately, mercury poisoning due to fungicide-treated seed fed swine is now uncommon. Occasionally, however, old mercury-treated seed may be included in animal feeds and can then produce toxicity. Mercury is a potent nephrotoxin and produces nephrosis and eventually uremia. At necropsy the firm shrunken kidneys are obvious. Although the Reinsch test may be used for laboratory confirmation of the presence of mercury in the feed, treatment is not of benefit due to the degree of damage present when a diagnosis is usually made.

An occasional, but when observed, destructive intoxication is that produced by the chronic ingestion of fluoride salts. Most frequently this results from industrial effluents deposited on pastures and consumed with the forage. The condition is chronic and characteristically produces bone, teeth, and hoof abnormalities. Calves may be born with teeth or bone lesions if the mothers were exposed to fluorides during pregnancy. Severe cases of fluorosis will also exhibit a chronic diarrhea difficult to differentiate from Johne's disease. No effective treatment is available, but the addition of aluminum to the diet will reduce the absorption of fluoride from the digestive tract.

**PETROLEUM PRODUCTS**

A variety of oils, greases, benzenes, hydrocarbons, and other petroleum products are used on and around domestic animals. Some are employed directly on machinery to which livestock have access; several are utilized as solvents for sprays and materials applied to animals; others are formulated for application to buildings, and animals lick or otherwise contact the applied product; or animals may directly consume the petroleum products by gaining access to storage areas housing opened containers of these materials. In certain areas of the United States, oil wells and oil storage tanks provide the potential for cattle consuming the crude petroleum product. Proper care and precautions are frequently not taken to assure that animals are protected from exposure. In complete fencings of crude oil holdings or storage areas may permit inquisitive cattle to satisfy their curiosity. Ignorance of the potential toxicity of these products leads owners to utilize various petroleums directly on livestock as therapeutic aids.

Petroleum products produce a characteristic sequence of clinical signs. If applied to the skin, irritation and thickening commonly results. Photosensitization is frequent, especially in white-haired or light-skinned individuals. If consumed by mouth, the petroleum material produces digestive disturbances, may be inhaled with resulting pneumonia, and after several days can produce liver, kidney, and bone marrow dysfunction. Pregnant animals may abort and a poor-doing individual, continual-
ly losing weight and eventually dying, is a usual outcome. Poisoning due to petroleum products is a complicated and varied intoxication. Its occurrence could largely be prevented by owner education and by assuring that proper precautions were taken to prevent animal access to these materials.

DRUGS AND OTHER THERAPEUTIC AGENTS

Errors in the choice of a therapeutic compound, in its route or mechanism of application, and in its dosage are common lapses of judgment that result in poisoning. The faulty selection of a worming preparation, the administration of a new chemical by an other-than-recommended route of administration, and the overdosage of an anesthetic are dangerous and frequently fatal errors. The mistaking of propylene glycol for mineral oil and the administration of 1 gallon of the wrong material to horses has resulted in a characteristic toxic reaction. Not only are such errors subject to frank poisoning, but adverse reactions and a multitude of possible acute and chronic variations may also occur.

The oral and topical application of anthelmintics and insecticides can produce toxicity in farm animals. In such instances, overdosing, misuse, or administration to debilitated animals are the usual causative factors. Careful reading of the product-container label and following of instructions is important. The label also contains a listing of contraindications and suggested therapy. In an emergency, the manufacturer will often be able to provide the most recently available therapeutic information.

Antibiotics, hormones, trace elements and minerals, vitamins, and parasiticides are added to rations to increase feed efficiency and/or animal health. Only rarely are these compounds toxicity problems to consuming livestock if recommended levels are present in the ration. These chemicals, however, are prominent regulatory concerns if their use results in residues in animal products intended for human consumption.

DRUG INTERACTIONS

The interaction of several foreign chemicals in a biological system is a fascinating new area of study. Many mechanisms are postulated for these potential efforts. The process(es) may produce toxicity by increasing the biological effect of a certain chemical or may produce a lower than expected effect by interfering with drug action. Several drugs may be present that simultaneously affect the same target organ, such as a combination of anthelmintics and anesthetic agents. The combination of organophosphate insecticides and phenothiazine compounds may result in decreased enzyme activity. Barbiturates and chlorinated hydrocarbons can produce increased enzyme activity and greatly alter the expected "normal" response. A recognition of the role age and species differences play in affecting biological functions and activities is important when attempting to predict the potential for these chemical interactions.
PROBLEMS OF CHEMICAL RESIDUES

Because of the Food and Drug Administration standards for food purity, foreign chemicals are not permitted in animal and human foods in excess of guideline or tolerance levels. Residues have been a common problem in past years with the chlorinated hydrocarbon insecticides and has led to the drastic reduction in their use on and around animals intended to human food production. In general, insecticides should not be used on meat-producing animals within 60 days of slaughter and should not be used at all on milk-producing animals. Obvious exceptions to this exist.

Chemical residues in foods are a hazard due to potential sensitivity reactions, the establishment and transfer of bacterial resistance, cancer-producing potential, effects upon the fetus, toxicity in more sensitive individuals, and various subtle adverse effects in a portion of the population. Residues may also affect the biological response to other foreign chemicals by participating in chemical interactions and, among other mechanisms, contribute to enzyme induction or inhibition.

ENVIRONMENTAL CHEMICALS

The occurrence of air, water, industrial, and other pollutions are just as much animal hazards as they are for humans. A good correlation may be observed between animal and human toxicity problems caused by chemicals polluting the environment; animal epidemiology is frequently identical to that observed for humans.

Air pollution usually results from industrial fumes released to the atmosphere, and animals in the vicinity are exposed to sulfur and nitrogen oxides, heavy metals such as zinc and lead, hydrocarbons, and various forms of particulate matter. Since most industrial plants are located in suburban and rural areas, livestock grazing surrounding pastures are increasingly likely to assume body burdens of these chemicals or to exhibit biological responses to their inhalation. Other common hazardous gases and air contaminants are hydrogen sulfide, carbon monoxide, and fluorides.

Water pollution is a special problem for rural areas utilizing streams and wells as municipal water sources. This is in contrast to larger cities that utilize upland reservoirs many miles distant from the consuming population. The sewage discharge of upstream communities and industrial complexes and agricultural enterprises (feedlots, fertilization) may result in a variety of toxic materials being present in the water used by a downstream stockman or community for drinking purposes. The same waters may enter wells supplying other farmsteads or communities.

Recent interest in nitrate concentrations in water supplies have resulted in speculation as to the potential hazard of the continuous ingestion of low-level nitrate waters. While the influence of nitrates on animal and human health has only partially been defined, there is little doubt that other foreign chemicals present in water supplies due to industrial and agricultural pollution are indeed capable of producing sig-
significant toxicity. These chemicals include arsenic, mercury, petroleum products, salt, insecticides, herbicides, and fungicides.

Other pollutants can produce toxicities and under circumstances that are frequently unique to the situation, Carbon monoxide is a special problem during winter weather in animals confined in tightly sealed quarters. Tractors or improperly vented heating equipment utilized within such facilities may produce lethal concentrations of this gas. Coal-tar (pitch, clay pigeon), chlorinated naphthalene, and various phenols (creasote) are capable of entering the animal's body and producing poisoning because of their presence in the environment. The severe toxicity of the various rodenticides and their wide use presents a hazard for livestock and companion animals alike because of their contamination of the environment. As with most pollution problems, prevention is a matter of education and regulation, and prophylactic efforts are immeasurably more rewarding than the treatment of poisoned individuals.

CONCLUSIONS

The variety of foreign chemicals capable of producing farm animal toxicities is almost infinite. As long as such potentially toxic materials exist and are utilized, hazards for animals, human and domestic alike, will be a prominent concern of the health community and of toxicologists particularly. Persons responsible for animal care must be aware of the hazards associated with the use of these foreign chemicals. They must be instructed and encouraged to use good judgment in the application of these compounds on and around our animal population. Forceful and continuing education is required to assure that safe and sensible use of toxic materials is practiced. Only if the persons handling and applying these chemicals recognize their responsibility can their use be made relatively safe, not only to the users, but also to their neighbors and to the animals in their care. User error or ignorance is the underlying cause for most farm animal poisonings, and effective education together with the requirement for good common sense is the prime method of reversing the increasing incidence of animal toxicities.

SUMMARY

Farm animals are constantly exposed to a wide variety of foreign chemicals, many of which are potentially toxic and some which result in clinical poisonings. Pesticides are applied on or around animals for the control of insects and rodents. These chemicals may be placed in areas without regard for accessibility to domestic livestock. Insecticides, herbicides, and fungicides are routinely and haphazardly applied to animal and environmental surfaces alike with apparent disregard for differences in absorptive capability. Fortunately, the newer herbicides and fungicides are relatively non-hazardous. Drugs are considered to have therapeutic effects; but the lay and professional person's disregard for species differences and variations in recommended dosages can result in poisonings. Adverse reactions may be misnomers for errors in judgment. Failure to provide satisfactory storage facilities for animal feeds and the
improper preservation and handling of feedstuffs allow the growth and development of a variety of mycotoxins. Ignorance on the part of animal owners and livestockmen can result in a number of unusual and sometimes fatal clinical syndromes. The dependence of domestic animals upon their owners for the total environment makes these animals extremely susceptible to environmental pollutants. Exposure to noxious gases, irritating and hazardous industrial materials and wastes, water contaminants, and casually discarded compounds of man's own use can and frequently do result in animal illnesses and death. Persons responsible for animals may be unaware of the potential hazard or lack good judgment in the use of these chemicals. Forceful and continuing education for the safe and sensible use of all foreign compounds on and around animals is needed. Persons handling and applying these materials must recognize their responsibility, not only to themselves but also to their neighbors and the animals in their care.

REFERENCES

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15. Schmutz, E. M., B. N. Freeman and R. E. Reed: Livestock-


REPORT OF THE COMMITTEE ON PHARMACEUTICALS

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Co-Chairman: Dr. Fred J. Kingma, Washington, D.C.

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Dr. Gail B. Smith, Somerville, N.J.

OTHERS PRESENT
G. Dean Lindsey, Carmel, Ind. (Dr.)
E. S. Brokken, Mexico, Mo. (Dr.)
R. Keith Farrell, Moscow, Idaho (Dr.)
Ray Reiker, Kansas City, Mo. (Dr.)
Grover D. Cloyd, Richmond, Va. (Dr.)
J. E. Fox, Ashland, Ohio (Dr.)
Walter Lewis, Boston, Mass. (Dr.)
J. N. Huff, Denver, Colo. (Dr.)
H. Clyde Wilson, Cedar Rapids, Ia. (Dr.)
R. D. Grant, Ballwin, Mo. (Dr.)
C. D. Van Houweling, Rockwell, Md. (Dr.)

Dr. R. Keith Farrell, Pioneering Research Laboratory, Western Region, Agricultural Research Service, U.S.D.A., Pullman, Washington, reported on his recent studies of use of dichlorvos (DDVP) impregnated flea collars on cats. Dr. Farrell found that laboratory cats (111, cats including controls) exposed to commercial flea collars (2,2-dichlorovinyl dimethyl phosphate [DDVP]) had a 42% incidence of an ataxia-depression syndrome with an 8% mortality. Whole blood cholinesterase was significantly (p<0.01) reduced in all DDVP collared cats. Cervical dermatitis occurred in 74% of DDVP collared cats.

Similarities between the flea-collar ataxia-depression syndrome and signs described for other organic phosphates were discussed.

Dr. Grover Cloyd, A. H. Robins, Co., Richmond, Va. reported that adverse reactions for the flea collars marketed by his firm were of a relatively low order, according to response from pet owners, ranging from 1 per 68,000 collars in 1968 to 1 per 38,000 in 1972. Dr. Cloyd also described the difficulty he encountered in attempting to produce clinical toxicity in cats.

The Committee reviewed a proposal to establish peer group review of products which a drug producer might consider inadequately evaluated by the F & DA. The drug producer at his request would have his NADA
referred to an ad hoc advisory committee composed of knowledgeable representatives from an appropriate livestock producer group, pertinent veterinary practice oriented group, National Academy of Science, Industrial Veterinarians Assn., Bureau of Veterinary Medicine (FDA), and the drug firm. The petitioner would be obligated to pay a fee toward the support of the advisory committee. The Pharmaceuticals Committee supports the concepts of peer review as proposed, with modifications as may be determined by more thorough review.

A proposal to develop new criteria for conducting and interpreting toxicity tests to establish the safety of animal drug residues was favorably received. In general, it permits a stronger input of scientific judgment in determining the safety or hazard related to carcinogens and other potentially toxic substances. The details are included in Appendix A.

The Committee reviewed the recent action of the Drug Enforcement Administration to retain the intensive security requirements of Schedule I drugs for etorphine (M99) and diprenorphine (M55), while reclassifying it as a Schedule II drug. Because of the hardships these security requirements would impose on veterinarians, and because of the absence of an equivalent substitute product for use on large wild animals and zoo specimens, the Committee recommends that the Drug Enforcement Administration apply the less strigent Schedule II security requirements to etorphine and diprenorphine.

The Committee supports and commends the Food and Drug Administration on their expanded program to inform veterinarians of the need to use drugs in strict compliance with other measures intended to minimize persistence of drug residues in animal food products. Information on withdrawal times as applied to cattle, swine, sheep and poultry is available on request from the Bureau of Veterinary Medicine, U.S. Food and Drug Administration, Rockville, Maryland 20852.
## PHARMACEUTICALS

During the past year the following new drug entities have been approved for animal use by the Bureau of Veterinary Medicine, United States Food and Drug Administration:

<table>
<thead>
<tr>
<th>DRUG</th>
<th>RT. OF ADMINISTRATION</th>
<th>SPECIES</th>
<th>CLAIM</th>
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<tbody>
<tr>
<td>Orcotein</td>
<td>Injection</td>
<td>horses</td>
<td>Treatment of Soft tissue inflammation</td>
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<tr>
<td>Carbadox</td>
<td>Oral</td>
<td>swine</td>
<td>Increase in weight gain, improve feed efficiency; control of dysentery, bacterial enteritis and necrotic enteritis</td>
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<tr>
<td>Gentamicin sulfate</td>
<td>Aseptic infusion</td>
<td>horses</td>
<td>Control of bacterial infections; improve conception in mares</td>
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<tr>
<td>Robenidine hydrochloride</td>
<td>Oral</td>
<td>chickens</td>
<td>Prevention of coccidiosis</td>
</tr>
<tr>
<td>Banbermycins</td>
<td>Oral</td>
<td>chickens</td>
<td>Increased weight gain and improved feed efficiency</td>
</tr>
<tr>
<td>Pyrantel tartrate powder</td>
<td>Oral</td>
<td>horses &amp; ponies</td>
<td>Removal and control of infections from parasites &amp; worms</td>
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<td>Levanisole hydrochloride</td>
<td>Oral</td>
<td>cattle</td>
<td>Anthelmintic</td>
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<td>Mebendazole</td>
<td>Oral</td>
<td>horses</td>
<td>Treatment of infections caused by roundworms, strongyles, pinworms</td>
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<tr>
<td>Ipronidazole hydrochloride</td>
<td>Oral</td>
<td>turkeys</td>
<td>Treatment of blackhead</td>
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<td>dogs, cats</td>
<td>X-ray visualization</td>
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<td>Monensin sodium</td>
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<tr>
<td>Diprenorphine hydrochloride &amp; Etorphine Hydrochloride</td>
<td>Injection</td>
<td>wild animals</td>
<td>Immobilization &amp; reversal</td>
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<td>Ticarbodine</td>
<td>Oral</td>
<td>dogs</td>
<td>Removal of roundworms, hookworms, tapeworms</td>
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<tr>
<td>Pralidoxime Chloride</td>
<td>Injection</td>
<td>dogs, cats, horses</td>
<td>Cholinesterase Reactivator (Antidote for organophosphate insecticide poisoning).</td>
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### CONDUCTION AND INTERPRETATION OF TOXICITY TESTS TO ESTABLISH THE SAFETY OF ANIMAL DRUG RESIDUES

#### CONDUCTION

1. Carcinogenicity testing should be required only when there is a valid reason to assume or suggest that the compound may be carcinogenic.
2. Substances which may become components of food should be tested by admixture of the material in the diet of the test animals.
3. Where possible, test species should be selected on the basis of the similarity of their known metabolic pathways with that of man where such information is available or can be obtained approximately.
4. In long-term animal toxicity studies, the maximum dosage level should not exceed the physiological capacity of the animal to metabolize and/or eliminate the substance in a fashion consistent with that observed at lower doses.

5. Where over-dosage produces a plateau in the levels of residues in edible tissues of the target species, this information should be taken into consideration in setting the dose levels for the species used in carcinogenicity studies.

INTERPRETATION

1. Isolated reports of carcinogenicity and other serious toxicities should be viewed with scientific skepticism and judgment withheld until the data are confirmed. Results yielding positive findings by the responsible Agencies should be amenable to duplication in a second laboratory.

2. Positive results obtained in a species in which the metabolism of the chemical is quite dissimilar from man should be considered as inconclusive, and, if negative data are found in a more appropriate species, inconsequential. Where human biological effect information is available or can be obtained in an appropriate manner, this information should be used in assessing the effect of possible residues in human food.

3. A substance should not necessarily be classed as a carcinogen where data are available to indicate that the increase in tumor incidence is simply a reflection of the physiological effect of the compound at doses far in excess of those which might be consumed as residues. Not every increase in tumor incidence is a sufficient basis for classification of a compound as a carcinogen. The total biological information available should be considered in making such a decision.

4. A modified Mantel Bryan statistical approach to evaluate the results of carcinogenicity testing and thus to establish the required sensitivity of the residue assay method was proposed in the Federal Register of July 19, 1973, under “Compounds Used in Food Producing Animals.”

This statistical approach may not always be practicable and the final regulations should be sufficiently flexible to permit the Commissioner to accept alternative approaches to arrive at conservatively acceptable assay methods.

It should be emphasized that these statistical procedures are not intended to establish population exposure levels, since actual residue levels are expected to be less than the sensitivity of the assay methods. Also, in any ultimate evaluation of safety, the benefit-to-risk ratio involved in the increase and improvement of the food supply must be considered in evaluating the proposed use of a particular substance.

5. The Food and Drug Administration should support research designed to improve the capability for assessing the carcinogenic hazard for man on the basis of data obtained in laboratory animals.
A REPORT ON THE EXOTIC NEWCASTLE DISEASE SITUATION IN THE UNITED STATES

R.E. OMOHUNDRO' AND J.W. WALKER²

INTRODUCTION

Exotic Newcastle disease or viscerotropic velogenic Newcastle disease (VVND) was discovered in Southern California in November 1971, and rapidly spread over eight counties in California. Parts of two southwestern Arizona counties were also found infected probably due to another introduction. A national emergency was announced March 14, 1972, and a task force was quickly assembled to eradicate VVND before it spread over the entire United States. The last infected flock found was depopulated July 3, 1973. On August 30, 1973, the last area quarantine in California was removed.

A symposium on VVND was held at a joint session of the United States Animal Health Association and American Association of Veterinary Laboratory Diagnosticians Meeting in 1972. Most of the technical aspects of the eradication program and a status report was published.¹ This paper will summarize damages and costs to date and provide an up-to-date summary of the most important events which have occurred during the past year.

DAMAGES DUE TO EXOTIC NEWCASTLE DISEASE

Table I summarizes the number of flocks and birds depopulated because of VVND. Most of the exposed flocks were backyard flocks near commercial flocks. Chickens from backyard flocks could be found loose beneath the layer cages in some commercial flocks. There were more than 10 times as many birds destroyed in inflected flocks as in exposed flocks.

Table II summarizes the total eradication cost to date. Indemnity costs have been approximately 15 percent higher than operational costs.

ERADICATION ACTIVITIES

The eradication program was based upon recommendations of a group of internationally recognized poultry disease scientists.² The most important activities required to eradicate exotic Newcastle disease can be

¹ Director, Emergency Programs, VS, APHIS, USDA, Federal Center Bldg. #1, Hyattsville, Maryland
² Senior Staff Veterinarian, Poultry Diseases, VS. APHIS, USDA, Hyattsville, Maryland
³ Symposium on VVND, Proceedings of 1972 Annual Meeting, USAHA, Miami, Florida
⁴ Drs. B. S. Pomeroy (Chairman), R. Bankowski, R. Hanson, S. Hitchner, H. Goldstein, L. Grumbles; later Dr. F. Craig was added to the group to contribute from the poultry industry.
summarized in four categories: (1) Quarantine, (2) Disease surveillance, (3) Depopulation, cleaning and disinfecting, and (4) Epidemiological disease prevention.

**Quarantine**—All types of domestic and pet birds were quarantined. An embargo was placed on virtually all avian imports. Movement of birds and hazardous products were stopped within the entire area except for those permitted by the task force. This included commercial and backyard chickens, racing pigeons, turkeys, pet birds, pheasant, quail, and all potentially infected or contaminated products. No hatching eggs were allowed to leave the area and table eggs had to be cleaned and sanitized before they could leave. No chicken manure could be moved without permit. All quarantines were actively enforced at well-marked check stations and with unmarked roving patrols. As the size of the area under quarantine was decreased, permit inspection and roving patrols became the method of enforcement. Birds were inspected before they were given permits to move to slaughter.

**Disease Diagnosis and Surveillance**—During the early stages of the outbreak reporting of sick birds by owners served as the primary method of detection and surveillance. Owners called a regulatory veterinarian or submitted sick birds to the state diagnostic laboratory at San Gabriel. One of the first major campaigns undertaken by the task force was repeated vaccination of all commercial layers, backyard flocks, and other species of birds maintained in confinement against Newcastle disease. After the first round of vaccination, four other rounds of vaccinations were completed but program supported vaccination was restricted to commercial poultry flocks. As soon as protective levels of antibodies developed, the amount of clinical disease was reduced but infected flocks continued to be a source of reinfection. As a result of the decline in clinical symptoms due to high levels of vaccination, disease reporting by owners was reduced. When susceptible birds were moved into infected flocks they soon became infected and in many instances died. It was quickly realized that an Epidemiological Necropsy Surveillance Program (ENSP) and sentinel bird program must be developed to detect inapparent infected flocks. Sentinel birds purchased as specific pathogen free by the task force from SPAFAS were placed in all commercial flocks and in approximately 10 percent of the backyard flocks to detect inapparent infected carriers. The SPAFAS sentinels were placed in commercial flocks at the rate of one sentinel per 1000 flock birds with a minimum of 30 sentinels per flock. Five sentinels were placed in each backyard flock selected. All sentinel birds were inspected weekly and checked for Newcastle antibodies 14 and 30 days after placement.

Even this extensive sentinel bird program did not detect all of the infected flocks. Apparently some flocks of birds were infected but not shedding viruses at the level detectable by sentinel birds during the 30-day period the sentinels were maintained under close surveillance.

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8 Specific Pathogen Free Avian Supply (SPAFAS), Inc., RFD #3, Norwich, Conn.
The final, and perhaps the most effective, disease surveillance system ever developed and implemented in an animal disease eradication program was the Epidemiological Necropsy Surveillance Program (ENSP) or “the dead bird pickup program.” All birds dying during a 24-hour period each week were systematically collected from all commercial poultry ranches in the outbreak area. Flock owners saved all birds found dead during the 24-hour period preceding the scheduled pickup. Attempts were made in the laboratory to isolate exotic Newcastle disease virus (NDV) from each bird, and a selected sampling of these were necropsied. The VVND virus was found in some well-vaccinated flocks before the flock owners or managers suspected any problem. However, in most instances some clinical manifestations and reduced production could be associated with introduction and spread of exotic Newcastle disease even in the best vaccinated flocks.

Laboratory support for the disease investigations and surveillance proved to be extremely important. The California Diagnostic Laboratory at San Gabriel served as the primary laboratory facility and a temporary laboratory was established at March Air Force Base near Riverside, California. From March 14, 1972, until October 1, 1973, a total of 48,237 laboratory submissions consisting of 422,368 specimens were processed.

**Depopulation, Cleaning and Disinfecting**—During the early phase of the eradication program, the number and size of the infected flocks were overwhelming. Contracts were negotiated for these activities conducted on commercial layer flocks, because the volume of work was greater than the available personnel could handle. Task force personnel were utilized for depopulation, cleaning and disinfection activities on premises containing infected or exposed backyard flocks, aviaries, hatcheries, and all other premises except commercial layer flocks.

Early in 1973, when the volume of work decreased, task force personnel were utilized for all depopulation, cleaning and disinfections. Infected birds were destroyed quickly, and the movement of personnel between contaminated and clean premises was better controlled.

**EPIDEMIOLOGICAL DISEASE PREVENTION**

As the incidence of disease was reduced, it became apparent that a disease prevention program was needed to inform poultry owners as to how the disease can be spread and actions which they and the servicing industries must take to reduce disease spread. As a result the epidemiological disease prevention program (EDPP) was initiated. Suggested practices for preventing the spread of poultry diseases were given to poultry ranchers, egg processors, feed suppliers, and service companies. Task force personnel worked directly with the poultry owners and other members of the industry to point out and recommend disease prevention practices.

While this program was prompted by observations made as to the manner in which the VVND virus was spread, the suggested procedures are applicable in the prevention of any diseases that are transmitted by movement of people, poultry and equipment.
DISCUSSION

The cost of eradicating VVND from the southwestern United States was significant in terms of dollars spent and damages to the poultry industry; however, without an excellent eradication program both figures could have been much higher. The outbreak area in southern California alone, was densely populated with many species of birds including 34.6 million layers, 2.1 million broilers, 2 million turkeys, 1.2 million backyard chickens, and other avian industries such as racing pigeons, commercial aviaries, wholesale and retail pet bird outlets. The practical eradication of VVND from this densely populated area without reducing the bird population of the area more than 15 percent was a remarkable achievement. The more than 11 million birds destroyed represent slightly less than 25 percent of the total; however, these were destroyed over an 18 month period and a layer can be in full production 6 months after hatching. Although many individual flock owners, egg processors, feed companies, hatcheries, and other related segments of the industry had individual problems, practical eradication of VVND was achieved without seriously disrupting the poultry industry in the area. Southern California was a table egg surplus area during the entire program.

The exotic form of Newcastle disease introduced into California is present in many other countries, but available records do not indicate this disease has ever been eradicated from any other densely poultry populated area. Perhaps the cost figures, total impact on the industry, and technical developments can be used by other countries to decide if eradication is feasible in their country. Although everyone associated with this effort to eradicate VVND from the continental United States can justifiably be proud of the accomplishments, the disease still exists in Puerto Rico and the threats of reintroductions of the disease from Puerto Rico and many countries throughout the world are constant.

Since the meeting of the USAHA in Miami, Florida, in 1972, in addition to the California report, five cases of VVND have been confirmed in Texas, Tennessee, the USDA Clifton, New Jersey, Quarantine Station and the ASPCA facilities at JFK Airport New York. One of these cases was confirmed on January 23, 1973, in a small backyard flock near the Mexican-U. S. border in Los Ebanos, Hidalgo County, Texas. This flock and exposed flocks consisting of 849 birds were promptly depopulated. Intensive surveillance including the use of sentinel birds was conducted with no evidence of spread. On May 3, 1973, State and Federal quarantines on southwest Hidalgo and southeastern Starr Counties in Texas were released.

On September 19, 1973, VVND was confirmed in the USDA Quarantine Station at Clifton, New Jersey, from fighting chickens imported from Martinique. The infected birds were depopulated and the building was cleaned and disinfected. Shipments into and out of the quarantine station were discontinued. All birds which remained in other facilities at the quarantine station and those which had gone forward were placed under quarantine and intensive surveillance. As of October 10, 1973, all of these have remained negative for VVND.
On September 24, 1973, two other cases of VVND were diagnosed. One case was confirmed in baby chicks from a farm in Clarksville, Tennessee. These chicks were hatched at a Somerset, Kentucky, hatchery from 27,540 hatching eggs imported from Hungary. Of these 27,540 eggs, only 14,633 chicks hatched. All but 6,734 of these chicks were euthanized at the hatchery by the hatchery owner and the remaining chicks were moved to the Clarksville, Tennessee, farm. The 6,734 chicks were moved to a Clarksville, Tennessee grower operation where two other groups of pullets were being raised. The chicks began dying on the second day and eventually were destroyed by the owner. Tissues from these chicks were sent by the C. E. Kord Laboratory to NADL where VVND was found. The hatchery was depopulated and cleaning and disinfection was completed on October 3, 1973. The two groups of pullets were moved to three farms in Kentucky before the VVND diagnosis was made. Those flocks are under State and Federal quarantine and placed under a 90 day period of surveillance. As of October 14, 1973, all remain free of evidence of VVND.

Also on September 24, 1973, VVND was diagnosed in a parrot from a Nashville, Tennessee, pet shop. It was determined that this parrot was purchased from a pet broker in Hayward, California. The remaining birds at the pet shop were destroyed. The premises were cleaned and disinfected and the birds of the pet broker are under surveillance.

On October 9, 1973, exotic Newcastle disease was confirmed in a parrot that originated in Columbia and imported into the United States on September 29, 1973, under the personally-owned pet bird provision. This bird was placed in the ASPCA facility at JFK Airport, New York. It died on September 30, 1973, and was submitted to the laboratory on October 1, 1973.

Possibly exposed birds have at this time been either destroyed or placed under State quarantine and surveillance.

NATIONAL SURVEILLANCE PROGRAM

In an effort aimed at more rapid detection and diagnosis of exotic Newcastle disease to aid in preventing possible dissemination of the infection into domestic poultry populations, a national surveillance program for exotic Newcastle disease was initiated on October 1, 1973. This surveillance program will include participation by poultry disease diagnostic laboratories, poultry breeding companies, integrated poultry operations, and other segments of the poultry industries. The program will make special effort to improve working liaison between State and Federal poultry disease agencies and the poultry and pet bird industries.

Each State is asked to set up and maintain its surveillance program with training and advisory assistance being provided by the USDA regional poultry epidemiologists.

The program will consist of at least one or more contacts monthly between each State's coordinator and key laboratory and industry people.
for rapid detection and diagnosis of any unusual sickness or death patterns in poultry populations and prompt field investigations by a State or Federal diagnostician, if circumstances warrant.

As long as exotic Newcastle disease exists in Puerto Rico, the U. S. poultry population will remain in danger. The best justification for the eradication of the disease from Puerto Rico is that the disease does not exist in any other part of the U. S. and poses a constant threat to all U. S. poultry.

If the effort in California had failed, there would be little justification for an eradication program in Puerto Rico.

After 2 years' experience in an eradication program, we have the scientific knowledge and the experience to deal with the problem wherever it may exist.

### TABLE I

**Depopulations Due to Exotic Newcastle Disease in Southwestern United States—March 14, 1972, to September 8, 1973**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected Flocks Destroyed</td>
<td>371</td>
</tr>
<tr>
<td>Exposed Flocks Destroyed</td>
<td>950</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,321</td>
</tr>
<tr>
<td>Birds Depopulated in Infected Flocks</td>
<td>10,627,400</td>
</tr>
<tr>
<td>Birds Depopulated in Exposed Flocks</td>
<td>950,923</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>11,578,323</td>
</tr>
</tbody>
</table>
TABLE II

Cost of Eradicating Exotic Newcastle Disease from Southwestern United States—March 14, 1972, until September 8, 1973

Federal Funds
A. Indemnity for 11,578,323 Birds in 1,307 Flocks
   1. Indemnity at Depopulation $21,658,324
   2. Supplemental 4,967,324

   $26,625,648

B. Operational Cost
   FY 1972  $10,535,392
   FY 1973  10,382,464
   FY 1974  252,095

   $21,169,951

Total  $47,795,599

In addition, the State of California Operational Cost during this period was approximately $2.5 Million.
INTRODUCTION

The New England Poultry Health Roundtable (NEPHR), a group of poultry disease workers from New England and New York from industry, regulatory agencies, and universities has voted to attempt eradication of infectious laryngotracheitis (ILT) from New England.

ILT, an easy disease to recognize early, does not spread (from a practical standpoint) through the air. It is not transmitted through the egg, and safe vaccines are available for its prevention. Very few foci of infection presently exist in New England. The New England Poultry industry is ready for an ILT eradication program and is anxious to cooperate in the implementation.

DEFINITION

Infectious laryngotracheitis is an acute viral disease of chickens, pheasants, and turkeys characterized by severe dyspnea, coughing, and rales with subsequent high mortality due to suffocation. The incubation period under experimental conditions is 2 to 4 days; under field conditions the incubation period is 10 days with a variation of 8 to 12 days.

TRANSMISSION

Transmission of ILT is primarily by man, fomites, and recovered carriers from an outbreak. Carcasses from birds which have died from the disease are a source of virulent virus for several days and in very cold weather, the virus remains viable for a longer period.

SIGNS AND LESIONS

The most typical attitude of an affected chicken is to extend the head and neck while gasping, called "pump-handle breathing". Birds will shake their heads, choke, cough up clots of blood, exhibit cyanosis, and eventually suffocate. There will be a 10-20% decrease in egg production. The beaks, the walls of the pen, and the submission boxes may be blood stained. Mortality is usually 5-15% but may run to 50%. Chicks (2-6
weeks of age) show gasping, lacrimation, and conjunctivitis. The disease spreads rapidly from bird to bird within a pen, but slowly from pen to pen or floor to floor. The course of the disease within a pen in about 2 weeks.

The trachea will show bleeding, mucus, and a yellow caseous plug at necropsy. Snoeyenbos et al. reported isolates of infectious laryngotracheitis capable of producing pneumonia and airsacculitis.

**DIAGNOSIS**

A clinical diagnosis can be made by a history of a sudden onset with high losses in specific pens, accompanied by rales, pump-handle breathing, and the presence of bloody cores in the tracheas of dead birds. Early in the disease of an individual bird, intranuclear inclusion bodies can be found in the tracheal epithelium. These are pathognomonic for ILT. Virus isolation in the CAM of 10-day embryos, producing white opaque plaques containing intranuclear inclusion bodies, will also confirm the diagnosis. A fluorescent antibody test is available for determining the presence or absence of ILT, infectious bronchitis, and Newcastle disease.

**PROPHYLAXIS**

Prevention of this disease is essential for poultrymen who raise birds in an ILT enzootic area. Two types of commercial vaccines are available. The first type used for many years, is a fully virulent vent vaccine administered on the surface of the cloaca with a wire brush. Not all birds in a pen would become immune by the cloacal method. Usually 5% would encounter the virulent virus via the respiratory route, and become recovered carriers of the disease thereby contributing to the enzootic nature of ILT. This practice has helped the disease establish itself in certain areas of the country.

A second type of ILT vaccine more recently available is the eye-drop product. It is an attenuated live virus vaccine, and does not appear to produce disease from pen to pen. Eye-drop vaccine will spread to unvaccinated birds in the same pen immediately after vaccinating the flock, but it appears that carriers are not produced.

Immunity with both types of vaccine is not as long-lived as once thought. Cellular immunity is involved and when new cells are replaced, immunity wanes. Birds which are vaccinated under 6 weeks of age with the eye-drop vaccine must be revaccinated at about 15 weeks of age to ensure an immunity level necessary to prevent an outbreak. The use of the eye-drop modified live virus ILT vaccine through the drinking water is being tried with some success.

**ERADICATION**

The ease or difficulty of eradication of a poultry disease depends on several major factors: egg transmission; carrier state; rapid or slow spread; safe vaccines available; and present level of infection. ILT is not egg transmitted, does produce carriers, and spreads slowly as it does not
appear to be airborne. We now have a safe vaccine, and the present level of infection in New England is very low.

A subcommittee of NEPHR has suggested the following model for eradication of ILT. This model has been accepted by the members and the state veterinarians concerned. The main points of this model program follow:

There must be recognition and programming for three different categories of birds: commercial flocks, show flocks, and backyard flocks. There is general agreement that for this program to be successful, the state veterinarians must recognize the above categories of birds and adhere to the following basic principles:

1) Identify each and every foci of ILT infection.
2) Inspect and quarantine the premises of all outbreaks.
3) Control bird and people movement to and from each infected premise.
4) Use proper isolation principles and security management on all farms, but especially ILT vaccinated and infected birds.
5) All vaccination must be done on a permit basis with the modified live virus eye-drop vaccine. Vent type vaccine is prohibited in New England.

As each group of birds is somewhat different, the following requirements are stated for each group:

1. Commercial
   a. By permit, inspection, and quarantine, control all bird movement from state to state and identify foci of any infection. Maintain a telephone system and written follow-up of all ILT outbreaks between each state veterinarian’s office. All Roundtable members should be informed of the size and the location of each outbreak.
   b. Use the modified live virus ILT vaccine by permit only where exposure to field virus now exists.
   c. Do not move recently vaccinated pullets for at least 4 weeks.
2. Show Birds.
   a. Promote the use by permit of ILT modified virus vaccine on premises of show birds and booster vaccination just prior to going to poultry shows. It is not felt that carrier birds are produced with this practice. It should prevent show birds from bringing virulent ILT virus back to their residence.
   b. Allow interstate movement of show birds by permit only. Include the ILT vaccination history.
   c. It is recommended that education be used rather than regulation in the disease control of show birds.
3. Backyard Flocks
   a. Promoting total depopulation of field virus infected small flocks under official quarantine and isolation procedures. It is recommended that state compensation be provided for the destruction of these small groups.
ERADICATION OF INFECTIOUS LARYNGOTRACHEITIS

NEPHR will declare an ILT-free state when:
1. No known foci of field virus (active outbreak or carrier flocks) is present in commercial birds.
2. Show birds and backyard flocks are under the control of each state regulatory agency.
3. A cooperative permit-reporting system of interstate movement of flocks and birds is satisfactory to all cooperating state regulatory agencies.

CONCLUSIONS
The members of the New England Poultry Health Roundtable feel very strongly that infectious laryngotracheitis eradication should be accomplished with very little difficulty if complete cooperation is received from the regulatory agencies, the poultry industry, and the diagnosticians.

REFERENCES
Chairman, Dr. W. K. Butterfield

During the past three years the literature has awakened many people in the avian disease field to the prevalence of avian influenza.

Beard and Helfer\(^3\) isolated influenza from turkeys in Oregon in 1970 and 1971 on sites approximately 70 miles apart. A/turkey/Ore/70 produced mortality in mature turkeys and was antigenically similar to A/turkey/Wisc/66 by hemagglutination-inhibition (HI) and to A2/Sing/57 by neuraminidase-inhibition. Egg production declined from 60% to 30%, but no decline in fertility or hatchability rates were noted.

A/turkey/Ore/71 did not produce disease signs or mortality in chicks, but did cause a very low mortality in mature turkeys. Egg production was reduced in turkeys from 65% to 20% during a two week period, returning to 50% within the next two weeks with no problems in hatchability or fertility. Chickens immunized with A/turkey/Ore/71 survived challenge with fowl plague virus.

A/turkey/Ore/71, essentially avirulent has the hemagglutinin antigen of virulent fowl plague virus and the neuraminidase of another virulent virus A/tern/S. Africa/61. Beard and Easterday\(^2\) state that virulence may be the only reasonable criterion of the seriousness of an avian influenza isolate as is true with Newcastle disease virus. The A/turkey/Ore/71 isolate may have potential use as a fowl plague vaccine.

Dr. Bankowski reports that turkey flocks in California have been suffering severely from influenza infections and that the isolate crosses by HI with A/turkey/California/meleagridis/64 and A/turkey/California/AC-3/65. There is some mortality and a marked affect on hatchability and poult yield. A severe hemorrhagic tracheitis is produced and the lungs are literally filled with blood. Turkeys are also found to have hemorrhagic enteritis.

Since June, 1971 several influenza accessions have been received at the Plum Island Animal Disease Center for confirmation. These isolates reacted with F.A.O. prepared sera to A/duck/England/62 and A/chicken/Scotland/59. An isolate from imported exotic birds isolated in California in April, 1972 reacted strongly by HI to antiserum produced against A/mynah/Mass./71. Influenza virus isolations have been made from several species including mynah birds, finches, weavers, owlets, bluebirds, and an oriole. The birds were imported from India, Taiwan, and Thailand.

Dr. Pomeroy reports that turkey flocks in Minnesota were infected with influenza virus in the fall and winter of 1972. After a brief respite, the weather change seems to be triggering the infection again this year.
Seasonal marketing and housing are involved in the disease process also.

Slemons, Johnson, and Malone\textsuperscript{12} isolated influenza viruses from finches held at an importers holding facility that was undergoing a viscerotropic velogenic Newcastle disease outbreak. The birds were imported from Taiwan in July, 1972.

Slemons, Cooper, and Orsborn\textsuperscript{11} isolated and identified 15 type A influenza isolates from 15 cases involving 12 different species of exotic birds from January to July, 1972. All isolates were inhibited by antisera against A/mynah/Mass./71, and all but two were inhibited by A/duck/England/56 antiserum. The isolates were not pathogenic for chickens. Two hemagglutinating isolates were not inhibited by antisera to Newcastle disease virus or type A influenza viruses. All avian species were imported from Thailand or were in contact with birds from Thailand.

Another paper entitled "Type A influenza viruses isolated from wild free-flying ducks in California" by Slemons, Johnson, Orsborn, and Hays\textsuperscript{13} indicates that from October to December, 1972, 41 type A influenza virus isolates were made from free-flying wild ducks, and seven isolations were made from domestic ducks in Southern California. These isolations support the hypothesis that wild birds may play an important role in the dissemination of type A influenza viruses and may provide optimum conditions for genetic interaction of type A influenza viruses.

Winkler, Trainer, and Easterday\textsuperscript{15} determined the prevalence, distribution and types of influenza antibody in several Canada goose populations from 1966-69. Antibody was found in 4.7% of 1401 geese tested by HI with an increase of reactors each year. Antibodies were found against A/turkey/Wisc/66, A/turkey/Wisc/68, A/turkey/Canada/63 and A/turkey/Alberta/69/66.

Slemons and Easterday\textsuperscript{16} attempted to determine the response of different avian species to the same influenza virus (A/turkey/Ontario/7732/66). Factors considered were antibody response, virus shedding, and overt signs of disease. While no signs of disease were observed among pheasants and ducks, Turkeys had a severe fatal disease. One of 19 pigeons and three of 20 quail died. There was a marked antibody response in pheasant and quail, but a poor response in ducks. Virus recovery varied considerably.

In another study these same authors determined responses in the same five species to A/turkey/Wisc/68 that is closely related antigenically to A/turkey/Ontario/7732/66. There were no deaths or signs of disease in any of five species. Antibody levels (HI) were highest in pheasants and turkeys. The difference between species in response to infection with avian influenza viruses should be taken into account in the design and interpretation of studies of the role of avian species in the natural history of influenza. It might certainly alter present influenza classification schemata.

Lang, Tumova, and Schild described an isolate from turkeys in 1967 that produced a marked fall in egg production with 2-3% mortality.
The isolate did not react with the seven known avian hemagglutinin subtypes so proposed an eighth. A/turkey/Ontario/6118/67 would be classed as Hav8Nav4.

With increased incidence, or at least awareness, of influenza isolations and the affects on various avian host species, I feel that we should mention literature from areas of the world other than North America in the possibility that these agents may be introduced by free-flying species.

Higgins and Schild studied three isolates from influenza outbreaks in domestic ducks during 1969 in Hong Kong. A/duck/Hong Kong/46/69 and /120/69 contained hemagglutinin and neuraminidase antigens closely related to A/turkey/Wisc/66 which in turn is closely related to those of human Asian influenza viruses. A/duck/Hong Kong/826/69 contained hemagglutinin and neuraminidase antigens related to those of A/chicken/Scotland/59 and A/tern/S. Africa/61, respectively.

Slepuskin and coworkers in Russia studied the relationship between influenza strains found in man and birds by testing migratory birds from Southeast Asia and China, the regions from which human influenza pandemics seem to originate. Hemagglutination-inhibition activity occurred most frequently to A/turkey/Wisc/66 and A/quail/Italy/65. Reactions with human viruses were negligible, but did occur with equine and porcine antigens.

Russian workers isolated four viruses from chickens and ducks in the USSR and Poland. One chicken strain (A/chicken/USSR/68) was closely related to fowl plague virus and also reacted by HI to A/tern/S. Africa/61 and A/chicken/Scotland/59. Another isolate (A/chicken/USSR/314/67) reacted with A/chicken/Scotland/59 while two duck strains (A/duck/Poland/1891/67 and A/duck/Poland/3362/67) reacted only with antisera to duck influenza isolates. These authors suggest that broad strain variations in the antigenic structure of the surface antigens of influenza virus may account for the one-way and two-way cross reactions detected between the isolate and other members of the chicken and duck type A influenza viruses.

Downie and Laver isolated an influenza virus from a shearwater with a hemagglutinin subtype of Hav6, but the neuraminidase was not related to that of any known virus and represents a new type Nav5. The isolate would be designated A/shearwater/E. Aust./1/72 (Hav6Nav5).

Laver and Webster found that sera collected from some species of shearwaters and terns inhibited the neuraminidase of the Asian/57 (H2N2) strain of human influenza virus.

In reviewing these recent publications we have touched on many problems such as:

1. Increased prevalence or incidence of type A influenza viruses and antibody in domestic and free-flying wild birds, both native and imported.
2. Genetic interactions and isolation of new viral subtypes.
3. The relationship of type A influenza subtypes to different avian species as to the infection and disease manifestations.
4. The relationship of type A avian influenza subtypes to those of human or mammalian origin or hosts.
5. The problem of unidentified hemagglutinating agents, and finally—
6. What can and should be done to protect the poultry industry from these field infections of avian influenza?

REFERENCES

RAPID DIFFERENTIAL DIAGNOSIS OF 
NEWCASTLE DISEASE VIRUS

L. S. Cram

ABSTRACT
Several strains of Newcastle Disease Virus (NDV) have been isolated from chickens in the United States; most strains are relatively harmless and result only in loss of production. Recent outbreaks (March 1972 to present) of a new and virulent strain that produces death in less than 6 days have necessitated a rapid differential test for identification of this particular strain. The plaque assay designed to differentiate velogenic strains of NDV requires 7-10 days for completion. We are developing a rapid differential assay that requires about 2-3 days to complete.

INTRODUCTION
Because only small differences exist among Newcastle Disease Virus (NDV) strains, we are not using immunological techniques to classify the causative agent but are measuring the effect of NDV infection on cellular systems. Highly virulent strains of the virus reportedly produce more cell fusion than do less virulent strains. Quantitation of cell fusion using the microscope is both slow and difficult. Flow microfluorometry (FMF) techniques have the advantage of being able to measure rapidly and quantitatively the extent of cell fusion which results in more DNA peaks than normal in the fluorescence spectrum obtained by FMF measurements. A correlation between degree of cell fusion and virus virulence is being sought.

Our semiannual report for July-December 1972, describing our initial experiments that demonstrated the feasibility of quantitating cell fusion with the LASL flow microfluorometer, is given in Appendix A. A detailed protocol for infecting chick embryo fibroblasts (CEF) and/or human amnion (FL) cells with NDV, dispersing, fluorescent staining, and measuring the degree of cell fusion is included therein.

PROGRESS TO DATE
As outlined previously, additional NDV strains have been obtained and grown up for stocks, and virus titer has been determined by plaque assay. About one dozen well-characterized NDV strains are now available in addition to six field strains that were obtained from Dr. H. A. McDaniels of the U. S. Department of Agriculture, Riverside, California.

CEF or FL cells were infected with a strain of NDV at a multiplicity of infection (MOI) of 100. After 16 hours of incubation, the cells were dispersed and fixed with formalin, and the DNA was stained using an acriflavine-Feulgen technique. Cell fusion induced by five strains of NDV grown on CEF cells is illustrated in Fig. 1, where cell number is plotted on a log scale to permit easy observation of the degree of cell fusion. The DNA distribution of control or noninfected CEF cells (fifth passage level)
is illustrated by the solid line in Fig. 1 and is typical for randomly growing cells. CEF cells in the G1 phase of the life cycle are recorded in channel number 13; cells in the G2 + M peak are a few counts, arising from cell clumps (channel number 40) and tetraploid cells (channel number 54). Curves illustrating the DNA distribution of CEF cells infected with the different strains of NDV show an increased number of cells in S and G2 + M and a considerable number of polyploid or fused cells in channel number 33 and higher. It is clear from Fig. 1 that, of the NDV strains used, Fontanna (the most virulent) produced the greatest amount of cell fusion. The relative degree of fusion of other NDV strains is easily visualized. Preliminary data indicate a good correlation between virus virulence and degree of cell fusion.

To understand the variables involved in making cell fusion measurements, several experiments were conducted to determine the dependence of the degree of cell fusion on parameters such as MOI, time of virus incubation, and cell density at time of virus infection. Figure 2 illustrates the increased number of fused FL cells produced by increasing the MOI (Texas GB strain of NDV) from 10 to 200. Additional measurements were made using an MOI of 500 and 1000, which are not included as they superimpose with the top curve (MOI of 200). These data indicate that, provided a MOI of greater than 50 is used, the exact virus titer is not a critical parameter. The experiment illustrated in Fig. 2 will be reproduced for additional strains of NDV to determine if the phenomenon holds for mesogenic and lentogenic strains of NDV.

Following infection of FL cells with the Texas GB strain of NDV (MOI = 100), cells were incubated for 4, 12, 16, and 20 hours before dispersal and fixation. Figure 3 illustrates the fact that cell fusion reaches a maximum level at approximately 16 hours.

The degree of cell fusion also depends on other factors such as cell density, as illustrated in Fig. 4. FL cells were infected with the Texas GB strain of NDV (MOI of 100) when cell densities between 1.8 x 10⁶ and 7.2 x 10⁶ cells per culture flask were reached. The top curve illustrates the results obtained for two cell densities, 4.8 x 10⁶ and 7.2 x 10⁶ cell per culture flask, indicating that at very high cell densities a maximum level of fusion is reached. Consequently, all experiments utilize confluent cell monolayers.

Figures 5 and 6 illustrate that some strains of NDV induce different levels of cell fusion relative to one another when allowed to fuse different cell lines. For example, Roakin induces slightly more fusion on FL cells than does Fontanna; the reverse is true on CEF cells. This differential sensitivity of a particular virus to cause cell fusion will be very useful in categorizing the virus and in studying its epidemiology.

Six NDV field strains from Riverside, California, currently are being studied using the techniques outlined above to determine their relative capacity to cause cell fusion. The results of this study will be compared to the known history of each strain.

A commercial flow microfluorometer, Cytofluorograph (Bio/Physics
RAPID DIFFERENTIAL DIAGNOSIS

Systems, Inc.), has been purchased and is being modified to perform rapid cell fusion analysis measurements.

SUMMARY

Flow microfluorometry has been used to demonstrate a positive correlation between the capacity of several strains of NDV to cause cell fusion and their virulence as determined in embryos. The relative capacity of a strain of NDV to fuse CEF or FL cells can be determined easily and rapidly using the rapid cell-analysis techniques that the Los Alamos Scientific Laboratory has developed. Three variables (i.e., multiplicity of infection, post-infection incubation time, and monolayer density) have been shown to have minimal effect on the results, provided the following conditions are met: (1) a MOI of greater than 50, (2) 16 hours incubation time, and (3) a confluent cell monolayer. The result is a simple, easily controlled system for determining the virulence of NDV strains.

APPENDIX A

SEMIANNUAL REPORT TO U. S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICES
LASL PROJECT R-081
(USDA PROJECT SYMBOL 382-9113-59513
RAPID DIFFERENTIAL DIAGNOSIS
OF NEWCASTLE DISEASE VIRUS
July-December 1972
Biomedical Research Group
Los Alamos Scientific Laboratory
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Project Manager: D. M. Holm, Ph.D.
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INTRODUCTION

In response to a request for help from the Emergency Disease Task Force of the U. S. Department of Agriculture, an effort is underway to investigate the applicability of the Los Alamos Scientific Laboratory's instrumentation for rapid differential diagnosis of Newcastle Disease Virus (NDV). Outbreak of a velogenic strain of the virus in this country has resulted in the disease being declared a national emergency. A differential diagnosis must be made in the laboratory to distinguish endemic strains of the virus from exotic or velogenic strains. Current diagnostic procedures employ the red/white plaque assay which is difficult to perform in the laboratory and very time-consuming (9-14 days).

Poste et al. (1) have reported that the degree of polyploidy in chick embryo fibroblasts infected with NDV can be correlated with the virulence of the virus. These data arise from manually counting the percent
polykaryocytosis on a microscope slide. A much faster and more accurate way of evaluating polykaryocyte formation is the technique of flow microfluorometry (FMF). Quantitative DNA staining of cell suspensions has been developed and substantiated at Los Alamos using the fluorescent-Feulgen technique. Thus, a rapid and quantitative measurement of polyploidy with the FMF instrument can be made on cells infected with NDV.

PROGRESS TO DATE

A. Instrumentation

Several trips to the California Newcastle operation have been made for recommendations and critical review of the program. Many of our recommendations have been accepted, assisting in effective resolution of the emergency. Specialized instruments were developed and provided, and others are in development. LASL developed and provided the California San Gabriel Laboratory an instrument for plaque counting, reducing to half the time (and thus personnel) required for performing the count. LASL provided and shipped a spectrometer for assessment of CA fluid turbidity in the laboratory at the request of staff at the San Gabriel Laboratory. Several "off-the-shelf" instruments were recommended by LASL, purchased by Emergency Programs, and put into use to varied extent in California.

B. Biological Investigations

A method of infecting and preparing cells for analysis has been developed that yields single cells (no clumps due to poor dispersion) or multiple fused cells in the case of cells infected with NDV. Confluent monolayers of chick embryo fibroblasts (CEF) or human amnion (FL) cells are infected with NDV at a multiplicity of less than 100. After cell fusion has occurred (10-16 hours), the cells are treated as follows:

1. Dispersed with trypsin and fixed in 10% formalin overnight.
2. DNA is stained by the fluorescent-Feulgen technique using acriflavin as the fluorescent dye.
3. Cells are rinsed, and their DNA distribution is measured on the FMF.

Figure 1A illustrates the type of data obtained with FL cells infected with the NJ-Roakin strain of NDV. The DNA distribution for control FL cells is typical for randomly growing mammalian cells, displaying a large number of cells in the G1 phase of the life cycle, followed by a continuum of cells with increasing amounts of DNA (S phase) and a second peak consisting of cells in the G2 and M phases of their life cycle. Beyond the G2 and M peaks are counts consisting of a few clumped cells and some small amount of brightly fluorescent debris which amounts to 1.4% of the total number of cells counted. Infected cells have many more cells in S and in G2 and M; in addition, the presence of polyploid cells shows up as a large increase in number of cells falling in channels 50-100. Distribution above channel 50 results from different combinations of cells in all phases of the life cycle fusing with one another.

Additional strains of NDV have been acquired and are being grown up
RAPID DIFFERENTIAL DIAGNOSIS

for virus stocks. Table IA lists the strains that will be available and their characteristics. Until facilities are completed and biological security is approved, experiments are being designed around the Roakin strain of NDV. As soon as possible, the experiment illustrated in Fig. 1A will be repeated with the NDV strains listed in Table IA. A comparison will then be made between the virulence of the virus and number of polyploid cells (i.e., relative number of cells with greater than G2 and M amount of DNA). Several control experiments will be performed to ensure that the degree of polykaryocytosis is an accurate measure of virulence.

Additional differences exist between the strains of NDV that might be useful diagnostically; one interesting difference is neuraminidase levels in infected embryos. Ideas such as this are being noted in hope of providing a complete assay capability that would cover all possible deficiencies in any one particular test.

Fig. 1. Fluorescence distributions of normal and NDV-infested chick embryo fibroblasts.
Fig. 2. Fluorescence distribution of normal and NDV-infected FL cells at different multiplicities of infection: (---) MOI = 10; (-- --- --) MOI = 50; (---) MOI = 100; and (--- ---) MOI = 200, 500, and 1000.
FL cells infected with Texas GB strain of N.D.V. (2-289)
Fig. 4. Fluorescence distribution of normal and NDV-infected FL cells at concentrations of $1.7 \times 10^6$, $2.8 \times 10^6$, $4.8 \times 10^6$, and $7.2 \times 10^6$ cells per flask.
Fig. 5 Fluorescence distribution of control and NDV-infected chick embryo fibroblasts.

RAPID DIFFERENTIAL DIAGNOSIS
Fig. 6. Fluorescence distribution of control and NDV-infected FL cells.
Figure 1A. Fluorescence distribution of control and NDV-infected FL cells (polyploid).

REFERENCE
TABLE IA
AVAILABILITY OF NEWCASTLE DISEASE VIRUS
AND THEIR CHARACTERISTICS

<table>
<thead>
<tr>
<th>Strain</th>
<th>Classification</th>
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<tr>
<td><strong>University of Wisconsin and USDA, Ames, Iowa</strong></td>
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<tr>
<td>Texas GB</td>
<td>Velogenic-Neurotropic (U.S.)</td>
</tr>
<tr>
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<td>Lentogenic (U.S.)</td>
</tr>
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</table>
REPORT OF THE COMMITTEE ON TRANSMISSIBLE—DISEASES OF POULTRY

Chairman: R. A. Bankowski, Davis, Calif.
Co-Chairman: H. W. Towers, Dover, Del.

ASIATIC NEWCASTLE DISEASE

The committee commends the U.S.D.A. and the California Department of Agriculture on its progress toward eradication of Asiatic New Castle disease from that state.

The Newcastle Task Force working with the California State Department of Agriculture, the U.S. Public Health Services, the various military services and the Task Force Advisory Committee have demonstrated to the poultry industry of this nation that a most complex disease entity can be eradicated. The committee commends the California Poultry Industry for their efforts in cooperating to prevent spread of this exotic disease to other parts of the country.

Dr. Claude Pfow, APHIS Poultry Diseases Staff, Hyattsville, Maryland, gave this committee an up to date report on the national Asiatic New Castle situation. The committee is pleased to hear that only one premise in California remains under quarantine at this time. This quarantine is expected to be lifted by the first of November, 1973. Because of the widespread incidence of Asiatic New Castle Disease in the Commonwealth of Puerto Rico, a quarantine was imposed on January 11, 1972, and still remains in effect.

On August 29, 1973, 25 fighting cocks arrived at the U.S. Department of Agriculture's Clifton Animal Import Center, Clifton, New Jersey, from Martinque. Within the next week, several of the birds sickened and died. On September 11, 1973, Asiatic Newcastle Disease was confirmed at Veterinary Services Diagnostic Laboratory, Ames, Iowa. The rest of the shipment of fighting cocks were destroyed. However, seven lots of birds that were in the quarantine station during the time the fighting cocks were there were released from quarantine. There were no gallinaceous birds in these lots. Nevertheless, all of these lots have been placed under a 90 day State order and from those that can be, cloacal and tracheal swabs will be collected at 30 day intervals and sent to the laboratory for virus isolation attempts.
A more potentially dangerous situation occurred during the month of August when 27,000 hatching eggs from Budapest Hungary, accompanied by a U.S. Import Permit, were received by a hatchery in Kentucky. Severe losses followed the placement of chicks hatched from these eggs on a farm in Tennessee. On September 4, 1973, specimens were forwarded to VSDL, Ames, Iowa, where Asiatic New Castle disease was confirmed. Started pullets that were on the Tennessee farm at the time the infected chicks were dying have been quarantined to the three premises to which they had been shipped. Similarly, the chicks that were hatched at the hatchery during and after the time the eggs and hatched chicks from Hungary were in the hatchery have been placed under quarantine on nine premises in three states. There has been no evidence of disease among any of these birds.

The most recent Asiatic New Castle disease isolation was made on October 9, 1973, from a Columbian parrot which died in the holding facilities at J.F.K. International Airport. There were seven lots of birds classified as exposed. These have either been destroyed or are under surveillance.

A more detailed history of these most recent Asiatic Newcastle disease outbreaks will appear in the association proceedings.

In the light of these most recent developments, the committee recommends strongly that U.S.D.A. facilities at both Miami and Clifton be adapted to an “all in—all out” procedure for the quarantine of the three orders of birds accepted there in order to prevent the chance of possible infection by exposure such as occurred at the Clifton, New Jersey station.

The committee recommends that U.S.D.A. make every effort possible to implement the proposed regulations for the quarantine stations to allow for the legal entry of pet bird importations.

Because certain species of birds are known to exhibit symptoms other than those typical of Asiatic Newcastle disease in chickens, the committee recommends that sentinel birds, and other diagnostic aids be incorporated into the quarantine procedures as added precautions.

In an effort to further stimulate State-Federal cooperative efforts, it is recommended that State officials be notified immediately of the importation of all avian species and their products where there is a risk of introducing exotic diseases.

The committee further recommends the prompt reporting by U.S.D.A. of suspected as well as confirmed cases of exotic diseases to the regulatory officials of the states involved in order that immediate action can be taken on the local level to stop the further movement of infected or exposed birds.

Finally, the committee recommends that U.S.D.A. modify its regulations to include the interstate movement of poultry with emphasis directed toward combating the spread of transmissible diseases of poultry.
DUCK VIRUS ENTERITIS

Dr. Pfow also reported to the committee on Duck Virus Enteritis outbreaks during the past year. Previously, this disease had been confined to the East Coast flyway. However, on January 19, 1973, duck virus enteritis was reported in mallard ducks and Canada geese at the U.S. Department of the Interior's Lake Andes National Wildlife Refuge in South Dakota. It was estimated, approximately 30,000 birds died in this outbreak.

So far this year, there have been eight Duck Virus Enteritis outbreaks. These have occurred in the states of South Dakota, Minnesota, Wisconsin, New York and Pennsylvania. Three of the outbreaks were in migratory waterfowl and five were in captive waterfowl.

Eight DVE suspect cases were investigated and proven negative by laboratory tests during the year.

The entire report on this expanding disease will appear in the proceedings.

MYCOPLASMA

The committee reaffirms its position toward the need for the eradication of mycoplasma from our nation's poultry flocks as a goal and opposes the use of any viable agents as a method of control.

During the fiscal year 1973, Veterinary Services (VS) made 285 shipments of reference material to 52 laboratories in 35 States and Canada. Also during FY 1973, VS conducted two schools for 14 State employees, and 12 Industry employees for training in techniques for conducting mycoplasma tests.

Four commercial laboratories have licenses to commercially produce and/or distribute plate and tube agglutination M. gallisepticum plate antigen in the United States. No laboratory is presently producing tube antigen.

M. gallisepticum plate antigen is available from only two sources. At the present time, one laboratory is producing M. synoviae plate antigen. Commercial antigen for both M. gallisepticum and M. synoviae were at short supply several times this year.

Infectious sinusitis occurred in turkey flocks in several States this year. It appears the infection originated in source flocks in one state.

STATUS REPORT ON THE PULLORUM/TYPHOID COOPERATIVE ERADICATION PROGRAM FOR TURKEYS

In 1969, the National Turkey Federation's disease control and eradication committee proposed a pullorum/typhoid eradication program for turkeys.

Recommendations by the U.S. Animal Health Association and the American Association of Avian Pathologists are the basis for the development of Veterinary Services Memorandum 565.1, Standard Procedures for Eradication of Pullorum Disease and Fowl Typhoid in
Turkeys and Recognition of States Participating.

The Administrator of Agricultural Research Service on April 30, 1971, determined the provisions in the memorandum and those of the National Turkey Improvement Plan were not totally compatible.

At the annual meeting of the Transmissible Diseases of Poultry Committee, USAHA, on October 25, 1971, revisions were made to make it compatible with the National Turkey Improvement Plan. At the biennial meeting of the National Plans in Denver, in July 1972, the provisions, as changed, were accepted unanimously.

Since then Minnesota, Iowa, Oregon, and North Dakota have been declared pullorum disease and fowl typhoid free for turkeys by the Secretary of Agriculture. Utah is in Phase 1 of the program.

The committee commends the regulatory officials, National Plans officials and industry of those states for achieving this enviable status.

The committee desires to reaffirm its previous recommendations regarding the eradication of Pullorum Disease and Fowl Typhoid. The Committee directs your attention to the progress made by the Turkey Industry when provided with a recognized eradication program with uniform methods and rules.

The committee has had its attention directed to the desires of the North Eastern States regarding a similar program for chickens and other poultry.

The committee is of the opinion that the U.S.D.A. has been derelict in not providing leadership in delegating this authority to a single service within its department. This committee recommends to the Secretary of Agriculture that all poultry disease programs be assigned to APHIS in an effort to coordinate all disease control and eradication efforts.

It is further recommended that the U.S.D.A. recognize the desires of the North Eastern States and provide Uniform Methods and Rules for the total eradication of Pullorum Disease and Fowl Typhoid as outlined by this committee.

AVIAN INFLUENZA

The committee recommends that the influenza report given by Dr. Butterfield be edited and included in the proceedings of the U.S.A.H.A.
TRANSLOCATION OF HEAVY METALS 
IN HUMAN FOOD CHAINS

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65201.

Presented at the Seventy-Seventh Annual Meeting of the United 
States Animal Health Association, October 14-19, 1973 in St. Louis, Mo.

This work was supported by contract 68-02-0092 from the En-
vironmental Protection Agency.

The translocation of heavy metals from one level of a food chain to 
another is an ongoing process that can affect animal and human health. 
This translocation of heavy metals into food products at the end of man's 
food chain is often unrecognized by the general public. When findings of 
chemicals in food are publicized, the public response is sometimes one of 
alarm. It should be commonly understood, however, that food safety is 
not determined by the total absence of potentially toxic, naturally 
occurring elements, but rather it is a question of how much is present in a 
particular food. The purposes of this report are to describe some of the 
determinants of translocation, provide measurements of cadmium (Cd), 
copper (Cu), lead (Pb), and zinc (Zn) translocation in a lead mining area of 
Missouri, review permissible level regulations and point out some unan-
swered questions that require future considerations.

DETERMINANTS OF TRANSLOCATION

Source of Contamination

Probably the greatest influence on the food chain end-point, whether a 
person or a cow, is the sum total of environmental contamination from all 
sources. These contaminants, from airborne, water borne, or solid 
waste sources, result in soil concentrations that exceed the levels nor-
mally found as background in the earth's crust. The background con-
centration is the reason that most soils, plants and food products contain 
some heavy metals. The relative amount of the heavy metal present will, 
therefore, be determined by the amount of contamination, plus the 
background concentration.

The contribution by multiple sources of contamination at any level of 
the food chain is additive. Even though some sources may contain very 
little of an element, the overall accumulation may be considerable. In 
recent years, very sensitive methods for detecting small amounts of an 
element, such as atomic absorption spectrophotometry, and neutron ac-
tivation, have become more widely used, and even trace amounts of 
heavy metals can now be measured.

Metallic Element and Compound Differences

Each element differs in its biological availability. Also the chemical 
form affects the uptake at each step of a food chain. For example, PbO is
readily absorbed by plants and animals, and the Pb ion easily enters into chemical reactions. However, PbS (galena) is a tightly bound, stable compound and undergoes chemical reaction less readily. Absorption on surfaces of insoluble compounds in the gastrointestinal tract of animals, presence of oxidizing and reducing compounds, and compounds that prevent tissue uptake and utilization of elements are also important factors affecting biological availability.\(^2\)

**Routes of Translocation**

The routes of translocation may be very complex and, for many elements, all the operable routes are probably not known. The following simplified model represents the translocation of airborne heavy metals in food chains of man.

One or another of these routes may not be involved for certain population groups. For example, vegetarians would not knowingly ingest animal food products. Some of the routes operate in both directions, especially the soil-water pathway. Also, some abnormal pathways are not shown, such as soil pica of children and domestic animals which can have serious consequences.

**Physical and Chemical Effects**

There are marked differences in the physical and chemical characteristics of air, water and soil that affect translocation. Vertical temperature structure of air, wind patterns and topography are major influences on the dispersal and fallout of airborne compounds.\(^3\) Soil and water pH, abundance of interacting chemical elements, physical condition of the soil as indicated by its tilth, temperature and moisture content of soil are also important influences.\(^4\)

**Species Differences**

Genetic differences between plant species and between animal species also affect translocation. Some plants accumulate metals, e.g. black gum absorbs unusual amounts of cobalt,\(^5\) and others are indicator species by growing luxuriantly in the presence of high soil concentrations of an element where other plants do not grow, e.g. ragweed is a good indicator plant for zinc excess.\(^6\) Plants also differ in their ability to mobilize and transport elements between their leaves and roots.

In animals, there are marked species differences in their intake and tolerance of heavy metals. Herbivores ingest vegetation which in some instances has been extensively exposed to airborne contaminants, while carnivores in the same area receive translocated compounds at a dif-
ferent trophic level of the food chain. Horses are thought to be more sen-
sitive to lead than cattle.6-7

Diet

Within a species, there may be variation in the translocation concen-
trations due to the composition of their diet. If animals, raised in a
contaminated area, are fed home-raised crops, their intake and tissue
concentrations will be higher than animals raised in the same area but
receiving feed that was prepared from crops raised outside the area.
Likewise, human beings eating garden-raised vegetables in such an area
will have a higher intake than persons who purchase all their vegetables
at a grocery store.

Food preferences and eating habits of carnivores will also affect their
diet and consequently affect heavy metal translocation. For example,
organ tissues are usually higher than muscle, thus a preference for organ
tissues would increase the intake of some elements. Lead, zinc, strontium
and other metals are deposited in bone more than in other tissues, thus
reducing the translocation of these elements in carnivore food chains.

TRANSLOCATION OF CADMIUM, COPPER,
ZINC AND LEAD IN THE
NEW LEAD BELT OF MISSOURI

A study was conducted in the New Lead Belt of southeast Missouri to
determine the levels of Cd, Cu, Pb and Zn in air, soil, vegetation roots
and unwashed tops, blood, milk, washed hair, meat and other tissues of
cattle. The study design and the sample collection and analysis have been
previously reported. The four elements (Cd, Cu, Pb and Zn) were se-
lected because of their content in the ore mined in the area. The lead ore
concentrate contained approximately 70.0% lead, 1.5% zinc, 0.5%
copper and 0.2% cadmium.

Four cows were identified as test cows on a privately owned farm
located approximately 800 meters north of a lead smelter and adjacent to
a highway used for trucking the ore to the smelter. Four control cows
were identified on another privately owned farm in the same
geographical region, but located outside the lead production area and,
therefore, free of these sources of contamination. The various com-
ponents of the food chain were sampled every four months and one test
cow and one control cow were selected at the end of a one year study
period for slaughter and collection of biological samples.

All analyses were performed by atomic absorption spectrophotometry
with a Perkin-Elmer 403 instrument. The metal content in the samples
were calculated on a dry weight basis, except for blood and milk samples
which were on a wet weight basis.

The concentrations of lead at each level of the food chain were com-
pared to derive some estimates that might be used in evaluating the con-
sequences of increased environmental contamination on the lead levels in
meat and milk produced in such an area. A translocation model was
developed for the components of the ecosystem under study, and
the corresponding lead concentrations were abstracted from the data for the
test farm (Figure 1) and the control farm (Figure 2). The entire study
period (i.e. four samplings) was used for determining the lead concentrations in air, soil, vegetation, and water that would have contributed to the cow’s body burden, i.e. the amounts in hair, blood, milk, liver, kidney cortex, muscle and bone of the cows slaughtered at the end of the study period. Soil and vegetation samples collected at 220 ft. from the highway were used in these calculations because they represented, better than 60 ft. and 140 ft. samples, the overall levels that would contribute to the cow’s body burden. Additional samples of soil and vegetation were collected on both the test and control farms at 440 ft. from the highway. These samples, from near the centers of the fields, were very close to the 220 ft. values thus indicating that the 220 ft. site was far enough from the highway to represent the general level in the pasture.

Based on data presented in Figures 1-2 and Table 1, the difference between dustfall Pb on test and control farms of approximately 10 fold, corresponded to approximately three times higher muscle Pb concentration and five times higher milk Pb concentration for the slaughtered test cow than for the slaughtered control cow. A similar relationship was found when suspended lead values from the air filter data were substituted for the dustfall values. The mean of the suspended lead values (2.17 ug/m³) on the test farm for the four sampling periods was approximately 15 times higher than the mean of the sampling periods on the control farm (0.14 ug/m³).

Soil samples would be more easily obtained than dustfall or air filter samples for predicting the muscle and milk lead levels of cattle grazing on the soil. The five times higher mean soil Pb concentration for all samplings on the test farm than on the control farm corresponded to the previously described three times higher muscle Pb and five times higher milk Pb on the test farm than on the control farm. On an equivalent basis, the test cow’s blood Pb concentration was 1/246 the test farm soil concentration and the control cow’s blood Pb concentration was 1/187 the control farm soil concentration. This relationship should not, however, be expected to be the same in other situations involving markedly different Pb contamination sources. For example, in the models developed for the test and control farms, water appeared to have very little contribution to the cattle’s intake of lead. The pH of the soil, type of vegetation, use of fertilizers and lime, rainfall and other meteorological conditions would also affect the relationships between air and soil lead levels and the levels found in animal food products, for human consumption, produced in a given area.

On the control farm, there was a general dilution in the lead concentrations at each step in transport between soil and biological tissues (Figure 2). The roots had a lower concentration than soil, unwashed vegetation tops lower than roots, and all body tissues were lower than the tops. The bone concentration of lead was higher than other body tissues sampled, and milk was less than 1/4 the blood lead level.

On the test farm, a different pattern was observed. The concentration of lead in roots was 2.6 times higher than soil, unwashed vegetation tops
were 2.6 times higher than roots, and finally all body tissues were less than the tops. Milk had between 1/3 and 1/4 the concentration of lead found in the blood.

The high lead concentrations found in unwashed vegetation on the test farm obviously reflected the airborne contamination because the washed vegetation tops were only 84.5% the level for unwashed vegetation tops (i.e. the washings contained 79 ug Pb/gm or accounted for 15.5% of the unwashed tops value). The consistent findings in all seasons of higher lead concentrations in roots than in soil on the test farm, but not on the control farm, indicated that under these conditions of relatively high levels of contamination, there was bioconcentration of lead in the roots. These observations also lend support to the view that much of the lead in vegetation, foliage and roots, enters the plant by foliar absorption.10-11

It is commonly thought that in an area of lead contamination, horses are more likely to develop lead poisoning than cattle grazing on the same pasture.12-14 In fact, that relationship has been observed in the test farm area as documented horse deaths due to lead poisoning occurred, but no cattle deaths have been diagnosed as lead poisoning. It has been suggested that the grazing habits of the horse may be the reason for the greater apparent susceptibility of the horse, versus the cow, to lead poisoning.15-17 Horses occasionally pull forage out by the roots and eat the roots and attendant soil along with foliage. The data reported here show that the soil and root lead concentrations are much less than unwashed vegetation tops, so a horse would have less intake if the roots were substituted for some of the tops in its diet. Therefore, it seems that the basis for observations of horses being afflicted more than cattle by lead poisoning in an area of contamination is for other reasons than their grazing habits, probably a lower biological tolerance on the part of the horse.

There were very low cadmium concentrations in the samples from the control farm (Table 2). The cadmium levels in air and hair samples from test and control farms had greater differences than the other types of samples. The test cow’s hair Cd concentration was 1/260 the test farm soil concentration. A relatively high value, 3.7 ug/gm, was found in the kidney of the test cow.

The copper levels in all of the nonbiological samples were higher on the test farm than on the control farm (Table 2). The detectable copper levels in all of the biological samples, except hair, were in reverse order, i.e. the test cow samples were lower than the control cow samples. The largest difference was between liver samples; the test cow liver was 1/10 that of the control cow. The mean copper concentration of six samples of normal bovine liver measured by Peden was 55 ppm16 and 13 bovine livers examined by O Cuill. et al.16 had a mean concentration of 44 ppm. Therefore, the control cow appeared to have a normal amount of copper while the test cow, which had greater exposure to environmental contamination by copper, had a value (7.25 ug/gm) below all but one of the values reported by Peden,16 and below all 13 values reported by O Cuill et al.16 A well known relationship between molybdenum (MO) and Cu
results in reduced storage of Cu in the liver with increased intake of Mo. It is possible that a similar relationship exists between Pb and Cu. An interaction between toxic amounts of Pb and Zn was recently reported. The copper content of washed hair was higher in the test cow than the control cow (Table 3). This observation seems to contradict the hypothesis that Pb interferes with Cu deposition. However, an airborne trace element measurement in hair samples, after thorough washing, may not exclude exogenous sources. Therefore, the higher values in hair of the test cow does not negate the possibility of reduced liver deposition of copper.

The zinc concentrations in nonbiological and biological samples are shown in Table 4. There was little difference between test and control farm samples except for dustfall, water and unwashed vegetation tops. The levels of zinc found in the liver, muscle and bone exceeded by approximately two fold the values in the soil and were approximately the same order of magnitude as the vegetation.

PERMISSIBLE LEVELS

Some of the permissible levels of lead in foods are shown in Table 5. The U.S. standards for fruits were promulgated by the Food and Drug Administration as part of the pesticide program because of the use of lead arsenate spray in orchards. The standards for England and Wales are more extensive and were enacted in 1961. It is interesting to note that the test cow's liver and kidney were in excess of the England and Wales standards, and that one milk sample from the test farm in January and all the samples from the four test cows in April exceeded the standard of 0.2 ppm. Consuming meat and milk from the test farm rather than the control farm, would result in an added 123 ug lead per day for an 8-10 year old boy. The garden crops from the test farm area were also analyzed and they were found to be higher than the same crops grown in other areas.

More concern about food chain pathways involving livestock will not only reduce the human consumer's intake, but also reduce the losses in weight gain and death resulting each year from lead poisoning. Based on an extrapolation from the New York State Veterinary College where the Ambulatory Clinic treats approximately four bovine lead poisoning cases per year, it was estimated that 20,000 cases occur in the United States per year for a total cost of $2,000,000 per year in cattle.

FUTURE WORK

There are many questions about translocation of heavy metals which need to be answered. Most environmental studies currently being performed only measure the total amount of the element being studied. This can be quite deceiving if a large portion of the element is tightly bound in chemical complexes that render the element nonavailable for plants or animals. Thus quantitative determinations of specific chemical forms are needed in environmental field studies as well as laboratory studies.
The best known heavy metal problems of livestock and man are those related to large exposures and acute illness. The recognized illnesses may be like the peak of an iceberg: many individuals may have unrecognized chronic changes. More research is needed to identify the chronic effects of heavy metal exposure and to associate nonspecific clinical changes with environmental exposure. One way to associate health effects and environmental exposures is to systematically measure temporal changes. Thorough disease reporting and continuous sampling of environmental materials when possible, are important parts of a surveillance system. Livestock and pet populations are in-place sentinels that can serve in this approach. The market food testing programs that are in existence were not designed to trace back unusually high, or dangerous, levels of a chemical to its origin. It would seem desirable to extend this methodology to answer some of the remaining questions about temporal changes in environmental contamination and their effect upon the food chain. It must be kept in mind that measurements made at one point in time may not be relevant a year or two in the future. During the one year of the study described in this report, the soil lead concentration more than doubled on the test farm.

The biological interrelationships of the metals and other elements are incompletely understood. The possible relationship between Pb exposure and reduced liver storage of Cu observed in this report is just one of the many interactions that should be examined more closely.

SUMMARY

The translocation of heavy metals in human food chains depends on the amount of contamination, chemical form of the metallic element, operable routes of translocation, physical and chemical effects on all levels of the chain, plant and animal species differences and diets. Translocation of Cd, Cu, Pb and Zn in a food chain involving air, water, soil, vegetation, cattle and man in the New Lead Belt in southeast Missouri was measured on a test farm and a control farm. Levels of all four of these metals, found in the Pb ore produced in the area near the test farm, were higher in the samples collected on the test farm than the samples collected on the control farm, except for Cu which was found in higher levels in samples from the test cows. These reduced Cu concentrations, especially that of the liver of the test cow, suggest that the presence of excess amounts of Pb may have interfered with Cu storage in the liver. There was a diminution in the concentrations of both Pb and Cd on the control farm from soil to vegetation to animal tissues, while Cu and Zn were higher or approximately the same levels in the animal tissues as in the plants and soil. Lead was concentrated in the forage roots which had levels higher than the soil in which they grew. There are presently no tolerances for Pb and Cd in animal food products in the United States. Future emphasis should be placed on (1.) quantitative determinations of specific chemical forms of these elements and their biological availability, (2.) chronic health effects resulting from low level exposures, (3.) improved surveillance of chemicals in foods, (4.) epidemiologic studies to determine
the health of populations where the environmental contamination is increasing, and (5.) gaining better understanding about complex inter-relationships that may exist between elements.

LEGENDS
Figure 1. Test Farm Lead Translocation Model
Figure 2. Control Farm Lead Translocation Model
### Table 1

Lead Levels in Food Chain Components on Test and Control Farms

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<th>Sample and Unit Of Measurement</th>
<th>No. of Samples For Each Farm</th>
<th>Mean Value*</th>
<th>Test Farm (a)</th>
<th>Control Farm (b)</th>
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<td>Dustfall (mg/m$^2$/mo)</td>
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<td>87.70</td>
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<td>Suspended Pb (ug/m$^3$)</td>
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<td>0.17</td>
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<td>Soil (ug/gm)</td>
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</tr>
<tr>
<td>Kidney</td>
<td>2</td>
<td>3.75</td>
<td>0.24</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>2</td>
<td>0.19</td>
<td>0.06</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>2</td>
<td>9.00</td>
<td>3.60</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hair (ug/gm)</td>
<td>2</td>
<td>63.50</td>
<td>0.55</td>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>

*Calculated on a dry weight basis, except for blood and milk values which were on a wet weight basis.
## Table 2

Cadmium Levels in Food Chain Components on Test and Control Farms

<table>
<thead>
<tr>
<th>Sample and Unit Of Measurement</th>
<th>No. of Samples For Each Farm</th>
<th>Test Farm (a)</th>
<th>Control Farm (b)</th>
<th>Ratio a:b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dustfall (mg/m²/mo)</td>
<td>12</td>
<td>0.935</td>
<td>0.145</td>
<td>6</td>
</tr>
<tr>
<td>Suspended Cd (ug/m³)</td>
<td>4</td>
<td>0.026</td>
<td>0.002</td>
<td>13</td>
</tr>
<tr>
<td>Soil (ug/gm)</td>
<td>12</td>
<td>0.785</td>
<td>0.394†</td>
<td>2</td>
</tr>
<tr>
<td>Water (mg/l)</td>
<td>8</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>1</td>
</tr>
<tr>
<td>Vegetation (ug/gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>12</td>
<td>2.09†</td>
<td>0.72*</td>
<td>3</td>
</tr>
<tr>
<td>Tops</td>
<td>12</td>
<td>5.43†</td>
<td>1.48*</td>
<td>4</td>
</tr>
<tr>
<td>Body fluids (ug/100 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>2</td>
<td>0.30**</td>
<td>0.30*</td>
<td>1</td>
</tr>
<tr>
<td>Milk</td>
<td>2</td>
<td>0.20**</td>
<td>0.20*</td>
<td>1</td>
</tr>
<tr>
<td>Tissues (ug/gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>0.90</td>
<td>0.24</td>
<td>4</td>
</tr>
<tr>
<td>Kidney</td>
<td>2</td>
<td>3.70*</td>
<td>1.35*</td>
<td>3</td>
</tr>
<tr>
<td>Muscle</td>
<td>2</td>
<td>0.10</td>
<td>0.10</td>
<td>1</td>
</tr>
<tr>
<td>Bone</td>
<td>2</td>
<td>0.05</td>
<td>0.05</td>
<td>1</td>
</tr>
<tr>
<td>Hair (ug/gm)</td>
<td>2</td>
<td>0.58</td>
<td>0.06</td>
<td>10</td>
</tr>
</tbody>
</table>

One value(*) two values (**), six values (†) and ten values (§) below lower detectable limit; the detection limit value was used for these samples in calculating mean values; all values were calculated on a dry weight basis, except for blood and milk values which were on a wet weight basis.
### Copper Levels in Food Chains Components on Test and Control Farms

<table>
<thead>
<tr>
<th>Sample and Unit Of Measurement</th>
<th>No. of Samples For Each Farm</th>
<th>Test Farm (a)</th>
<th>Control Farm (b)</th>
<th>Ratio a:b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dustfall (mg/m²/mo)</td>
<td>12</td>
<td>1.965</td>
<td>0.325</td>
<td>6.0</td>
</tr>
<tr>
<td>Suspended Cu (ug/m³)</td>
<td>4</td>
<td>0.028</td>
<td>0.007</td>
<td>4.0</td>
</tr>
<tr>
<td>Soil (ug/gm)</td>
<td>12</td>
<td>10.64</td>
<td>6.26</td>
<td>1.7</td>
</tr>
<tr>
<td>Water (mg/l)</td>
<td>8</td>
<td>0.047*</td>
<td>0.016*</td>
<td>2.9</td>
</tr>
<tr>
<td>Vegetation (ug/gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>12</td>
<td>22.25**</td>
<td>15.90†</td>
<td>1.4</td>
</tr>
<tr>
<td>Tops</td>
<td>12</td>
<td>12.48</td>
<td>8.11†</td>
<td>1.5</td>
</tr>
<tr>
<td>Body fluids (ug/100 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>2</td>
<td>67.5</td>
<td>126.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Milk</td>
<td>2</td>
<td>&lt;7.0</td>
<td>&lt;7.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Tissues (ug/gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>7.25</td>
<td>60.00</td>
<td>0.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>2</td>
<td>2.75</td>
<td>3.85</td>
<td>0.7</td>
</tr>
<tr>
<td>Muscle</td>
<td>2</td>
<td>1.30</td>
<td>1.45</td>
<td>0.9</td>
</tr>
<tr>
<td>Bone</td>
<td>2</td>
<td>0.58</td>
<td>0.74</td>
<td>0.8</td>
</tr>
<tr>
<td>Hair (ug/gm)</td>
<td>2</td>
<td>8.2</td>
<td>5.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

One value (*), two values (**), and five values (†) below lower detectable limit; the detection limit value was used for these samples in calculating mean values; all values were calculated on a dry weight basis, except for blood and milk values which were on a wet weight basis.
### Table 4

**Zinc Levels in Food Chain Components on Test and Control Farms**

<table>
<thead>
<tr>
<th>Sample and Unit Of Measurement</th>
<th>No. of Samples For Each Farm</th>
<th>Test Farm (a)</th>
<th>Control Farm (b)</th>
<th>Ratio a:b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dustfall (mg/m^2/mo)</td>
<td>12</td>
<td>9.48</td>
<td>1.23</td>
<td>8</td>
</tr>
<tr>
<td>Suspended Zn (ug/m^3)</td>
<td>4</td>
<td>0.51</td>
<td>0.41</td>
<td>1</td>
</tr>
<tr>
<td>Soil (ug/gm)</td>
<td>12</td>
<td>27.65</td>
<td>19.22</td>
<td>1</td>
</tr>
<tr>
<td>Water (mg/l)</td>
<td>8</td>
<td>0.66*</td>
<td>0.13</td>
<td>5</td>
</tr>
<tr>
<td>Vegetation (ug/gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>12</td>
<td>61.46</td>
<td>51.65</td>
<td>1</td>
</tr>
<tr>
<td>Tops</td>
<td>12</td>
<td>70.58</td>
<td>44.68</td>
<td>2</td>
</tr>
<tr>
<td>Body fluids (ug/100 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>2</td>
<td>420</td>
<td>355</td>
<td>1</td>
</tr>
<tr>
<td>Milk</td>
<td>2</td>
<td>230</td>
<td>230</td>
<td>1</td>
</tr>
<tr>
<td>Tissues (ug/gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>33.45</td>
<td>51.60</td>
<td>1</td>
</tr>
<tr>
<td>Kidney</td>
<td>2</td>
<td>17.75</td>
<td>19.60</td>
<td>1</td>
</tr>
<tr>
<td>Muscle</td>
<td>2</td>
<td>43.50</td>
<td>41.90</td>
<td>1</td>
</tr>
<tr>
<td>Bone</td>
<td>2</td>
<td>70.05</td>
<td>88.50</td>
<td>1</td>
</tr>
<tr>
<td>Hair</td>
<td>2</td>
<td>84.1</td>
<td>78.3</td>
<td>1</td>
</tr>
</tbody>
</table>

*One value below lower detectable limit; the detection limit value was used for this sample in calculating mean values; all values were calculated on a dry weight basis, except for blood and milk values which were on a wet weight basis.*
Table 5

Permissible Levels of Lead in Food, United States and England-Wales

<table>
<thead>
<tr>
<th>Country and Food</th>
<th>Permissible Level (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td></td>
</tr>
<tr>
<td>Citrus fruit</td>
<td>1.0</td>
</tr>
<tr>
<td>Apples, apricots and tomatoes</td>
<td>7.0</td>
</tr>
<tr>
<td>England and Wales</td>
<td></td>
</tr>
<tr>
<td>Non-alcoholic beverages (milk)</td>
<td>0.2</td>
</tr>
<tr>
<td>Beer</td>
<td>0.5</td>
</tr>
<tr>
<td>Apples and pears</td>
<td>3.0</td>
</tr>
<tr>
<td>Liver and other fresh meat</td>
<td>2.0</td>
</tr>
<tr>
<td>Canned meat and gelatin</td>
<td>5.0</td>
</tr>
</tbody>
</table>
REFERENCES


ADDENDUM
COMMITTEE ON PUBLIC HEALTH AND ENVIRONMENTAL QUALITY

Chairman: Richard L. Parker, Atlanta, Ga.
Co-Chairman: Robert H. Singer, Bryan, Tex.


The Committee considered a draft of a paper provided by Dr. R. Keith Farrell of Pullman, Washington, which dealt with toxic effects of flea collars on cats. The data suggests the possibility of serious toxic effects in cats from contact with such collars including dermatitis and an ataxia-depression syndrome. Several members of the Committee were aware of antecdotal accounts of humans developing a dermatitis from contact with this type of collar.

Dr. Robert Singer was appointed Chairman of a sub-committee to:
1. Review evidence of public health hazards from the use of (2,2-dichlorovinyl dimethyl phosphate (DDVP or dichlorvos). In addition to the use of such materials in pet flea collars the possibility of cumulative effects from other uses of the compound should be considered.
2. Report to the Committee at the annual meeting in 1974, or if the results of the sub-committee’s review seem to warrant a report should be made directly to the Committee Chairman prior to the 1974 annual meeting.

The Committee noted the report of the Committee on Rabies and the invitation of that Committee to include the entire question of pet animal populations. The Committee felt that this was of definite interest to us and authorized the Committee Chairman to explore with the Chairman of the Committees on Rabies and on Animal Welfare this area of consideration.

Dr. Bill Childers of Texas A&M University presented an interesting paper entitled “The Public Health Aspects of Aquaculture.” Dr. Childers informed the Committee that there has been a 50% increase in the consumption of fish and seafood in the United States in the past 10 years and that before this increase, consumption equalled natural United States production (catch). Last year the United States consumed 56 million tons of seafood and fish, of which 100,000 tons were produced by aquaculture ventures. Such production, both natural and in modified environments, has a possibility of rapid spread of disease causing organisms which can threaten both the animals themselves and man.

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Vibrio parahemolyticus represents probably the greatest threat to human health from aquaculture practices. It inhabits salt water and has been incriminated in a number of outbreaks of food-borne illness attributed to seafood. The organism causes gastroenteritis in man and is highly heat sensitive.

Salmonellosis has not been reported to be a problem in seafood, primarily because of the lethal effect of salt water. Botulism, type E, has caused a few deaths from consumption of underprocessed seafood products. Erysipelothrix insidiosa infection is an occupational hazard of fish handlers. Aeromonas liquefaciens is pathogenic for both man and fish.

Shellfish may concentrate viral agents from polluted water and transmit these agents to man. Marine dinoflagellates produce a toxin which is pathogenic to man.

Diphyllobothrium latum, a fish tapeworm, may be transmitted to man and other mammals by consumption of raw or undercooked fish.

Industrial pollution by mercury has resulted in mercury intoxication with some deaths reported from consumption of contaminated fish. Both fish and shellfish are capable of transmitting this poison to man resulting in central nervous system degeneration. Chlorinated pesticides and polychlorinated biphenyls are world-wide environmental contaminants which are stable and long-lived in nature. Pesticides are present in the environment in greater quantities because they have been deliberately spread, whereas PCB's have accidentally been introduced. Both compounds undergo food-chain amplification as they move upward toward man. The effects of these compounds on human health has not been clearly established. However, they have caused morbidity, mortality and reduced reproduction in both native and aquaculture marine animals. Their effect on aquaculture operations is potentially dangerous.

The Committee respectfully submits this report for approval.
WHOLESOME FOOD: INDUSTRY AND CONSUMER CONCERNS

The year of 1973 may well be remembered as the year of wholesome foods... the year that Clostridium botulinum, Staphylococcus, and Clostridium perfringens, Shigella, Vibrio parahaemolyticus, and especially Salmonella became household words.

This may be stretching the truth a bit... but certainly consumers have become increasingly knowledgeable and concerned about health, the environment, and the quality and safety of their food. This concern has been heightened in recent months by the recall of canned mushrooms which required a nationwide public warning and an all out campaign to remove the implicated products from the market.

MEDIA FEATURES FOOD SAFETY PROBLEMS

Food manufacturers are still reeling from the (GAO) General Accounting Office’s Report to Congress on Insanitary Conditions in the Food Manufacturing Industry... This is the report that revealed inspections of 97 establishments selected from many different industries, discovered “significant insanitary” conditions in 23 plants; “insanitary conditions” in 28 plants. Only 30 out of 97 plants were in compliance. This presented an inevitable question: How many of the 60,000 food plants, food warehouses, and similar establishments the Food and Drug Administration inspects are out of compliance, and how is the welfare of the consumer affected? Needless to say, resources became available for an intensified program to eliminate these insanitary conditions in the Nation’s 60,000 food plants.

The Department of Agriculture was also referred to in the Reader’s Digest article entitled “How Safe Is Food?” This article reported on the 1971 (GAO) report on inspections of poultry plants and it revealed: “The USDA’s inspection of poultry plants was woefully inadequate. After surveying 68 federally inspected plants, which account for nearly 1/5 of all the poultry slaughtered in the United States, the GAO found a dismaying list of insanitary and weak enforcement practices by the Department of Agriculture.”

Distributors, including food retailers, also got into the act; fortunately or unfortunately, this has been somewhat overshadowed in recent months by rising food prices. Retailers in some states, especially the cities of Chicago and Dayton, Ohio, will long remember their place in the “insanitary sun.”

A Chicago TV station, in its hamburger report, stated “filth or extraneous matter in samples collected from 18 of the 20 food stores... E-Coli (bacteria) in over half the samples.” The report further stated that “E-Coli bacteria, could indicate animal or human fecal matter.”

The Dayton Daily News, not to be outdone, headlined in their Sunday edition, “Hamburger: A Dirty Story” and followed it up with the following lead: “Hamburger bought at seven Dayton food stores was dirty... out of 14 samples tested... not a single one met the cleanliness...
INDUSTRY AND CONSUMER CONCERNS

guidelines used by the Ohio Department of Agriculture." The report further stated that “according to Consumer Report Magazine bacteria counts of 1,000,000 to 5,000,000 may cause mild intestinal stress.” Also of interest, was the statement that “there is widespread acceptance that 10,000,000 bacteria per gram is the point at which hamburger begins to decompose.” Four samples taken in the test exceeded that limit... one reached 380,000,000.

Moneysworth, The Consumer Letter gave the restaurant and food service industry some cause for concern by stating: “Have you ever dined out—and stayed out for days afterward with ptomaine, undulant fever, or a zapped stomach? It’s no secret that eating in the U.S. is a health hazard. In New York City, literally hundreds of restaurants were closed for health violations, most of them nauseating. Major health code violations were found in 86 percent of the restaurants in one mid-Manhattan district.”

MORE RESOURCES ALLOCATED TO FOOD SAFETY

Although some of those presiding over the regulatory areas are convinced that “today’s food supply is safer than it has ever been before,” the American consumers are apparently unconvinced. Consumers have raised their expectations to peaks never before known in this country and they are much concerned with their health and demand that food products be safe. They have made significant demands on the Federal Government and the Congress has responded with new and stronger laws and the Government agencies as well as industry groups are responding with new programs initiated to help alleviate the consumer’s concern about food safety.

Concern about food safety is not new... many agencies including FDA and the USDA have had ongoing programs of research, education, and inspection programs designed to achieve the objectives of providing wholesome foods to the consumers.

There is little question, however, that in recent years more attention and resources have been focused on the problems of food safety.

In 1964, the grocers in Massachusetts recognized the role of bacteriological control in supermarkets. The State Grocers’ Association, at that time, put on a vivid demonstration of aseptic meat cutting. Much to the amazement of most of the members, wrapped cuts of meat had their shelf life extended from two to seven days. It was pointed out to achieve this longer shelf life it would require radical changes in equipment used in meat cutting operations, training of personnel to understand the reason for better sanitation practices, and the use of special detergents and sanitizers.

At about the same time, my research and Extension colleagues at the University of Missouri were preparing their “Guidelines for Handling Prepackaged Meats in Retail Food Stores” in which they pointed out that packaged meat display life could be doubled by improved handling methods. They also provided the sanitation, temperature, and product...
care guidelines to achieve longer shelf life. Other colleagues in New Mexico and New Jersey tested the meat sanitation program and found that savings in products and increased net value of products more than covered the additional costs of the sanitation program.

The Supermarket Institute and National Association of Retail Grocers, as well as Extension and other private and public educational groups, brought this information to the attention of retailers at workshops, seminars, and through various bulletins and visual publications.

PROJECT CONSUMER CONCERN

In July 1972, Project Consumer Concern was introduced with its objective of effecting improvements in food handling, storage and distribution of food to insure wholesome food for consumers.

A well-known Extension technique was used in this project... the demonstration store. Demonstration supermarkets were established in Pennsylvania, North Carolina, and Ohio. This concept utilized the result demonstration principle in which improvements, based on research results, are established in one store of a firm, or a group of stores, and extended by various means to other stores within the firm.

PROBLEMS OF SANITATION IN SUPERMARKETS

In the development of the Total Store Sanitation Program for the demonstration stores, problems were defined that were affecting food safety. These included:

(1) A lack of commitment and follow through by top management.
(2) No uniform guidelines for the sanitation program.
(3) Disagreement among regulatory agencies as to the equipment, facilities, materials, and cleaning methods to be used.
(4) Confusion as to temperatures and how to check them.
(5) Ineffective use of code dates on perishables.
(6) Unawareness of effect of lighting on product temperatures.
(7) Careless handling of frozen foods.
(8) Perishables not refrigerated during transportation.
(9) Principles of safe food handling not understood by personnel handling prepared foods.
(10) Inadequate cooking or reheating temperatures for prepared foods.
(11) Potential for cross contamination and recontamination as same person handled uncooked and ready-to-eat products as well as money.
(12) Many potential hazardous products were not being protected in the bakery... left uncovered and unrefrigerated. Smoking was also observed in this department.
(13) Cooked and uncooked fish were being stored, processed, and displayed in common areas.
(14) At the checkout, water and blood from meat and poultry were wiped from belts, but belts were not sanitized.

Utilizing available research and accepted industry practices, pro-
cedures and checklists were developed for operators' use. These have been made available to the industry by the National Association of Retail Grocers.

FDA DEVELOPS MODEL SANITATION ORDINANCES FOR SUPERMARKETS

Of great interest to the food distribution and food service industries were the announcements by the (FDA) Food and Drug Administration that "they will give priority to regulatory initiatives which would substantially lessen the risks of food contamination in the distribution of human foods, especially the uniform food service sanitation ordinance and code, and a model ordinance for retail food stores and standards for controlling microbiological contamination in the transportation of foods."

In discussing the model ordinance for food stores, the FDA report states: "The model ordinance includes specific mention of cross contamination...it stipulates several practices which can reduce the spread of microbiological hazards, in the retail product. For example, the draft requires thorough cleaning of a meat grinder before switching from one meat to another."

The uniform food service sanitation ordinance will also include explicit mention of cross contamination and recontamination by potentially hazardous food.

FDA also plans similar model ordinances for those transporting human foods and animal feeds.

A recent contact with the FDA indicated that the food service and retail store ordinances would be published in the Federal Register prior to January 1, 1974.5

THE FIRST OF MAY IN OREGON

May 1, 1973, may also be a significant date for the food distribution industry as it was on this day that the new Oregon consumer law establishing bacterial standards for all meat at retail went into effect. Oregon is the first state to adopt such standards. Basically, the law states "meat food products will be deemed adulterated if the microbacterial level exceeds 5 million organisms per gram in fresh or frozen meat products and one million for meat products cooked or smoked...of if E-Coli organisms exceed 50 per gram in fresh and frozen meat products or 10 per gram in cooked or smoked."

Although a Supermarket News Report stated that some state officials here are hopeful that the Oregon meat bacterial standards law will serve as a model for the U.S. Department of Agriculture. The USDA is interested in bacterial standards and especially how they are established and how they are applied. In a paper on microbiological standards, a USDA scientist states "microbiological criteria can be applied in three ways: first, by taking a figure out of the air, this is, of course, the bad way; second, by determining the general microbiological levels in foods in commerce without regard to the effect of processing, this is a better way;
and third, the best, by determining what is a good manufacturing practice and then determining the microbial levels associated therewith. When a microbiological criterion is established, methods for sampling and analysis should be specified. The data both for establishing a microbiological criteria and for establishment of acceptability of given lots should be statistically significant; therefore, it is necessary to know the natural variation of levels of microbial groups within various commodities. If microbiological criteria require drastic changes in an entire industry, they should be applied with great discretion to avoid seriously damaging the industry."

Another source indicates that there is absolutely no way to relate bacterial number to safety and that putrification depends on many things, including types of organisms and temperature of storage as well as numbers. This statement is supported by a study of the bacteria of ground beef that also includes this challenging statement... "as hamburger is usually handled in meat markets it is inevitable that the meat will become contaminated with organisms capable of growing rapidly at ordinary refrigerated temperatures. The extent of the growth and the time required for spoilage in the consumer's possession will depend mostly on the degree of contamination, the time the meat is held in the store, and, of course, the temperature at which it is stored... thus, the purchaser receives a product already heavily innoculated and to prevent its rapid spoilage he must consume it promptly or freeze it." 

STOP AND SHOP'S ASEPTIC MEAT PROCESSING

In 1972, one firm finally challenged the "zero-home-life" of ground beef with a new concept of a central, refrigerated meat processing plant to prepare beef in an aseptic environment... a method that removes all surface bacteria from incoming shipments of beef. Following cleaning, the carcasses move through holding, cutting, and wrapping stages under constant low temperatures and in a close to sterile atmosphere. According to chairman Sidney R. Rabb of Stop and Shop Stores, Inc., "the greatest single investment ever made by the company will provide vacuum-wrapped cuts of beef and ground beef to all supermarkets that will end up in display cases as clean, fresh, aged, and uniformly trimmed cuts."

IMPOSSIBLE TO ELIMINATE SALMONELLA

Assistant Secretary of Agriculture, Clayton Yeutter, has noted that it is impossible to totally eliminate salmonella. "Steps can, however, be taken and others developed, through more research, to greatly reduce bacterial loads on animal and poultry carcasses. This, with lowering salmonella organisms in animal feeds and improved feed handling practices, will reduce salmonella as a significant health hazard."

Several research studies of particular interest to this audience are the USDA's Agricultural Research Service-Oregon State University study... "obtain information relative to the cost benefits implementing a total store sanitation program and improving the handling of returnable
bottles and containers."

Also of interest is a Northeast Regional research project with New Jersey, Massachusetts, New York, Pennsylvania, and Rhode Island participating with the objective of "developing programs and procedures to protect foods during preparation, manufacturing, distribution, marketing, and serving from potential quality losses and health hazards."

AMERICAN CONSUMERS ARE CONCERNED ABOUT SAFETY OF FOOD

American consumers today are the best informed and most militant in history. Scientific achievements have raised consumer expectations to a peak never before known in this country. Greater influence and broader educational opportunities have made consumers more concerned about their environment and about their health, and they are demanding that products be safe, effective, and honestly labeled and promoted.

A recent FDA study of consumers' opinions about the safety of foods revealed: "More then one-third of those interviewed said that foods were getting less safe; 76 percent believed that a dinner prepared fresh by the consumer himself is safer than ready-to-eat foods, such as frozen dinners or canned stew. Foods which are cooked or heated before serving were judged by 48 percent of the consumers to be safer than foods which are put directly on the table; in comparing fresh foods with dry, frozen, or canned, 68 percent of the consumers think fresh foods are safer; 86 percent believe that food containers are important in making foods safe. As for processing, 77 percent believe that the manufacturing process kills all or most of the germs which might contaminate the food; 72 percent of the consumers say that manufacturers do a good job of keeping impurities out of food. As to who does the most to make foods safe, the Government was given 45 percent of the credit by consumers; processors, 23 percent; consumer groups, 21 percent; and companies that sell food to the public, 5 percent." The concern of Government and the food industry is the belief by the public that food is getting less safe.

The Economic Research Service of the USDA is in the process of conducting a similar consumer study of homemakers' knowledge, opinion, and attitudes toward food safety on selected items. This study will concentrate on consumer food handling practices with special attention given to meat and poultry products.

USDA-FDA TO SPONSOR JOINT CONSUMER PROGRAMS ON FOOD SAFETY

At a recent USDA-FDA press conference on food safety, it was announced that the two Departments would expand and coordinate an intensive consumer educational campaign aimed at eliminating careless food handling practices in the home and in food service establishments. In addition to coordinating their respective consumer education activities, they will also be developing joint materials. The aim is to provide publications that can gain mass distribution through consumer
groups, educational institutions, and the food industry. The overall objective is to carry food messages to every household in the United States.

EXTENSION INCREASES FOOD SAFETY ACTIVITIES

The Extension Service is gearing up its activities to carry the food safety message in greater volume to 10 million families and 3 million youths it reaches each year in rural and urban America.

Yes, the years of 1972-73 may well be remembered as the years for more wholesome food . . . as food safety problems became better defined and better communicated to food industry groups, Government agencies, and to consumers. Also, the coordinated activities of these groups will bring about further improvements in the safe handling of foods . . . that is in the transportation, storage, and preparation especially in the supermarket, in food service operations, and in the home.

REFERENCES

As of June 30, 1972, USDA had withdrawn participation in the five year old cooperative State-Federal Salmonella Program. USDA reported a severe reduction in funding and a personnel ceiling for fiscal year 1972 which had come at a time when emergency situations had arisen in other animal disease programs. This situation made it imperative that USDA utilize available manpower to fewer, highest priority programs.

On the Federal side, the Food and Drug Administration has legal responsibility for regulatory activity regarding contamination of animal feeds, and is expected to carry on their work, at least at the present level. During these past five years FDA was involved in regulatory activities when Salmonella contaminated animal and marine processing plant and feed mill products were found.

Both the USDA and FDA recently completed extensive internal studies of present control programs for Salmonella.

Steps leading to what government officials said would greatly reduce Salmonella and other foodborne illnesses, were announced on August 14, 1973 at a joint press conference of the U.S. Department of Agriculture and Health, Education, and Welfare.

In releasing the reports of internal task forces within each agency, USDA and HEW officials advocated:

1. Expansion and coordination of an intensive consumer education campaign aimed at eliminating careless food handling practices in the home and food service establishments.

2. Development of a voluntary cooperative Industry-State-Federal program coordinated by the Food and Drug Administration to eliminate Salmonella from rendered animal by-products used in animal feeds. FDA is prepared to provide technical advice and assistance in the development of such programs.

   FDA’s limited inspectional resources will be re-directed from the present emphasis on rendering plants to the blenders who produced about 60% of the nation's animal by-products.

3. Modification of processing procedures and facilities in meat and poultry plants under USDA inspection to reduce bacterial cross-contamination of products and equipment.

4. Intensified support of industry and USDA-financed research aimed at controlling and eliminating Salmonella throughout the food chain, and

Speech given at the 77th Annual United States Animal Health Association before the Salmonella Committee on Oct. 17, 1973, in St. Louis, Missouri.
5. Development by FDA of model ordinances governing sanitation and food handling in retail stores, food service institutions, and standards for food transportation industry.

At this press conference, Assistant Secretary of Agriculture Clayton Yeutter noted that it is impossible to totally eliminate Salmonella since it exists throughout our environment. Meat animals and birds are therefore a common source of Salmonella organisms. Carryover contamination from the live animal and bird to raw meat and poultry occurs with some frequency.

Several steps can be taken, however, and others can be developed through more research, to greatly reduce bacterial loads on animal and poultry carcasses, Mr. Yeutter said. This, combined with lowering the level of Salmonella organisms in animal feeds, and improved food handling practices at the consumer level will reduce Salmonella as a significant human health hazard.

Noting that improper handling of food at the retail level and in the home is a major source of Salmonella infection, Dr. Charles C. Edwards, HEW's assistant secretary for Health, emphasized the importance of a joint educational effort by USDA and HEW. "We are convinced that an effective consumer educational program can accomplish more than millions of dollars spent on additional government regulatory programs," Dr. Edwards said. He also explained the actions FDA is undertaking to develop uniform ordinances for state and local governmental units, standards for the transportation industry, and the redirection of FDA's inspecional resources.

As a result of the adoption of the FDA Task Force Report and the joint USDA-FDA press conference announcement, the Commissioner's Office of FDA set into motion several charges for immediate action. One assignment being to establish voluntary cooperative Industry-State-Federal programs for the control of Salmonella in animal feeds. This particular assignment naturally fell to Dr. C. D. Van Houweling, Director of the Bureau of Veterinary Medicine in FDA. It was at that time that I was assigned the task of serving as Chairman and Coordinator for BVM/FDA in these activities.

On September 7, 1973 our first Salmonella Committee meeting was held at FDA with officials from the following organizations in attendance:

- United States Animal Health Association
- Association of American Feed Control Officials
- National Association of State Departments of Agriculture
- Virginia Department of Agriculture
- Animal Health Institute
- American Feed Manufacturers Association
- National Feed Ingredients Association
- Midwest Feed Manufacturers
- National Renderers Association
American Meat Institute Foundation
National Independent Meat Packers Association
National Fishmeal and Oil Association
National Broiler Council
National Broiler Marketing Association
Association of American Railroads
United States Department of Agriculture
United States Department of Commerce
Bureau of Veterinary Medicine/FDA
Division of Federal-State Relations, EDRO/FDA
Field Investigation Branch, EDRO/FDA
Division of Planning and Analysis, EDRO/FDA

The agenda of this first meeting was designed to acquaint all attendees with the previous USDA Cooperative State-Federal Salmonella Program activities and to discuss methods of developing new "Voluntary Cooperative Industry-State-Federal Salmonella Control Programs."

During this meeting it was decided to form a smaller so-called "working committee" from those in attendance, with the assignment to proceed in developing the necessary programs for Industry-State-Federal cooperation.

On September 25, 1973, the first working committee meeting was held, representatives of the following organizations serve as members:
National Association of State Departments of Agriculture (NASDA)
United States Animal Health Association (USAHA)
Association of American Feed Control Officials (AAFCO)
National Renderers Association (NRA)
National Fishmeal and Oil Association (NFOA)
American Meat Institute Foundation (AMIF)
American Feed Manufacturers Association (AFMA)
Association of American Railroads (AAR)
Poultry and Egg Institute of America (PEIA)
United States Department of Agriculture (USDA)
Food and Drug Administration (FDA)

It was decided the first and most logical step would be to attempt to pick up where USDA left off with its cooperative State-Federal Salmonella control program.

It was called to our attention during this meeting; that recommended guidelines had been unanimously adopted, by the Committee on Salmonellosis at the 1972 USAHA meeting. These guidelines were for use by Federal or State Governments or other supervisory agencies desiring to carry on uniform methods and rules for the elimination of Salmonella in animal and marine products intended for use in animal feeds.

This working committee, after reviewing several drafts coordinated through my office, presented the attached proposal of uniform methods and rules adopted by the USAHA Salmonella Committee. This proposal
serves as the initial step toward re-instituting control of Salmonella in animal feeds through voluntary cooperative efforts of Industry-State and Federal organizations represented by our committee. Your support in our efforts is greatly needed.

We recommended the adoption of our proposal by the Salmonella committee and by the general membership of USAHA during this meeting here in St. Louis.

UNIFORM METHODS AND RULES FOR A VOLUNTARY COOPERATIVE INDUSTRY-STATE-FEDERAL PROGRAM FOR THE CONTROL OF SALMONELLA IN ANIMAL AND MARINE 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, and all other animal and poultry rendered by-products or mixtures thereof for animal feed use.

B. Marine Products: Fish meal, crab meal, shrimp meal, fish liver and glandular meal, fish protein concentrate-feed grade, blended mixture thereof or other rendered marine products to be used in animal feeds.

C. Lot: A lot of animal and/or marine products shall be the amount of product for a single shipment or a day’s production, whichever is greater.

D. Official Sample Unit: At least one hundred (100) grams of a representative sample of animal and/or marine products collected by a State or Federal inspector or other authorized individual. 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 or other authorized individual.

F. Plant Sample: A random sample unit of a finished product, ready for shipment, collected by plant personnel in such a manner that it represents the contents of a total lot.

G. Positive Sample Unit: An official sample unit of an official sample from which Salmonella is recovered by an official laboratory using the Salmonella test procedures described in Part I (I). (ARS 91-68-1).

H. Negative Sample Unit: An official sample unit of an official sample from which no Salmonella is recovered by an official laboratory using the Salmonella test procedures described in Part I (I). (ARS 91-68-1.)

I. Salmonella Test: The laboratory examination of an official sample collected as outlined in D-above, and tested by the procedures and methods recommended by the United States Animal Health Association, United States Department of Agriculture and Food and Drug Administration for these cooperative program activities (ARS 91-68-1). (This will allow for composite sample testing subject to
approval of the American Association of Veterinary Laboratory Diagnosticians.) (AAVLD)

J. Rendering Establishment: An establishment that renders (cooks) animal and/or marine products as defined in Part I (A) and (B) for use in animal feeds, whether or not operated in conjunction with a slaughtering or processing plant.

K. Blending Establishment: An establishment that grinds, blends, mixes, or further processes animal, marine, or animal and marine products for the purpose of making such products suitable for use in animal feeds. A blending establishment does not render (cook) animal or marine products.

L. Quality Control Program: A test for Salmonella of a plant sample representative of not less than one of the lots produced each week.

M. 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 and/or marine products.

N. Phase II “Cleanup”: A program phase to designate a rendering or blending establishment producing Salmonella positive animal and/or marine products but is participating in the cooperative Industry-State-Federal Salmonella Program as described here in Part II (B).

O. Phase III “In Compliance”: A program phase to designate a rendering or blending establishment that has met and is maintaining the requirements for this designation as described here in Part II (C).

P. Finished Product: Rendered or blended material, ready for shipment, collected in the shipping or storage area, that will undergo no further processing in the plant where collected.

Q. Official Inspection: A plant inspection conducted by a State or Federal inspector or other authorized individual.

R. Official Laboratory: A State, Federal or other authorized laboratory following the guidelines as established in Part I (I).

Part II: Recommended Procedures

A. Phase I “Evaluation”

1. Objective: Determine the presence or absence of Salmonella in detectable amounts in the finished product produced by each rendering or blending plant in the State.

2. Procedure:
   a. If the presence of Salmonella is detected in one or more official sample units, the plant cannot remain in Phase I for more than 90 days after evaluation. If an official sample discloses positive results, the plant must, within this period, move into Phase II or be dropped from the program.
   b. If tests on official samples collected, not less than 30 days apart, are negative at the official laboratory, the plant can move directly into Phase III status.
B. *Phase II "Plant Improvement"*

1. **Objective:** Reduce the number of plants not in compliance.
2. **Procedure:** Official inspections and official samplings shall be conducted at intervals of not more than 180 days. Samples shall be collected in such a manner that 20 official sample units are collected each 12-month period, and not more than 10 official sample units are collected on any one inspection. If tests on official samples collected are negative the plant can move into Phase III.

(In general the same administrative procedural concept as used in the operation of the previous State-Federal Cooperative Program will serve as the guidelines for this program, at the present time.)

C. *Phase III "In Compliance"*

1. **Objective:** To give recognition to plants that demonstrate the capability of producing a product negative for Salmonella when sampled and examined by the procedures recommended in Part I (I).
2. **Procedure for maintenance:**
   a. A plant may maintain its compliance status each fiscal year provided two official inspections are conducted and not less than 20 consecutive negative official sample units are collected during a 12-month period. Not more than 10 official sample units are to be collected on any one inspection. Each official inspection and sampling not less than 90 days apart.
   b. In the event a Phase III plant fails to meet compliance, the plant must then obtain two successive negative officials samples within a 6-month operating period. Failure to achieve this, results in the plant being placed in Phase II.
   c. The plant maintains a Salmonella Quality Control Program which meets the approval of the cooperating State and Federal officials. Inspector examines self-monitoring records (culture results). (These results cannot be used to disqualify a plant.)
REPORT OF THE COMMITTEE ON SALMONELLOSIS

Chairman: H. G. Geyer, Washington, D.C.
Co-Chairman: John W. Walker, Washington, D.C.


The 1972 Committee submitted a Resolution expressing grave concern over the failure of the Federal Government to release appropriated funds to continue the cooperative State-Federal Salmonella Control program for rendering and fish meal plants. It also recommended that if the Secretary of Agriculture could not achieve reinstatement of these funds, “that a similar program be instituted as soon as possible by the Food and Drug Administration through financial cooperation with other Federal or State Agencies”.

In response to the latter recommendation, Dr. William B. Bixler, FDA, Bureau of Veterinary Medicine, presented a report on the status of the Voluntary Cooperative Industry-State-Federal Program. It was indicated that FDA has legal responsibility for regulatory activity regarding contamination of animal feeds, and is expected to carry on this work, at least at the present level.

Dr. Bixler presented for the Committee’s consideration a new “Proposed Uniform Methods and Rules (SUMR) for a Voluntary Cooperative Industry-State-Federal Program for the Control of Salmonella in Animal and Marine Products for use in Animal Feeds”. (Attachment 1).

After discussion the Committee approved these Salmonella Uniform Methods and Rules as the Program to be followed for the control of Salmonella in Animal and Marine Products Used in Animal Feeds.

The Committee makes the following recommendations:

1. That Veterinary Services, APHIS, USDA, make personnel available, that are experienced in various aspects of their former Cooperative State-Federal Salmonella Program, to the Food and Drug Administration to train and orient FDA personnel to aid them in getting the New Cooperative Industry-State-Federal Program under way in states where trained and personnel are not located. Dr. Bixler pointed out that FDA has no new funds for this program and will rely heavily on State Agencies and the rendering and fish meal industries for implementation of this program.

2. That the U.S. Animal Health Association inform the American Veterinary Medical Association and National Association of State
Departments of Agriculture of the new Salmonella Control Program under FDA Aegeus and encourage these organizations to aid FDA in obtaining adequate support to carry out the program.

3. That FDA distribute copies of the SUMR to State and Federal Animal Health Officials, the rendering and marine products industries, and FDA field personnel.

4. That the Chairman of the Salmonella committee request a report from the Resolutions Committee on what response was received from USDA on Resolution No. 6 as reported in the 1972 proceedings. This resolution dealt with funding for the Salmonella Control Program.

Since Salmonellosis may be caused by any one of the 1,300 known serotypes, means must be devised to assess and apply known technology through measures that can be expected to be practically effective for its control. The National Academy of Sciences, in its 1969 report, An Evaluation of the Salmonella Problem, recommended that "An attempt should be made to evolve a realistic assessment of the degree of hazard imposed by various foods, feeds, and drugs; and the quality-control requirements in relation to salmonella contamination should reflect the degree of hazard. Assessment of the potential hazard of a given product should reflect not only past history but also current status as determined by continuing product surveillance. Appropriate provision should be made for addition of new products to the "sensitive" group as well as for the removal of products as justified by improved industrial practices". The report further recommended that "A definite policy should be stated regarding compliance or non-compliance. The term "salmonella-free" should not be used regarding salmonella in relation to foods because it is not possible, with certainty, to assure complete absence. Limits of acceptability can be based only on the probability that salmonella are not present or are present at less than a statistically defined level. Sampling procedures should be clearly described and a "cut-off" established so that industry will have a reasonable base from which to determine if its products meet requirements." The Committee urges that these recommendations be taken into account in future action programs.

The following written reports were received from Committee members and constitute a part of this report:

1. Attachment No. 2: The Salmonella Problem: (A statement released on August 14, 1973, by Dr. Clayton Yeutter, Assistant Secretary of Agriculture, outlining the USDA's approach to the total salmonella problem and the U.S. consumer's responsibility to take certain precautions to protect himself against salmonella infections.) The furtherance of this recommendation is reflected in the paper presented at this meeting by Mr. Lewis F. Norwood.

2. Attachment No. 3: Salmonella Isolations from Livestock and Poultry (Fiscal Year 1973).

3. Attachment No. 4: A Summary of Salmonella Isolates from Food Animals and All Other Agricultural Sources Serotyped for Diagnostic Purposes (Fiscal Years 1967 to 1973).

THE SALMONELLA PROBLEM

INTRODUCTION

The goals of this report are threefold:

A. To recommend immediate practical steps to reduce the Salmonella load of raw meat and poultry.

B. To suggest long range steps which are not immediately practical but which could be established later.

C. To list research proposals designed to improve husbandry, processing and distribution practices.

Except for cooking, there is no easy answer to the Salmonella problem. Reduction of the incidence of salmonellae in raw meats and poultry is multifaceted. The changes in procedures suggested in animal husbandry, transport, processing, distribution, retailing and food service are only partially supported by scientific data. To measure small reductions or increases in salmonellae is extremely difficult because the incidence of carrier animals varies from 0 to 100 percent. These wide variations mask any minor changes in Salmonella level on the meat itself.

Furthermore, the number of salmonellae on a contaminated carcass is normally very low. Thus, the size of the sample analyzed affects the incidence.

On the other hand, the resistance of Salmonella is similar to that of many of the organisms always present on the surface of meat and poultry products. The pseudomonads, the coliform group, E. coli, etc., are similar to Salmonella in that they are washed away or readily destroyed by processing techniques. One must assume, therefore, that any process that will reduce the general bacterial load of commonly occurring species, will reduce the Salmonella load as well.

Thorough cooking, the best way to destroy salmonellae, is foolproof provided the workers do not recontaminate the cooked food from the raw product or from hands, equipment, etc.

The Department already has an extensive ongoing, consumer educational program directed primarily at the housewife and food service employees. Since most of the food poisoning outbreaks occur because of mishandling at this level, the Committee as one of its suggestions, urges expanded education efforts.

SCIENTIFIC BACKGROUND

Salmonella contamination and/or infection of the Nation's meat and poultry products begins with breeding flocks and herds, then continues in rearing areas where endemic infections spread from one animal to another (1,3). Contaminated feeds also contribute to the cycle (2,3). During transport from the farm to the slaughterhouse, and during feed-lot operations, crowding, poor sanitation, and physical stress increase the likelihood of infection among uninfected animals so that the incidence
of infected animals at slaughter is much higher than it is on the farms. Whereas inspectors sort out obviously sick animals at ante-mortem inspection, the typical adult carrier has no symptoms of illness. Thus, inspectors are powerless to prevent the entry of carrier animals into the slaughterhouse. Many investigators have described the varying incidence of asymptomatic carriers at slaughter (4, 5, 6, 7, 8). In addition, many surveys have demonstrated salmonellae in fresh and frozen domestic and imported raw meats (8a).

Raw red meats and poultry are a principal source of these organisms in the kitchen, but not the only source (8b). Although salmonellae readily die with cooking, poor kitchen sanitation sometimes transfers them from raw to cooked foods where they can multiply to a level that will cause illness on ingestion. Errors in food handling at food service establishments and homes account for about 80 percent of all foodborne disease, including salmonellosis. Raw poultry and raw red meat are frequently blamed for cross-contaminating cooked products (8b, 9, 10, 11).

During slaughter and further processing, the contaminated carcass spreads Salmonella to other uncontaminated carcasses. Common baths, such as the scald tank can cross-contaminate poultry carcasses (12, 13). Indeed, intestinal bacteria, including salmonellae, enter the lungs and air sacs in the scald tank (13).

Similarly, Galton (14) found the hog dehairing machine was a vehicle for the spread of salmonellae among carcasses.

Several authors have suggested modifications of processing procedures to reduce bacterial loads on animal carcasses. For example, scalding, scraping and singeing of hog skin before removal almost completely destroyed the organisms on the skin surface (15, 16, 17). Many of the processing procedures required by MPI reduce the microbial load of poultry carcasses and red meats significantly. For example, bacterial levels on the surface of poultry usually fall because of the MPI requirement for continuous overflow of scald tanks (18), and chillers (19, 19a). In a recent USDA study the incidence of Salmonella contaminated birds was not changed by the chilling process (4). However, sometimes, microbial levels increase during evisceration and handling (20).

Occasionally, the scientific and industrial literature may suggest improvements such as replacing a scald tank by a steam cabinet (12). These improvements usually require additional testing before MPI can require their use, and before establishments are willing to invest heavily in them.

In time, the flow of water and product, and the normal cleanup operations, usually remove all salmonellae from the processing plant. Timoney, et al. (21), demonstrated that the predominant serotypes isolated from poultry processing equipment changed with each new flock slaughtered. It is possible, therefore, that Salmonella-negative raw meat might be produced if the flocks and herds received for slaughter were free of Salmonella carriers.
A wealth of data is available concerning the effects of chlorination upon the microbial load of animal products (22, 23, 24, 25, 26, 27, 28, 29, 30). As long as chlorination or other steps reduce the general microbial load, one would expect that they would also reduce the Salmonella load.

Chlorination, however, cannot do the entire job because salmonellae may find their way into hair follicles, feather follicles and fatty layers. Chlorine cannot penetrate under the surface of organic foods. Surkiewicz, et al. (41, 42) recommended chlorinated water to reduce cross-contamination in washing and chilling.

The Chemistry Staff, Scientific and Technical Services, has been considering the possibility that chlorine may combine with organic materials to produce hazardous substances. Extremely heavy chlorination of meat (i.e., 10 percent by weight) has formed unknown compounds that appear as broad peaks on the GLC equipment. These studies should be extended to show what compounds are formed at the "parts per million" level.

In the meantime, the Committee has agreed to press for widespread chlorination to help solve the very real Salmonella problem, and to reconsider only if the chemistry investigations reveal compounds of a hazardous nature at the low levels (.01 percent) of chlorine proposed.

RECOMMENDATIONS FOR IMMEDIATE APPLICATION

A. General—Some proposals of a general nature apply to both meat and poultry:

1. *Microbiological Surveillance*—Continue and increase the surveillance of cooked foods to eliminate sources of *E. coli* and related organisms if inspection shows they are related to cross-contamination from raw products. Follow the NAS-NRC recommendations for sampling cooked foods for *Salmonella*, then take immediate action to remove from the market *Salmonella*-contaminated lots.

2. *Separation of Raw from Cooked*—Section 308.16 of the Manual of Meat Inspection Procedures, and section 81.132 of the Poultry Inspectors’ Handbook now require sanitizing of hands and equipment to preclude cross-contamination, and require separate storage areas for raw and cooked product. The Committee recommends (a) new wording to require, for new construction, a physical separation of raw from cooked product during both processing and storage, (b) more consistent enforcement of the washing and sanitizing clauses in the current manuals, and (c) publication of these requirements in the Federal Register.

3. *Chlorination*—MPI should require the chlorination of all water supplies used in meat and poultry plants. The amount added to the water should be adjusted so that at least one part per million residual occurs at each water tap within the plant. During cleanup, the chlorine residual should be raised to at least 100 per million.
4. Improved Plant Layout Requirements—MPI should require applicants for equipment and facilities approval to reveal the type of product to be processed and the route the product follows through the plant.

5. Air Movement—To reduce the possibility that dust or aerosols from hides or feathers will contaminate finished product, all new constructions should be designed to prevent air movement from the killing operation toward the finished product.

6. Salmonellosis, a Reportable Disease—The Department should encourage the States to require that salmonellosis be a reportable disease. Funds and manpower should be provided through Veterinary Services to assist the State Animal Health Officials in the investigation of outbreaks and providing expertise for corrective measures.

7. Salmonella-Free Feeds—The Department should encourage the Food and Drug Administration to continue the Cooperative State/Federal Salmonella Program with the animal and marine protein producers and extend the program to include the livestock and poultry feed manufacturing industry. When USDA stopped its program, there were 196 firms producing Salmonella-negative protein ingredients on a regular basis. An additional 545 had not reached this point. The Department should now request FDA to require finished feeds in interstate commerce to be Salmonella-negative.

8. Training of Inspectors—The Microbiology Staff should assist the Training Staff in developing a training program for inspectors so that they better understand the various aspects of the Salmonella problem.

B. Red Meats—In addition to the above, MPI should take the following specific steps in the red meat area:

1. Swine Dehairing—Swine dehairing machines should be flushed and sanitized at midshift. (This is in addition to present requirements for daily cleanup.) The machines should be constantly flushed during use, with water containing at least 100 ppm chlorine.

2. Casings—Natural casings for sausages should be so processed that they are Salmonella-negative before use in inspected product.

3. Calf Carcasses—Calf carcasses should be hot skinned and shipped in wrappers.

4. Sick Animals—All sick animals should be handled as suspects, killed only at the end of the kill day, and only under rigorous sanitary procedures. Such animals are five times as likely to be Salmonella carriers and should be bacteriologically monitored.

5. Swine carcasses should be sprayed with water containing at least 20 ppm chlorine after dehairing and prior to evisceration. Each finished carcass should be sprayed with water containing at least
20 ppm chlorine prior to leaving the official establishment.

C. Poultry—In addition to A above, MPI should require the following:

1. Counter-Flow in Evisceration Trough—The water in the evisceration trough should flow opposite to the direction of the product flow.
2. Bleeding Time—The length of the bleeding time should be sufficient to permit cessation of wing flapping and final defecation prior to entry into the scald tank.
3. Neck Trim—The neck stub should be trimmed far enough to remove exposed tissues contaminated by the slaughter cut.
4. All water used on the poultry lines from the exit of the scald tank to the chill tank exit should contain at least 20 ppm residual chlorine as measured at point of use before it contacts the birds.

IMPROVEMENTS FOR LONG RANGE APPLICATION

A. Continuing Surveillance—MPI should increase its microbiology facilities in order to expand existing surveillance programs on both cooked and raw products. As the steps proposed herein begin to reduce the Salmonella problem, certain firms will begin to produce Salmonella-negative foods and will thus represent good commercial practice. They should follow the developments by surveillance to make corrections in those firms that do not conform to good commercial practice. Parallel bacteriological studies and testing programs in the industry should also be encouraged.

B. Retail in Designated States—MPI should offer scientific and technical support to the State and local agencies responsible for overseeing sanitary practices at the retail level. This cooperation could be an effective tool for reducing salmonella contamination during this phase of product distribution.

C. Education—The Department should expand greatly its educational efforts in the area of food poisoning, particularly salmonellosis.

D. Live Animals and Poultry—Veterinary Services should expand its programs to include prevention of salmonellosis in flocks and herds. Suggestions for inclusion in the programs are:

1. Participate in cooperative programs to maintain breeding flocks and herds negative to the common serotypes considered to cause a carrier state in each species.
2. Participate in cooperative programs to monitor livestock and poultry for salmonellosis prior to slaughter.
3. Participate in cooperative programs with Extension Service, Environmental Protection Agency, and others to correct environmental conditions that contribute to Salmonella transmission in livestock and poultry, for example, sanitation at each stage in the life cycle of livestock and poultry, marketing and transportation prior to slaughter, waste management, etc.
RECOMMENDATIONS FOR RESEARCH

Determination of the efficacy of a given procedure to reduce or destroy salmonellae is difficult because of the variation in incidence of salmonellosis in the animal population. The Agricultural Research Service should compare the survival of bacteria in parallel slaughter lines using standard and experimental equipment under commercial conditions.

At this time, ARS should also investigate:

A. Changes in Eviscerating Procedures for Poultry—The opening cut is sometimes made in such a way that a damaged intestinal tract will permit fecal contamination of the surfaces. A few firms are experimenting with improved nonstandard procedures for opening and drawing the birds. These need investigation.

B. Scalder—Immersion scalding is the usual method, although some firms have installed steam chests for the purpose. Theoretically, these would be advisable because they eliminate the common bath.

C. The Pickers—To date no one has been able to develop an alternative to the rubber-fingered pickers, although this operation is a major cause for cross-contamination among carcasses.

D. Hog Dehairing—There may be alternatives to the hog dehairing machine. It spreads salmonellae over the surfaces of most carcasses. Skinning may be the answer.

E. Sanitation—The economics of sanitizing all equipment and hands between contacts with carcasses should be studied.

F. Hot Water or Steam Jacket Washes—Heating the surfaces of animal or poultry carcasses just before packaging will certainly destroy most salmonellae. We need data to show its antimicrobial effectiveness, and its effect on the color of the meat or poultry. We should also learn from parallel tests the effectiveness and utility of chlorine in hot water.

G. Destruction by Chemicals—ARS should continue to investigate chlorine and other chemicals to destroy salmonellae on meats.

H. Wrapping Carcasses—The surfaces of red meat carcasses support high bacterial loads because of contamination and long storage. Contamination could be reduced by wrapping in appropriate materials for shipment. Investigators should determine whether this is feasible and what kinds of materials would be suitable. ARS has already determined that this approach shows promise (33).

In addition, MPI should conduct certain investigations after consultation with ARS for input and assistance:

A. Boning Areas—Scientific studies have shown that both raw (12, 31) and cooked (32) boning sometimes contribute heavily to bacterial increases. Investigation by MPI personnel may reveal the need for more frequent cleanup or other corrections.

B. Pork Lymph Glands—It has been reported that certain lymph glands remaining with pork carcasses contribute to Salmonella contamination of the meat. USDA should try to confirm this finding,
either in MPI labs or in those of ARS.

C. Pasteurization of Byproducts — In rendering plants, the problem with Salmonella is compounded because animal byproducts usually contribute to cross-contamination of the final product. It would be technically feasible to require pasteurization of animal byproducts before they leave inspected meat and poultry plants.

CURRENT RESEARCH

The current research projects on Salmonella which are underway in USDA, or supported by USDA, are numerous. Some are particularly pertinent, and MPI should follow them carefully. All of these projects except the first are listed under CRIS number 230400. Some were completed recently, and reports should be available at an early date. The Projects are:

A. Tissue Glue — Research at Los Alamos Scientific Laboratories, AEC, to determine whether a tissue glue inserted into the rectum at slaughter can prevent feces from leaving the gut during slaughter.

B. Poultry Pasteurization — Study at ARS in Albany and Athens of effects of physical and chemical pasteurization on poultry meat.

C. Salmonella Detection — Research at University of Wisconsin's Food Research Institute (FRI) to shorten the time needed for Salmonella detection in the laboratory.

D. Salmonella in Foods — Research at FRI to learn the fate of Salmonella in cured meats and cheese, including effect of pH, temperature, redox potential and water activity on survival of Salmonella.

E. Salmonella Control in Poultry — Research at Athens to test chemical agents for their effectiveness in destroying salmonellae. Study effects of heat, gamma and uv irradiation and of processing operations and equipment on Salmonella contamination of carcasses.

F. Further Processed Turkey Products — Research at Iowa State University to relate incidence of Salmonella, staphylococci, and other bacteria on raw meat to that of finished products.

G. Salmonella-Free Poultry — Research at University of Massachusetts on litter management to optimize salmonellacidal activity of used litter.

REFERENCES

2. Nape, W.F., 72nd Ann Meeting USLSA (1968)
8a. USDA files
23. Patterson, J. T., Br. Poul. Sci. 9:129 (1968)

SALMONELLA ISOLATIONS FROM LIVESTOCK AND POULTRY
(FISCAL YEAR 1973)

HISTORIC BACKGROUND

This is a report of the serotyping of cultures of Arizona and Salmonella isolated from livestock and poultry and their environment by the cooperating animal diagnostic laboratories in the United States.

Our predecessor, the Animal Disease Eradication Division, Agricultural Research Service, began providing serotyping services for Salmonella cultures isolated from nonhuman sources in 1957. Both the serotyping and the compiling of the annual report was done by the very capable microbiologist and pioneering Salmonella serologist, Alice Moran, at the Center for Disease Control, Atlanta, Georgia, until the unit was moved to the new National Animal Disease Laboratory, Ames, Iowa, in the summer of 1962.

The Salmonella Surveillance Report was started by CDC in 1952. Since that time, this report has been included in that publication and constitutes the major portion of the isolations from nonhuman sources.

In 1966 the request for a Laboratory Report for Salmonella Serotyping (ANH Form 10-3) was designed for machine processing. The first
SALMONELLOSIS

national report of Salmonella in Livestock and Poultry was produced from the data processing printouts and distributed (ARS 91-48-5) in September 1967.

During fiscal year 1967 through 1972 a brief summary of this activity was included in the Status Report of the Cooperative State-Federal Salmonella Program.

With the fiscal year 1973 report, we will not duplicate that made by CDC but will attempt to make other uses of this data in keeping with the original purpose of this report; such as, to measure the scope and economic loss due to salmonellosis in livestock and poultry and to gather information of epidemiological value.

To do this, it is most urgent that the request form (ANH Form 10-3), especially Item 9 (number in the flock or herd) and Item 10 (number dead), be filled in as accurately as possible. If more than one submission is made from the same case, this should be noted in Item 15 (comments), e.g., 1 of 3 cultures from this case; 2 previous submissions have been made; etc. Also submissions from nonclinical cases should be noted in Item 15. This information will help to prevent multireporting of single cases and lessen the chance of reporting nonclinical cases as salmonellosis.

To increase the reporting efficiency of this Salmonella data we are asking all of our serotyping laboratories to return the incomplete request forms and withhold serotyping until the completed ANH Form 10-3 has been received.

REPORT ON THE DILLON BEACH PROJECT
COOPERATIVE AGREEMENT ON
SPECIFIC PATHOGEN FREE TURKEYS

The Dillon Beach Project was developed as a cooperative effort with the Nicholas Turkey Breeding Farms, Inc.; California Department of Agriculture, Division of Animal Industry; the Regents of the University of California; United States Department of Agriculture, Agricultural Research Service, Animal and Disease Parasite Research Service (now Livestock and Veterinary Sciences), and Animal Health Division (now Veterinary Services, Animal and Plant Health Inspection Service). The first objective was to determine the feasibility of establishing turkey breeder flocks free of Salmonella, Arizona, and Mycoplasma disease organisms and to maintain primary breeder flocks free of these organisms.

This constitutes a brief progress report of the Dillon Beach Ranch Salmonella and Arizona work and includes all activities from April 1969 through June 30, 1973 (see attached table). There was only one known Salmonella isolation, Salmonella tennessee, from 1969 to 1972 until the fourth generation poult was hatched in 1972. S. heidelberg and S. infantis were isolated from hatch debris. Subsequently, S. heidelberg was isolated from brooder house litter and S. infantis from one pool of five cull poult. S. heidelberg and S. infantis were isolated from GI tracts at
market time from both pedigree and line flock breeders. *S. san diego* was later isolated from GI tracts of roaster-fryer cull poults from the processing plant. Since there was no previous isolation of *S. san diego* from any source at Dillon Beach, the nine *S. san diego* isolations may have been processing plant contaminates.

We have been unable to determine definitely the source of Salmonella at Dillon Beach in 1972. *S. infantis* was recovered from one feed sample.
### TABLE I - (Continued)

**SALMONELLA SEROTYPES REPORTED FROM DOMESTIC ANIMALS OF THE UNITED STATES**

**FISCAL YEAR 1973**

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<th>Other Avian</th>
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<th>Cattle</th>
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| S. albany                     | 6      | 10      | 0           | 1     | 0      | 2             | 0                   | 19    |
| S. amager                     | 2      | 1       | 0           | 0     | 0      | 0             | 0                   | 3     |
| S. amstercord                  | 0      | 0       | 0           | 0     | 0      | 0             | 0                   | 1     |
| S. anatum                     | 66     | 12      | 4           | 10    | 22     | 11            | 73                  | 198   |
| S. bareilly                   | 0      | 0       | 2           | 0     | 1      | 0             | 3                   | 6     |
| S. bareilly var 14             | 0      | 1       | 0           | 0     | 0      | 0             | 0                   | 1     |
| S. blockley                   | 2      | 26      | 1           | 2     | 0      | 0             | 2                   | 33    |
| S. born                        | 0      | 2       | 3           | 0     | 1      | 3             | 9                   | 9     |
| S. bovis-sorbiificans         | 0      | 0       | 1           | 0     | 0      | 0             | 0                   | 1     |
| S. bronchii                   | 0      | 2       | 1           | 0     | 1      | 0             | 3                   | 3     |
| S. brenday                    | 26     | 37      | 0           | 6     | 3      | 5             | 7                   | 84    |
| S. california                 | 1      | 18      | 0           | 1     | 0      | 0             | 2                   | 22    |
| S. corso                      | 1      | 9       | 0           | 0     | 0      | 0             | 2                   | 12    |
| S. cluster                    | 22     | 3       | 0           | 1     | 0      | 2             | 3                   | 31    |
| S. cholera-suis               | 0      | 0       | 1           | 0     | 0      | 0             | 0                   | 1     |
| S. cholera-suis var kunzendorf | 0      | 1       | 0           | 278   | 10     | 1             | 0                   | 290   |
| S. cornellis                  | 1      | 0       | 0           | 0     | 0      | 0             | 1                   | 1     |
| S. cubica                     | 4      | 9       | 0           | 2     | 0      | 1             | 27                  | 43    |
| S. derby                      | 15     | 2       | 4           | 11    | 2      | 1             | 6                   | 41    |
| S. chamaeleon                 | 0      | 0       | 0           | 0     | 0      | 0             | 2                   | 2     |
| S. drypool                    | 5      | 7       | 1           | 0     | 2      | 0             | 2                   | 17    |
| S. dublin                     | 1      | 0       | 1           | 0     | 46     | 3             | 0                   | 51    |
| S. cimicbuetel                | 3      | 10      | 0           | 0     | 0      | 2             | 17                  | 32    |
| S. citricidids                | 2      | 20      | 5           | 7     | 7      | 19            | 3                   | 63    |
| S. gallinarum                 | 1      | 12      | 0           | 0     | 0      | 0             | 0                   | 13    |
| S. gaminara                   | 0      | 0       | 0           | 0     | 0      | 1             | 0                   | 1     |
| S. good                       | 0      | 0       | 0           | 0     | 0      | 1             | 2                   | 3     |
| S. glvo                       | 5      | 5       | 0           | 1     | 0      | 9             | 3                   | 23    |
| S. grumpensis                 | 2      | 0       | 0           | 0     | 0      | 0             | 0                   | 2     |
| S. habano                     | 1      | 3       | 0           | 0     | 0      | 6             | 0                   | 10    |
| S. halmstad                   | 1      | 0       | 0           | 0     | 0      | 0             | 0                   | 1     |
| S. hartford                   | 0      | 0       | 1           | 0     | 0      | 0             | 0                   | 1     |
| S. heldeberg                  | 156    | 89      | 5           | 7     | 5      | 8             | 7                   | 277   |
| S. hennepin                   | 0      | 0       | 0           | 0     | 0      | 1             | 0                   | 1     |
| S. indiana                    | 53     | 4       | 0           | 3     | 0      | 1             | 1                   | 62    |
| S. infantis                   | 36     | 79      | 1           | 5     | 1      | 6             | 7                   | 134   |
| S. jawa                       | 2      | 0       | 0           | 0     | 1      | 4             | 0                   | 7     |
| S. johannsberg                | 2      | 3       | 0           | 1     | 0      | 0             | 1                   | 7     |
| S. kentucky                   | 1      | 9       | 0           | 0     | 0      | 0             | 21                  | 31    |
| S. kentucky var jerusalem      | 0      | 0       | 0           | 0     | 0      | 0             | 3                   | 3     |
| S. kilmarley                  | 0      | 0       | 0           | 0     | 0      | 0             | 3                   | 3     |
| S. kollbus                     | 1      | 6       | 0           | 2     | 0      | 1             | 1                   | 11    |
| S. luxington                  | 0      | 0       | 0           | 0     | 0      | 1             | 5                   | 6     |
| S. illae                      | 0      | 0       | 0           | 0     | 0      | 0             | 2                   | 2     |
| S. illi.siyfield               | 1      | 2       | 0           | 1     | 1      | 4             | 0                   | 9     |
| S. livington                  | 0      | 4       | 0           | 0     | 0      | 0             | 0                   | 4     |
| S. lowman                     | 1      | 0       | 0           | 0     | 2      | 1             | 0                   | 4     |
| S. modella                    | 0      | 0       | 0           | 0     | 1      | 0             | 0                   | 2     |
| S. manhattan                  | 5      | 4       | 0           | 0     | 0      | 0             | 0                   | 9     |
| S. manila                     | 0      | 1       | 0           | 0     | 0      | 0             | 0                   | 1     |
| S. matoponl                   | 0      | 0       | 1           | 0     | 0      | 0             | 0                   | 1     |
| S. meloagridis                | 4      | 0       | 0           | 0     | 0      | 0             | 4                   | 8     |
TABLE 1 - (Continued)

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* Source not identified on one report.

The number of Salmonella reports from a State cannot be interpreted as a measure of incidence but may indicate one or more of the following:

- personal interest of the diagnostic community,
- the nature of the livestock industry,
- the occurrence of salmonellosis in the major livestock industry.

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It has been recommended that control programs should begin with an attack against the most prevalent serotypes. The "percent of total" column indicates that there is some variation in the geographic distribution of the ten most prevalent serotypes. Perhaps control measures that would be for Salmonella in general would be more appropriate on the national level.
### TABLE 5

**SUMMARY OF THE TEN MOST FREQUENTLY REPORTED SALMONELLA SEROTYPES AND PULLORUM-GALLINARUM ISOLATED**

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<td>52,488</td>
<td>3.7</td>
</tr>
<tr>
<td>Reading</td>
<td>85</td>
<td>6</td>
<td>86,600</td>
<td>4.9</td>
</tr>
<tr>
<td>Typhimurium var copenhagen</td>
<td>32</td>
<td>4</td>
<td>62,600</td>
<td>2.2</td>
</tr>
<tr>
<td>Pullorum-gallinarum</td>
<td>2</td>
<td>1</td>
<td>3,500</td>
<td>62.9</td>
</tr>
</tbody>
</table>

*Reporting efficiency 14.0%*

<table>
<thead>
<tr>
<th>FY 1973</th>
<th>CHICKENS</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype</td>
<td>Number Reports</td>
<td>No. Reports Showing Mortality</td>
<td>Number Chickens</td>
<td>Average Mortality</td>
</tr>
<tr>
<td>Infantis</td>
<td>79</td>
<td>26</td>
<td>428,970</td>
<td>2.7</td>
</tr>
<tr>
<td>Typhimurium var copenhagen</td>
<td>75</td>
<td>16</td>
<td>377,000</td>
<td>3.0</td>
</tr>
<tr>
<td>Montevideo</td>
<td>76</td>
<td>16</td>
<td>292,190</td>
<td>2.1</td>
</tr>
<tr>
<td>Worthington</td>
<td>72</td>
<td>13</td>
<td>266,500</td>
<td>2.0</td>
</tr>
<tr>
<td>Saint-Paul</td>
<td>29</td>
<td>12</td>
<td>294,200</td>
<td>3.7</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>96</td>
<td>12</td>
<td>158,595</td>
<td>3.5</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>89</td>
<td>11</td>
<td>230,100</td>
<td>2.0</td>
</tr>
<tr>
<td>Simsbury</td>
<td>52</td>
<td>11</td>
<td>264,200</td>
<td>2.9</td>
</tr>
<tr>
<td>Senftenberg</td>
<td>32</td>
<td>9</td>
<td>116,400</td>
<td>10.1</td>
</tr>
<tr>
<td>Agona</td>
<td>23</td>
<td>9</td>
<td>124,000</td>
<td>4.1</td>
</tr>
<tr>
<td>Pullorum-gallinarum</td>
<td>59</td>
<td>17</td>
<td>30,202</td>
<td>25.4</td>
</tr>
</tbody>
</table>

*Reporting efficiency 25.1%*

*Reporting efficiency is the percentage of the requests for serotyping that included the number in the flock or herd and the number dead.*
**TABLE 5** - (Continued)  
**SUMMARY OF THE TEN MOST FREQUENTLY REPORTED SALMONELLA SEROTYPES ISOLATED**

<table>
<thead>
<tr>
<th>FY 1973</th>
<th>SWINE</th>
<th>CATTLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype</td>
<td>Number Reports</td>
<td>No. Reports Showing Mortality</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td><strong>Salmonella</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cholera-suis var kunzendorf</td>
<td>278</td>
<td>137</td>
</tr>
<tr>
<td>typhimurium</td>
<td>82</td>
<td>27</td>
</tr>
<tr>
<td>typhimurium var copenhagen</td>
<td>26</td>
<td>7</td>
</tr>
<tr>
<td>saint-paul</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>anatum</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>newport</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>infantis</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>enteritidis</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>bredeny</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>blockley</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Reporting efficiency</strong></td>
<td>42.4%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FY 1973</th>
<th>CATTLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype</td>
<td>Number Reports</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td><strong>Salmonella</strong></td>
<td></td>
</tr>
<tr>
<td>typhimurium</td>
<td>719</td>
</tr>
<tr>
<td>typhimurium var copenhagen</td>
<td>170</td>
</tr>
<tr>
<td>newport</td>
<td>191</td>
</tr>
<tr>
<td>cholera-suis var kunzendorf</td>
<td>10</td>
</tr>
<tr>
<td>anatum</td>
<td>22</td>
</tr>
<tr>
<td>dublin</td>
<td>46</td>
</tr>
<tr>
<td>enteritidis</td>
<td>7</td>
</tr>
<tr>
<td>muenchen</td>
<td>4</td>
</tr>
<tr>
<td>saint-paul</td>
<td>11</td>
</tr>
<tr>
<td>senftenberg</td>
<td>1</td>
</tr>
<tr>
<td><strong>Reporting efficiency</strong></td>
<td>26.1%</td>
</tr>
</tbody>
</table>

*Reporting efficiency is the percentage of the requests for serotyping that included the number in the flock or herd and the number dead.*
## TABLE 5 - (Continued) - 3 -.

**SUMMARY OF THE TEN HOST FREQUENTLY REPORTED SALMONELLA SEROTYPES ISOLATED**

<table>
<thead>
<tr>
<th>FY 1973</th>
<th>SHEEP &amp; GOATS</th>
<th>HORSSES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serotype</strong></td>
<td><strong>Number Reports</strong></td>
<td><strong>No. Reports Showing Mortality</strong></td>
</tr>
<tr>
<td><strong>Salmonella typhimurium</strong></td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td><strong>anatum</strong></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>typhimurium var copenhagen</strong></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>san-diego</strong></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Reporting efficiency</em></td>
<td>46%</td>
<td></td>
</tr>
<tr>
<td><strong>Salmonella typhi</strong></td>
<td>82</td>
<td>9</td>
</tr>
<tr>
<td><strong>typhimurium var copenhagen</strong></td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td><strong>heidelberg</strong></td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td><strong>newport</strong></td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td><strong>muenter</strong></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>enteritidis</strong></td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td><strong>ordalieburg</strong></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>anatum</strong></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><strong>bredeney</strong></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><strong>good</strong></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>Reporting efficiency</em></td>
<td>9.8%</td>
<td></td>
</tr>
</tbody>
</table>

*Reporting efficiency is the percentage of the requests for serotyping that included the number in the flock or herd and the number dead.*
A SUMMARY OF SALMONELLA ISOLATES FROM FOOD ANIMALS AND OTHER AGRICULTURAL SOURCES SEROTYPED FOR DIAGNOSTIC PURPOSES – FY 1967-1973

I NUMBER OF ISOLATIONS

<table>
<thead>
<tr>
<th>ANIMALS</th>
<th>NO. OF ISOLATES</th>
<th>PERCENT OF TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TURKEYS</td>
<td>12,820</td>
<td>25.3</td>
</tr>
<tr>
<td>CHICKENS</td>
<td>10,499</td>
<td>20.7</td>
</tr>
<tr>
<td>SWINE</td>
<td>4,272</td>
<td>8.4</td>
</tr>
<tr>
<td>CATTLE</td>
<td>3,676</td>
<td>7.2</td>
</tr>
<tr>
<td>SHEEP</td>
<td>247</td>
<td>0.4</td>
</tr>
<tr>
<td>ALL OTHER SOURCES</td>
<td>19,111</td>
<td>37.7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>50,625</td>
<td>99.7</td>
</tr>
</tbody>
</table>

FROM ANH FORM 10-3
I SEROTYPES OF MOST FREQUENTLY REPORTED SALMONELLA ISOLATIONS FROM CATTLE – FY 1967-1973

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>NUMBER</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhimurium*</td>
<td>2,436</td>
<td>66.2</td>
</tr>
<tr>
<td>S. newport</td>
<td>439</td>
<td>11.9</td>
</tr>
<tr>
<td>S. dublin</td>
<td>279</td>
<td>7.5</td>
</tr>
<tr>
<td>S. anatum</td>
<td>79</td>
<td>2.1</td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>77</td>
<td>2.0</td>
</tr>
<tr>
<td>S. saint-paul</td>
<td>68</td>
<td>1.8</td>
</tr>
<tr>
<td>SUBTOTAL</td>
<td>3,378</td>
<td>92 %</td>
</tr>
<tr>
<td>TOTAL</td>
<td>3,676</td>
<td></td>
</tr>
</tbody>
</table>

*INCLUDES VAR. COPENHAGEN

From ANH Form 10-3
**SALMONELLOSIS**

III. **MOST FREQUENTLY REPORTED SALMONELLA ISOLATES FROM CHICKENS - FY 1967-1973**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhimurium*</td>
<td>1,832</td>
<td>17.4</td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>1,503</td>
<td>14.3</td>
</tr>
<tr>
<td>S. infantis</td>
<td>965</td>
<td>9.1</td>
</tr>
<tr>
<td>S. thompson</td>
<td>937</td>
<td>8.9</td>
</tr>
<tr>
<td>S. blockley</td>
<td>672</td>
<td>6.4</td>
</tr>
<tr>
<td>S. saint-paul</td>
<td>481</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>6,390</td>
<td>61%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10,499</td>
<td></td>
</tr>
</tbody>
</table>

*Includes var. Copenhagen

From ANH Form 10-3

IV. **SEROTYPES OF MOST FREQUENTLY REPORTED SALMONELLA ISOLATES FROM HORSES - FY 1967-1973**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhimurium*</td>
<td>352</td>
<td>63.4</td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>42</td>
<td>7.5</td>
</tr>
<tr>
<td>S. newport</td>
<td>34</td>
<td>6.1</td>
</tr>
<tr>
<td>S. anatum</td>
<td>19</td>
<td>3.4</td>
</tr>
<tr>
<td>S. saint-paul</td>
<td>11</td>
<td>1.9</td>
</tr>
<tr>
<td>S. oranienburg</td>
<td>11</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>469</td>
<td>84%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>555</td>
<td></td>
</tr>
</tbody>
</table>

*Includes var. Copenhagen

From ANH Form 10-3
### SEROTYPES OF MOST FREQUENTLY REPORTED SALMONELLAE AND ARIZONA ISOLATES FROM SHEEP - FY 1967-1973

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhimurium*</td>
<td>74</td>
<td>29.9</td>
</tr>
<tr>
<td>Arizona 26:30</td>
<td>55</td>
<td>22.2</td>
</tr>
<tr>
<td>S. saint-paul</td>
<td>35</td>
<td>14.1</td>
</tr>
<tr>
<td>Arizona 26:29,30</td>
<td>17</td>
<td>6.8</td>
</tr>
<tr>
<td>S. newport</td>
<td>13</td>
<td>5.2</td>
</tr>
<tr>
<td>Untypable Group B</td>
<td>5</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>199</td>
<td>80 %</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>247</td>
<td></td>
</tr>
</tbody>
</table>

*Includes var. copenhagen

From ANH Form 10-3
<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. CHOLERA - SUIS</td>
<td>2,126</td>
<td>49.7</td>
</tr>
<tr>
<td>VAR. KUNZENDORF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. TYPHIMURIUM*</td>
<td>732</td>
<td>17.1</td>
</tr>
<tr>
<td>S. DERBY</td>
<td>259</td>
<td>6.0</td>
</tr>
<tr>
<td>S. HEIDELBERG</td>
<td>152</td>
<td>3.5</td>
</tr>
<tr>
<td>S. ANATUM</td>
<td>121</td>
<td>2.8</td>
</tr>
<tr>
<td>S. SAINT-PAUL</td>
<td>98</td>
<td>2.2</td>
</tr>
<tr>
<td>S. INFANTIS</td>
<td>81</td>
<td>1.8</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>3,569</td>
<td>83%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>4,272</td>
<td></td>
</tr>
</tbody>
</table>

*Includes Var. Copenhagen

From ANH Form 10-3
VII. SEROTYPES OF MOST FREQUENTLY REPORTED SALMONELLA ISOLATIONS FROM TURKEYS - FY 1967-1973

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. HEIDELBERG</td>
<td>2,323</td>
<td>18.1</td>
</tr>
<tr>
<td>S. SAINT-PAUL</td>
<td>1,685</td>
<td>13.1</td>
</tr>
<tr>
<td>S. SAN-DIEGO</td>
<td>1,397</td>
<td>10.8</td>
</tr>
<tr>
<td>S. TYPHIMURIUM*</td>
<td>886</td>
<td>6.9</td>
</tr>
<tr>
<td>S. ANATUM</td>
<td>660</td>
<td>5.1</td>
</tr>
<tr>
<td>S. SENftenBERG</td>
<td>584</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>7,535</strong></td>
<td><strong>59%</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12,820</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Includes var. copenhagen

From ANH Form 10-3

VIII. THE NUMBER OF SALMONELLA ISOLATES FROM COMPANION ANIMALS SEROTYPED FOR DIAGNOSTIC PURPOSES - FY 1967-1973

<table>
<thead>
<tr>
<th>Animal</th>
<th>No. of Isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>555</td>
</tr>
<tr>
<td>Dog</td>
<td>228</td>
</tr>
<tr>
<td>Cat</td>
<td>97</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>880</strong></td>
</tr>
</tbody>
</table>

From ANH Form 10-3
### Material Cultured for Salmonella and Arizona Species

**Dillon Beach Activity Report - April 1969 to June 1973**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter PH</td>
<td>0/144&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0/543</td>
<td>0/166</td>
<td>0/259</td>
<td>ND</td>
<td>ND</td>
<td>a) Disinfection trial study</td>
</tr>
<tr>
<td>LH</td>
<td>0/594&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/120</td>
<td>0/180</td>
<td>0/108</td>
<td>0/176&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td>ND</td>
<td>0/180</td>
<td>0/149</td>
<td>0/152</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BH</td>
<td>ND</td>
<td>0/571</td>
<td>0/388</td>
<td>6/23&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0/56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glaucal Swabs PH</td>
<td>ND</td>
<td>ND</td>
<td>0/97</td>
<td>0/48</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BH</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0/1230&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI Tract PH</td>
<td>0/24</td>
<td>ND</td>
<td>ND</td>
<td>23/73&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>14/147&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BH</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>11/400&lt;sup&gt;f&lt;/sup&gt;</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hatchery Debris</td>
<td>ND</td>
<td>0/8</td>
<td>0/4</td>
<td>20/499&lt;sup&gt;g&lt;/sup&gt;</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead Germs (10 day)</td>
<td>0/1600</td>
<td>0/1918</td>
<td>0/1123</td>
<td>0/60&lt;sup&gt;h&lt;/sup&gt;</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryos</td>
<td>ND</td>
<td>0/678</td>
<td>0/486</td>
<td>0/28</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sexing Debris</td>
<td>ND</td>
<td>0/6</td>
<td>0/10</td>
<td>0/5</td>
<td>ND</td>
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**Remarks**
- a) Disinfection trial study
- b) *S. heidelberg*
- c) 123 pools of 10 each
- d) 22 *S. heidelberg*; 1 *S. infantis*
- e) 14 *S. heidelberg*
- f) 9 *S. san diego*; 2 *S. heidelberg*; 5 Ariz. paracolon
- g) 5 *S. heidelberg*; 15 *S. infantis*
- h) 4 pools of 15 each
- i) 5 *S. infantis*; 5 per pool
- j) 5 *S. infantis*; all samples pooled, 5 per pool
- k) 5 samples per pool
- l) *S. tennessee*
- m) *S. infantis*
- n) undetermined
- o) *S. bornum* (fish meal)
- p) *S. typhimurium v. copenhagen*

* Number positive/Number negative
** ND (Not Done)
THE STATUS OF THE STATE-FEDERAL HOG CHOLERA ERADICATION PROGRAM

INTRODUCTION

Fiscal year 1973 turned out to be a very critical period in the State-Federal Hog Cholera Eradication Program. It began on a note of optimism because of the achievements gained the previous year. It also ended with optimism when incidence of the disease was at an all-time low during the last 4 months of the fiscal year. The interval between these successes proved to be very trying and provided a costly lesson in disease eradication.

Disastrous outbreaks of hog cholera in the Midwestern, Northeastern, and Southeastern sections of the United States prompted the Secretary of Agriculture to declare hog cholera a National Emergency on October 11, 1972. On that same day Federal regulations were amended to allow the Federal government to pay a greater share of indemnities in Phase III States.

The declaration of emergency allowed for an intensified effort nationally in the form of needed manpower and funds to detect and stamp out the spreading infection, and resulted in the desired effect on hog cholera incidence. This report will record the progress achieved and problems which remain.

PROGRAM STATUS
(Figure 1)—Map Attached

By July 1, 1973, only one State remained in Phase III, which represents elimination of outbreaks, compared to three, 1 year earlier. Forty-nine States and Puerto Rico had attained Phase IV, protection against reinfection, status; 45 of these enjoyed Free status.

During Fiscal Year 1973, two States, Mississippi and Rhode Island, advanced to Free status. Seven States, Georgia, Indiana, Kentucky, Nebraska, Ohio, Tennessee, and Virginia suffered outbreaks involving spread of the disease and lost their Free status. All of these except Indiana and Virginia returned to Free status during the year. South Carolina, which lost Free status during FY 1972, regained it again in May 1973.

New Jersey lost Phase IV status during the year and regained it along with Indiana, North Carolina, and Virginia.

All these phase changes represent a net gain of one hog cholera Free State, and two States moving from Phase III to Phase IV.

Significant advances have taken place since July 1973 such as Virginia returning to Free status and Texas returning to Phase IV status, thus putting all 50 States and Puerto Rico in Phase IV, with 46 Free States.

In retrospect, it appears we have just regained the enviable position we were in at the beginning of FY 1973. There is a solid belt of hog cholera Free States across the Nation with the exception of Indiana in the North Central States. New Jersey, North Carolina, and Texas, which
also remain in Phase IV, all lie on the periphery of the country.

INCIDENCE

Hog cholera incidence increased from the program low of 76 positive cases in FY 1972 to 163 in FY 1973. (fig. 2).

Figure 2—Bar Graph

During the spring months prior to the beginning of FY 1973, hog cholera had again appeared in States in the Southeastern United States. As the summer months continued, the disease simmered undetected, then spread rapidly to Midwestern and Northeastern States during August and September. This rapid spread was attributed to several factors. Marketing practices which were in violation of program standards had gone undetected and allowed the spread of the disease.

Illegal movement of "feeder sows" spread the disease to one Midwestern State and illegal practices involving marketing of slaughter swine introduced the disease in a separate part of that same State, and an adjoining State.

Efforts at tracing movement of slaughter swine through markets and back to the herds of origin were thwarted by the lack of identification of these swine. Another impediment was the fact that nonapproved markets may legally ship slaughter swine interstate and in many cases are not required to keep records of such transactions. Diversion of slaughter swine back to farms for feeding and breeding purposes continued to be a problem.

Slaughter swine were marketed from herds later detected as positive herds, thus putting virus in the pork supply. The chain of infection was allowed to continue by failure to enforce regulations requiring proper heat treatment of garbage fed to swine.

Another factor contributing to the increased spread of the disease has been the reluctance of some States to depopulate exposed herds. This was a major consideration prompting the change in Federal regulations to allow the Federal share of indemnities paid in Phase III States to be increased to 75 percent.

Reporting and investigation of suspicious cases were increased from the previous fiscal year mainly due to increased incidence of the disease. However, only 523 suspicious reports were submitted to Veterinary Services during the last quarter of the fiscal year. This already dangerously low level of reporting has continued to decrease to this date.

It is also disturbing to report that a much smaller proportion of the positive cases this year were detected as the result of the reporting of sick swine by owners, veterinary practitioners, county agents, and other interested persons. Thirty-nine percent of the cases were found by State or Federal personnel in surveys around infected premises.

Subsequent to the declaration of emergency and in accordance with recommendations of this association, remedial actions were initiated in several areas. Market review teams inspected all markets approved to
handle swine for interstate shipment in 39 States on a priority basis. Deficiencies were detected in 29 percent of the 1,406 markets inspected. The few markets which were either unwilling or unable to correct the deficiencies had their approval removed.

Inspections were made of food waste feeding establishments in Connecticut, Delaware, Maryland, Massachusetts, New Jersey, North Carolina, Pennsylvania, South Carolina, and Rhode Island. Many instances of noncompliance with cooking standards were noted and corrected. On-the-spot technical advice was given to garbage feeders by an Agricultural Research Service (ARS) engineer to bring cooking equipment up to standards necessary to properly heat treat the product.

Temporary livestock inspectors were hired in several States to meet additional manpower needs. They assisted in inspection activities in markets and on garbage feeding premises and in the search for new garbage feeding premises. They were also utilized in task force operations in outbreak areas in Georgia, Indiana, Kentucky, New Jersey, North Carolina, Ohio, Pennsylvania, South Carolina, Tennessee, Texas, and Virginia.

The number of counties in which hog cholera was diagnosed in FY 1973 increased by 38. The number of States which experienced the disease increased by 10. The disease was diagnosed in 77 counties in 18 States and Puerto Rico. (fig. 3).

Figure 3—Map Attached

Seventy-one percent of the infected herds were reported by five States. (fig. 4).

Figure 4—Map Attached

Although the disease appeared in 18 States and Puerto Rico during the past year, incidence has been sharply reduced. Only 16 of the 163 positive cases diagnosed during FY 1973 occurred during the last 6 months of the year. This represents a 90 percent reduction of incidence.

Through extreme effort on the part of the States and the swine industry, these epizootics were brought under control and stamped out. As in the past, the task force approach was greatly relied upon. Many States placed embargoes on swine from States where infection was present. Larger area quarantines were utilized in many States to attain better control of swine movements. In December 1972, joint State-Federal quarantines were placed on the entire States of New Jersey and Pennsylvania because infected or exposed swine had moved through markets. After swine movements had been traced, these quarantined areas were quickly reduced in size to include only the area where infection was located. This decisive action effectively prevented further spread of the disease.

EPIDEMIOLOGY

(Figure 5)—Bar Graph Attached

Figure 5

For the first time since inception of the program, vaccination was not
incriminated as the source of a single case of hog cholera. More important is the fact that hog cholera incidence is down considerably from prior years when the swine industry was highly dependent on vaccination. This serves as a testimonial to the commitment of the industry and veterinary profession to total hog cholera eradication instead of hog cholera control.

The movement and marketing of swine continued to be a major problem as mentioned before. Movement of swine within States accounted for 15 percent of the cases reported and swine movement from State to State accounted for 8 percent.

Area or neighborhood spread was determined to be the source of 39 percent of the cases. However, this category is very closely related to interstate and intrastate swine movements. Approximately two-thirds of the cases attributed to area spread occurred in Indiana, in two separate outbreak areas. Hog cholera was introduced into both areas by the interstate movement of swine.

Recent insect-vector research has proven that hog cholera can be transmitted mechanically by mosquitoes, tabanids, stable flies, and house flies. Epidemiology indicated that insect vectors played an important part in the area spread of hog cholera encountered in Indiana last year, stressing the importance of vector control.

The feeding of raw or improperly cooked garbage was responsible for 21 percent of the cases reported during FY 1973 and was considered the source of cases in nine States and Puerto Rico. Due to the large numbers of swine in these herds, almost one-half of the total program indemnity was paid to the owners of these herds. Emphasis is made in this area because it is a well-known fact that proper heat treatment of the product destroys hog cholera virus.

The source of 17 percent of the cases reported was not established. Although more determined efforts were made to determine the source of all cases, epidemiology continues to become more difficult. As the disease incidence decreases, a greater proportion of the cases diagnosed are of a chronic, long-standing nature, making epidemiology more obscure and challenging. Despite this, the ability to determine the means of spread of hog cholera has continued at a high level.

OUTLOOK

The last two cases reported in the country occurred in Texas on May 2, 1973, and in Indiana on June 28, 1973. This makes a total of only two cases in the past 6 months through September 30, 1973.

Progress in this program has been most gratifying. Incidence of the disease is at a new low. Many of the obstacles in the path toward eradication have been removed, but some remain.

We must again guard against complacency. The goal of eradication was almost within our grasp at this time 2 years ago but we let it slip away. A total commitment is needed by the swine industry, veterinary profession, regulatory officials and allied industries to follow through and complete the task. Reporting of sick swine must be stimulated. We must
still “suspect hog cholera first.”

The program has progressed despite being hampered by the lack of mandatory swine identification. Regulations governing interstate movement of swine have been relaxed as States attain Phase IV and Free status, no longer requiring the separation of feeder and breeder swine from slaughter swine nor that slaughter swine be sold first when both classes of swine are in a market on the same day in these States. The relaxation of these regulations has allowed new inroads of transmission and caused several Phase IV and Free States to become reinfected. There is continued danger of this happening again. The program standards, however, have not been relaxed, and still dictate that these sound eradication measures be followed.

The movement of infected and exposed swine through markets and the feeding of improperly cooked garbage to swine continue to be the greatest threat to hog cholera eradication.

As eradication is progressing toward achievement, the prevention of reintroduction of the disease from other countries becomes more paramount. One year ago our import regulations were amended to prohibit the importation of live swine from countries not known to be Free of hog cholera. Pork and pork products from these countries were also prohibited entry unless processed in a manner that will destroy hog cholera virus. Increased border surveillance is also being initiated to prevent entry of the virus.

We possess all the necessary know-how to keep the disease at a low level and eradicate it totally. However, we still need total effort and a complete dedication to the cause. It is still necessary to report sick swine, identify all swine, separate feeders and breeders from slaughter swine in markets, and properly heat treat all garbage fed to swine. If present program standards are fully implemented and executed, there should be no deterrent to reaching our goal in the near future.
HOG CHOLERA ERADICATION PROGRAM

Cooperative State-Federal

Hog Cholera Eradication Program

JULY 1, 1973

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HOG CHOLERA REPORTED
Fiscal Year 1973

77 Counties in Which One or More Cases Were Reported
18 States and Puerto Rico

71% of Positive Hog Cholera Cases Found in Five States
FISCAL YEAR 1973
SOURCES OF HOG CHOLERA CASES

% OF POSITIVE CASES

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1/ Completed investigations
1973 REPORT OF THE USAHA COMMITTEE ON NATIONWIDE ERADICATION OF HOG CHOLERA

Chairman: D. L. SMITH, Indianapolis, Ind.
Co-Chairman: J. B. TAYLOR, Montgomery, Ala.


At this juncture, the Committee feels that surveillance is more important than ever. To implement and assure better surveillance—(1) field investigations and reporting should be continued in accordance with recommended State and Federal guidelines. This may call for more direct communications—reporting by telephone—and other simplifications of reporting procedures.

The Committee again recommends—(2) that all swine tissues submitted to State diagnostic laboratories be screened by the fluorescent antibody (FA) technique. Also, this screening data should be promptly reported by the States and thereby become available for national surveillance, evaluation and reporting.

On another aspect of surveillance, the Committee again recommends—(3) increased surveillance and other program activities in high risk areas of the United States—especially along the U.S.-Mexico border.

The Committee also recommends—(4) that surveillance be broadened by the increasing of personal and direct communications with: veterinary practitioners, swine producers, extension specialists, vocational agricultural leaders, 4-H officials, news media representatives, and other interested persons. The aim is to achieve better reporting and understanding of swine diseases.

Increased personal and direct communications with meat inspection personnel by State and Federal veterinarians should also be stimulated, to encourage submission of tissue samples from diseased swine.

The feeding of raw or inadequately cooked garbage to swine continues to be a threat to the eradication of hog cholera. On this issue—(5) the Committee reemphasized the importance of adequate enforcement of State laws and regulations dealing with garbage.

The Committee—(6) reaffirms its position as to the urgent need for a National swine identification program, taking into account the continual problem of “offs,” “lights,” “culls,” and other “junk” swine which are often diverted from slaughter channels back to farms.

The Committee—(7) again recommends that hog cholera market stan-
dards currently prescribed for Phase II States be reinstituted and maintained in all States no matter what their present program phase may be. The Committee also reaffirmed its recommendation that these market standards—as contained in Memorandum 561.1 of July 23, 1971—be maintained for a period of three years after the last State in the Nation is declared free of hog cholera.

The Committee—(8) notes that its recommendation that all States should be regarded as “infected” or “free” has not been implemented and urges continued and prompt action toward this goal.

Lastly—(9) the Committee recognizes the importance of crediting the industry for its outstanding achievement in attaining a “hog cholera free” Nation—possibly by May 1974. While recognizing this achievement, however, the Committee urges the continuing of program activity at present levels and a continuous future surveillance. Complacency must not be allowed to negate the achievement.
A STUDY OF SWINE VESICULAR DISEASE IN ENGLAND*

Dr. Tobin, Distinguished Guests and Colleagues;

You have heard this morning about the laboratory and research aspects of Swine Vesicular Disease. I would now like to tell you of some field observations concerning the eradication of this disease from Great Britain. As you know, this disease was first reported in Italy in 1966, and later in Hong Kong in 1970. It was a disease which clinically resembled foot-and-mouth disease, but which only attacked swine. Great Britain first reported the disease as a foot-and-mouth disease outbreak on December 11, 1972. It was later found to be the same disease as that in Italy and Hong Kong.

A meeting was held at the Food and Agriculture Organization (FAO), in Rome, on January 9, 1973, concerning this disease and it was found that Poland, Austria, and France, also had the disease. It was recommended at this meeting that the disease should be made notifiable and that stamping out procedures be put into effect by the slaughter of affected and in-contact pigs. Thirteen cases were reported by Great Britain in 1972, and by April 1, 1973, there were 86 cases reported. Because this was a new disease and the United States had no experience with control or eradication of Swine Vesicular Disease, I was sent to Great Britain for 60 days, to observe their eradication program. I arrived in London on April 7, 1973.

To give you an idea of the impact this disease had on Great Britain, I should tell you that England, Wales, and Scotland comprise 88,745 square miles of land which is a little larger than the State of Kansas. It has a swine population of 8,600,000 pigs. As of August 21, 1973, 6.4% of these pigs had been destroyed because they were affected with or had contact with Swine Vesicular Disease (SVD). A recent communication has indicated 4 additional outbreaks were confirmed on October 11 & 12, 1973. Thus, Great Britain has experienced a total of 107 outbreaks since December 11, 1972.

ORIGIN

The disease was first reported in garbage fed pigs in Staffordshire, England. There were five primary cases which were confirmed from December 11 to 19, 1972. All of the primary cases were garbage fed and the source was thought to be imported pork of common origin. After the initial primary cases, those cases which were later attributed to garbage feeding were thought to have been due to recycling of the infection from infected animals which had gone to slaughter.

*Presented at USAHA meeting in St. Louis, Missouri, October 19, 1973.
Presented by: Gary P. Combs, D.V.M.
Principal Staff Officer, Technical Support
USDA, APHIS, Veterinary Services, Emergency Programs.
DIAGNOSIS

If a reported case had vesicular lesions and was associated with a known outbreak of SVD, diagnosis was made clinically. On the other hand, if the case was vesicular but there was no known association with another SVD case, laboratory tests were needed to differentiate from foot-and-mouth disease (FMD). Where doubt existed as to diagnosis, the more severe FMD restrictions were placed on a described area and no movement could take place except by permit for cattle, sheep, or swine. Once the disease had been diagnosed as SVD, the restrictions were applied only to swine. The investigator of the suspect case telephoned a report to the Veterinary Services Headquarters, located at Tolworth, where a decision was made as to the need for laboratory testing and as to the diagnosis of the case. Clinical signs observed were a temperature reaching 104-106°F. Lameness and tenderness of the feet, vesicles on lateral aspect of the coronary band and separation of horn of foot often occurred. Five to ten percent of the pigs had snout vesicles, and there was a morbidity of 65-90%, with negligible mortality.

REPORTING

One of the unusual aspects of the disease is that although many lesions or vesicles may be present, the swine do not show overall as severe a lameness as they do with foot-and-mouth disease. Consequently, farmers and inspectors quite easily missed seeing the early signs of the disease in their herds. As a result, a greater percent of the cases were found by traceback and those cases which were reported by owners, had been present for a good while. Veterinarians knowing the infection was present in a herd often missed affected pigs.

EPIDEMIOLOGY

One of the major factors of concern about this disease is the resistance of the virus outside of the host. It takes a pH of less than 2 or greater than 12.5 to inactivate the virus. A slurry of water and manure was held at 4°C since December 15, 1973, and as of June 1st the virus titer had not decreased 1 log.

FACTORS OF SPREAD

The major means of dissemination of the virus appears to be direct contact with infected animals or contaminated materials. Aerosols although produced by affected pigs do not appear to be significant from the standpoint of this disease. Of all the cases which have occurred, there was only one case where lateral spread might have taken place. Of the first 90 cases; 5 were garbage fed primaries, 13 were due to the movement of pigs to market (assembling), 18 were market contacts with infected pigs, 19 cases concerned movement of pigs in previously contaminated trucks, 7 cases involved direct movement from infected premises,
2 cases involved contact with infected pigs in trucks, 16 cases incriminated the feeding of garbage (49 of the 90 fed garbage), 1 case each was due to personnel movement and local spread, 3 cases were due to a re-occurrence of the disease after restocking and 5 cases were obscure.

It is interesting that swine hauled in trucks as long as three days after infected swine had been hauled, came down with the disease, even after a cleaning of the vehicle had occurred. Essentially, the disease spreads as does hog cholera except that there is very little area spread. However, because of the durability of the virus, the chances of reoccurrence after restocking and the need for more attention to cleaning and disinfection are greater for SVD than has been necessary for hog cholera.

**DISPOSAL**

Swine are destroyed humanely by the use of gun or captive bolt pistol. They are then pithed by inserting a metal rod into the brain through the hole made by the firearm. The swine are then either burned, buried, or sent to a rendering establishment depending upon the circumstances of each case. The use of the burial method may cause a future problem if swine continue to have contact with the area where burial takes place. After a complete clean up and before final release of quarantine, earthworms were picked up from an infected premises near where infected swine had been buried. These earthworms were found to harbor the virus both on the outside their body and in their gut, indicating mechanical contamination with contaminated soil.

**CLEANING AND DISINFECTION**

Great efforts were expended in the cleaning of infected premises. This requires hard work over a long period of time to properly clean an infected premises. Initially there was not much known about what disinfectants should be used with the disease. When I left the procedure was to have a light spraying initially of grossly contaminated material with FAM, a locally produced disinfectant, then a burning of straw, hay and combustible materials, and the burial or rendering of manure, carcasses, garbage, etc. A very complete clean-up and washing was then done, with several sprayings and soakings with FAM. A final spraying with 1% NaOH (lye) was done. Organic matter still left was subjected to the use of a flame gun or blow torch. After a period of time (8 weeks after completion of initial C & D), a limited restocking took place, and the premises were allowed to function normally.

**WHAT SHOULD WE DO?**

I feel that since this disease is now in several countries of Europe and since a major eradication program is only being conducted by Austria and Great Britian, and because the virus of SVD is so durable outside of the host, that it is only a matter of time before we will be faced with an introduction of this disease. I believe that swine fed on waste food present the greatest hazard for the introduction of this disease into the
United States and believe that inspectors regulating these premises should be acutely aware of the clinical signs of SVD. An education program urging prompt reporting should be aimed at both our Veterinary Practitioners and swine raisers.
First of all, this morning I propose to bare my soul and confess that I plan to explore with you a different orientation in the health area. I do not propose to deal with cause, effect and cure for different individual diseases. I do plan to look at swine health from the standpoint of management—the one who owns the pig.

Our goal in the development of a business is—pure and simple—the returns to capital and our own efforts. Since we will be applying systems thinking, perhaps we should start by merely mentioning in passing that the organization and structure of the business is always, to us, of first concern in the development of a swine production unit. After the decision is made as to type of organization preferred—such as the corporate entity, we would move to the internal structuring of the business. After decisions such as possible divisions of the company would come the choice of enterprise or enterprises. For us, this would be swine production which would be thought of as a means of converting energy in the form of feed grains into a product in higher demand, pork.

Whether feed grains are purchased or produced is beside the point; feed conversion remains the central part of the enterprise and focuses attention on the importance of this conversion process. My position is—and I can’t take time to substantiate it this morning—is that health level is of greatest importance in feed conversion. Health level then, becomes the measuring stick to analyze the system and each component. This comes first in consideration.

Because of our goal of returns to capital, we must have real efficiency in the business, starting with favorable feed conversion; we must also have sufficient volume to permit enough capital to be used to make it a really worthwhile business. This suggests to me the importance of a swine system, then, that is essentially open-ended.

This means that we cannot be spending our life “putting out fires”, so to speak, in the health area. We need, in order to survive, to design a system where health is engineered into the system and disease problems out—as far as possible—when the business is in the planning stage. We refer to what we would define as real disease prevention. All too often disease prevention or preventive medicine is thought of as allowing disease to gain a foothold, but attempting to deal with it before it surfaces. This, to me, is in no way prevention, and a distinction needs to be drawn.

Because of the concept of designing health in—disease out—of the system we called it “a built-in approach” to health merely for want of a better description. We have discovered no great new basic truths, but
only are making this application of facts you all have known for years.
For logical discussion, we refer to a three point program; you will say we are over-simplifying. We do not intend to, but recognize that most of what we are aware of falls under these headings. They are:

1. Isolation
2. A Dry Environment
3. Lowered Stress, Physiological and Psychological

**ISOLATION**

By isolation we refer to the isolation of the pig from pathogenic organisms of the outside world, and also from the microflora of the herd itself. An explanation is not needed to this group regarding importance of maintaining health by using this tool. However, use of the principle, starting with a closed herd, has certainly been overlooked when we examine the economic impact of neglecting the concept. The cost is insignificant in the case of our business; the rewards are not only an opportunity for feed efficiency, but also an opportunity to plan a business which can run on a continuing basis, year after year, without the gyrations of introduction of disease. The impact of disease on the profit and loss statement is pretty well recognized by producers. There is a choice: fight bloody scours, T.G.E., each disease, step by step from scratch to immunization program with the economic consequences, or use this tool to deal with all transmissable diseases of the outside world.

We mentioned isolation from the microflora of the herd itself. In 1960 we measured the impact of turning sows out of farrowing crates to feed as a group—and to bring back a new mix of microflora each time. The differences were so startling that isolation by stage and from others in the herd was incorporated into the system. By use of the slotted floor the pig is even isolated to a degree from his own microflora when the floor separates it from its waste.

**A DRY ENVIRONMENT**

Even though we use isolation to avoid the introduction of pathogenic organisms, we recognize that we must deal with the microflora indigenous to the normal pig. A dry environment has been emphasized in order to bring about a situation under which bacteria simply do not thrive.

We will not turn this system into rule of thumb or a “how to do it” handbook; we feel that we must deal with concepts that will apply—if they are true—in any land under changing conditions. We are talking about as efficient a ventilation system as possible, but the total slotted floor comes back again in this point as a must; it is essential if we are to be successful in achieving the goal of a dry environment. We are not talking about any washing of pens or buildings whatever except possibly the farrowing pens or nursery pens. In this case our evidence is not overwhelming for or against washing; this might depend on the tailoring of system to individual cases. We are convinced, though, that a dry environment is the item of importance.
LOWERED STRESS

We lower physiological stress by providing optimum conditions of housing which avoid heat and cold extremes, as well as guarding against high humidity which we just referred to on another point. Reduced exertion also lowers stress and by lowering these physiologic stress items feed efficiency is enhanced; optimum conditions are provided for maintaining a high degree of health.

The need to reduce social or physiological stress has not been so generally recognized. It is seen in fighting, tail biting and stomach ulcers. Such stress is apparently involved in making pigs more vulnerable to certain diseases. What we are talking about is basically pig happiness. Because the economic consequences of stress of both kinds are totally unacceptable, the built-in-approach to health is used to design it out. Examples of application are the family unit, small numbers per pen, tethered sows.

HERE IS HOW THE SYSTEM LOOKS

First of all it is highly isolated with all its implications. New genetic lines are introduced by artificial insemination. Casual help is not used in the operation; only employees who can be trusted not to be involved in an isolation break. Employees change clothing on the farm for work there; step into work rubbers at different building entrances; drive farm truck to market and change to market rubbers when leaving the truck at the tailgate.

The building are all environmentally controlled with total slotted floors, with exception of the breeding barns where partial slots are optional.

The flow chart shows sows going from the breeding barn to the farrowing barn. The family unit is maintained here and at three week weaning the sows go back to the breeding barn for breeding at first heat. Weaned pigs maintain their family group as they move to the nursery. At approximately 50 pounds they go to the finishing group where an economic compromise can be made if desired by placing up to 20 or 30 pigs of the same size and age together; these would be litter mates as far as practical.

This sketch has shown only a few views as an illustration; the concepts of the systems approach are the vital ones.

HOW THE SYSTEM WORKS

Perhaps we should say the system—any system—does not work; it must be worked. If the concepts are understood and applied, it is a system where returns to capital can be achieved; it is relatively "open-ended". If basic principles are not understood, if instead, a how-to-do-it approach is attempted, it will not achieve its goal.

Because disease is designed out of the system as far as possible, feed conversion is enhanced. Diseases are not introduced, indigenous microflora are controlled, scours, for instance, are not a problem. The system permits full use of pens and buildings without emptying out and cleaning
up or cooling down.

The cost? What do we charge to health? If we consider housing to be necessary regardless, then the housing cost per hundred-weight marketed will not be higher, because of the greater usage permitted. Feed conversion is enhanced, and that is how we set out to achieve returns to capital.

This topic has been difficult to condense logically because of the many inter-relationships of the points which we could but briefly touch. What we attempted to point out was simply this:

The goal—returns to capital and our own effort.

How Achieved—convert feed grains into pork.

Desirable Feed Conversion—attained with high health level.

High Health Level—attained with a swine production system using built-in-approach to health.

Built-in Approach to Health—one that designs health in—disease out—as far as possible, when the system is planned.

This, then, is how management looks at swine production utilizing systems thinking.
ERYSIPELAS IMMUNIZING PRODUCT REVIEW

M. H. Bairey and J. H. Vogel

INTRODUCTION

There are 5 different immunizing products being manufactured under U. S. Veterinary Biologics License for the prevention of Erysipelas in swine.

Table 1

In the fiscal year 1973, three licensed manufacturers produced 51 serials of vaccine for market totaling about 10.6 million doses.

Of these vaccines, one is an injectable live avirulent vaccine. The second is an injectable live modified vaccine and the third is an oral live avirulent vaccine.

A killed bacterin was produced by 18 licensed manufacturers in FY-73 with a total of 262 serials and about 36 million doses.

Four licensed companies produced 106 serials of antiserum in FY-73 containing over 40 million milliliters.

EVALUATION PROCEDURES

Table 2

The Standard Assay method for Erysipelas vaccines, both avirulent and modified live culture, is a vaccination, challenge swine protection test. Four pigs are used for both vaccinated and control groups. A single vaccination is given 21 days prior to challenge inoculation and the animals are observed for 7 days postchallenge. For a valid test 75% of the controls must demonstrate signs of infection. A serial of vaccine is satisfactory if 75% of the swine are protected.

The standard assay method for evaluating erysipelas bacterins in swine is the same as for vaccines.

There is a standard assay method for evaluating erysipelas bacterins in mice. This is also a vaccination challenge procedure using groups of at least 16 mice and utilizing threefold dilutions of the bacterin to be assayed and comparing it with threefold dilutions of a Standard reference Bacterin. The challenge inoculation used is a 100 LD50 per-mouse dose. The validity of the test is dependent upon the dilutions being in the marginal protective range for mice with no more than two of three dilutions being either above or below 50% protection. The interpretation for acceptance or rejection is based on the performance of an unknown bacterin in comparison to the Standard Reference Bacterin. A bacterin being assayed is accepted as satisfactory if it protects no less than 87.5% of the protection produced by the Standard.

The assay for antiserum is a statistical two-stage procedure in mice. Antiserum is injected into groups of 40 mice each which are challenge inoculated 24 hours later with a live erysipelas culture that is 100% lethal for untreated mice within 7 days. Eighty five percent of the treated mice must survive for 14 days for the serial of antiserum to be
satisfactory. Second stage assays are conducted on serials that protect between 72.5% and 85% and the accumulated results are used to determine acceptance. The acceptance levels were established using a reference antiserum which protected 75% of serum treated swine against a 75% infective challenge given 24 hours after the serum injection.

EVALUATION RESULTS

Table 3

Over the past 4½ years we have evaluated 6 serials of vaccine for potency in swine. At least one serial of each type of vaccine and at least one serial from each producer. Five serials protected at least 3 of 4 pigs and one serial protected only 2 of 4 for 83.3% satisfactory testing rate.

During this same period of time 49 serials of bacterin were assayed in swine. Forty six of these serials protected at least 3 of 4 pigs while 3 did not. This was a 93.9% satisfactory testing rate. Twenty six serials of bacterin were assayed by the mouse test with 20 satisfactory and 6 unsatisfactory for only a 76.9% satisfactory testing rate.

One hundred thirteen serials of antiserum have been assayed in mice with 93 serials being satisfactory for a satisfactory testing rate of 82.3%.

The variation in results of the bacterin assay in swine as compared to the bacterin assay in mice stimulated a correlation test in 1971 using the same serials in both tests.

Thirteen different serials of swine erysipelas bacterin produced by 7 different manufacturers were examined. All serials were subjected to the swine protection test and the mouse protection test as described in the APHIS Standard Requirements.

Animals were determined to be susceptible to E. insidiosa by the use of unvaccinated controls obtained from the same source as the vaccinated animals.

All bacterins produced satisfactory protection in swine. According to the mouse protection test, only 7 of the 13 serials were satisfactory, a 53.8% correlation of testing procedures.

This work supported the host-animal swine protection test as an effective means of evaluating erysipelas bacterins. The lack of correlation in the mouse protection test cast doubts as to the significance of this test in evaluating swine erysipelas bacterins. Because assay by the mouse test only would disqualify many serials of bacterin that would protect swine against an infective challenge, the mouse test was discontinued by the Veterinary Biologics Laboratory.

However, because of the difficulty and expense of obtaining susceptible swine and the unavailability of sufficient testing facilities a laboratory animal assay procedure was advantageous to both the biologics industry and the Veterinary Biologics Laboratory. Work was continued by Veterinary Biologics Laboratory to develop a meaningful mouse test.

The mouse challenge culture was changed so that the same culture was used in mice that was used in swine. The dosage in mice was increased from 0.1 ml to 0.2 ml. A new reference bacterin was prepared and evalu-
ated in swine and in mice. The threefold dilutions were revised to include a 1:10 dilution where previously a 1:30 dilution had been the lowest dilution.

Table 4

The new reference bacterin protected at least 75% of the swine using either a 2 ml or 5 ml dose or a 1:2 dilution of the 2 ml dose. A 1:4 dilution of the bacterin protected between 25% and 50% of the swine and a 1:10 dilution of the bacterin protected no more than 1 out of 4 animals.

Table 5

This reference bacterin gave greater than 50% protection at a 1:10 dilution, 50% or more protection 2 out of 7 times at the 1:30 dilution; less than 50% protection 7 out of 7 times at the 1:90 dilution and no more than 20% protection 7 out of 7 times at the 1:270 dilution.

This information indicated to us that our reference bacterin did protect swine adequately but was on the weak side because it would give reduced potency when diluted as little as 1:4. Also the mouse potency test correlated with the swine potency test under our revised assay procedures.

One assay has been completed using 4 commercially manufactured serials of erysipelas bacterin in both the swine and mouse potency test. All 4 serials were satisfactory by both the swine test and the mouse test.

These correlation studies will be continued and if the correlation of the mouse test to the swine test can be repeated a significant number of times, it will be used to monitor serials of swine erysipelas bacterin for potency.

SUMMARY

From our data we would have to conclude that the immunizing products for erysipelas in swine are at least 80% effective.

Table 6

We were not able to evaluate vaccines and bacterins at as high of testing rate as would be desirable. The testing of only 2.6% of the vaccine serials and 4.2% of the bacterin serials may not be a good estimation of the potency levels of these products.

This fact can be attributed to not having a rapid economical assay method as we have for the antiserums where we tested nearly ¼ of the serials produced.

The development of a valid mouse potency test for the bacterins and vaccines should increase this testing percentage to a significant level for a more accurate monitoring of the efficacy of these products.
Table 1. Summary of Erysipelas Products Manufactured in Fiscal Year 1973

<table>
<thead>
<tr>
<th>Product</th>
<th>Number of Manufacturers</th>
<th>No. of Serials Marketed</th>
<th>No. of Doses Marketed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccines</td>
<td>3</td>
<td>51</td>
<td>10.6 million</td>
</tr>
<tr>
<td>Bacterins</td>
<td>18</td>
<td>262</td>
<td>35.9 million</td>
</tr>
<tr>
<td>Antiserum</td>
<td>4</td>
<td>106</td>
<td>40.15 million ml</td>
</tr>
</tbody>
</table>

Table 2. Assay Methods for Potency of Erysipelas Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Test Animal</th>
<th>Group Size</th>
<th>Assay Procedure</th>
<th>Acceptance Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccines</td>
<td>Swine</td>
<td>4</td>
<td>Vaccination/challenge</td>
<td>75% protection</td>
</tr>
<tr>
<td>Bacterins</td>
<td>Swine</td>
<td>4</td>
<td>Vaccination/challenge</td>
<td>75% protection</td>
</tr>
<tr>
<td>Bacterins</td>
<td>Mice</td>
<td>&gt;16</td>
<td>Vaccination/challenge of 3-fold dilutions</td>
<td>&gt;97.5% of protection of the Standard Reference Bacterin in mice</td>
</tr>
<tr>
<td>Antiserum</td>
<td>Mice</td>
<td>40</td>
<td>Serum treatment and challenge</td>
<td>85% protection in mice</td>
</tr>
</tbody>
</table>

Table 3. Results of Erysipelas Evaluations by Veterinary Biologics Laboratory

<table>
<thead>
<tr>
<th>Product</th>
<th>Serials Assayed</th>
<th>Serials Satisfactory</th>
<th>% Satisfactory of Serials Assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccines</td>
<td>6</td>
<td>5</td>
<td>83.3%</td>
</tr>
<tr>
<td>Bacterins (Swine)</td>
<td>49</td>
<td>46</td>
<td>93.9%</td>
</tr>
<tr>
<td>Bacterins (Mice)</td>
<td>26</td>
<td>20</td>
<td>76.9%</td>
</tr>
<tr>
<td>Antiserum</td>
<td>113</td>
<td>93</td>
<td>82.3%</td>
</tr>
</tbody>
</table>
**Table 4. Results of Reference Bacterin When Evaluated in Swine**

<table>
<thead>
<tr>
<th>Reps</th>
<th>Undiluted 5 ml dose</th>
<th>Undiluted 2 ml dose</th>
<th>1:2 dilution of 2 ml dose</th>
<th>1:4 dilution of 2 ml dose</th>
<th>1:10 dilution of 2 ml dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*4/4</td>
<td>3/4</td>
<td>3/3</td>
<td>1/4</td>
<td>1/4</td>
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<tr>
<td>2</td>
<td>3/4</td>
<td>4/4</td>
<td>2/4</td>
<td>1/4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3/4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Noninfected/Total Assayed

**Table 5. Results of Reference Bacterin When Evaluated in Mice**

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>Reps</th>
<th>Swine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:10</td>
<td>1:10</td>
<td>*13/20 18/20 17/20 18/20 Undil 3/4 4/4 3/4</td>
</tr>
<tr>
<td>1:30</td>
<td>1:30</td>
<td>5/20 4/20 7/20 7/20 10/20 4/19 12/20 1:4 1/4 2/4</td>
</tr>
<tr>
<td>1:90</td>
<td>1:90</td>
<td>1/20 3/20 0/20 0/20 1/20 1:19 3/20 1:10 1/4 1/4</td>
</tr>
<tr>
<td>1:270</td>
<td>1:270</td>
<td>1/20 3/20 0/20 0/20 0/20 0/20 0/20 0/20 0/20</td>
</tr>
</tbody>
</table>

* Survivors/Total

**Table 6. Summary of Product Testing by Veterinary Biologics**

<table>
<thead>
<tr>
<th>Products</th>
<th>% Satisfactory of Serials Tested</th>
<th>Estimated No. of Serials Produced</th>
<th>No. of Serials Tested</th>
<th>Testing %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccines</td>
<td>83.3%</td>
<td>230</td>
<td>6</td>
<td>2.6%</td>
</tr>
<tr>
<td>Bacterins</td>
<td>93.9%</td>
<td>1180</td>
<td>49</td>
<td>4.2%</td>
</tr>
<tr>
<td>Antiserums</td>
<td>82.3%</td>
<td>480</td>
<td>113</td>
<td>23.6%</td>
</tr>
</tbody>
</table>
ON THE CONTROL OF TRANSMISSIBLE GASTROENTERITIS OF SWINE
E. O. Haelterman, D.V.M., PH.D.

With the approaching eradication of hog cholera, transmissible gastroenteritis (TGE) emerges as the most important indigenous viral disease of swine. In spite of many years of effort, no entirely effective and safe method of immunization against TGE has been developed; and, in common with almost all other viral diseases, there is no effective chemotherapy against TGE virus. The current success of the hog cholera eradication program and the accomplished eradication of such diseases as foot and mouth disease and vesicular exanthema of swine emphasize the fact that organized control programs constitute the most effective and least expensive means of preventing losses of livestock from diseases susceptible to such an attack. Some of the known features of TGE suggest that a control program based on identification of infected herds and premises and their quarantine for relatively brief periods of time could greatly reduce the prevalence of TGE in the swine population.

Although there has been some controversy, it is now generally accepted that TGE is caused by a single virus which has the physico-chemical characteristics of coronaviruses. Characterization of isolants in a number of foreign countries including England (4) and Germany (17) and Japan (9) as well as a number of laboratories in the USA (2, 3) indicates that all natural strains are antigenically the same or very closely related. This facilitates serological identification of the virus in a control program and would obviate many of the problems associated with the control of diseases caused by viruses having several antigenic types.

TGE virus is relatively fragile outside of the host. It is rapidly inactivated at warm temperatures and by radiation (3, 8). Except during cold and cloudy weather it is unlikely to survive in contaminated premises for any extended time. This has been offered as an explanation for the relatively rare spread of TGE between herds in the warm-weather-months.

The natural host range of TGE virus appears to be very limited. Other than swine, only dogs and foxes (7) have been shown to be capable of being infected with TGE virus. Dogs do not become ill on infection with TGE virus but the virus has been recovered from their stools for 10 to 14 days after feeding them intestines of infected pigs. Since TGE-infected dogs do not develop diarrhea it would seem that the virus shed in solid stools of dogs would be unlikely to be consumed by swine. Thus dogs may have a rather minor role in the epizootiology of TGE, but this deserves further study. A recent report by Cartwright in England (5) indicated that rises in TGE virus neutralizing antibody titers were found in the sera of dogs which had experienced an episode of diarrhea and vomiting. Virus was not recovered in this case. In another study Norman, McClurkin and Stark (14) found TGE antibody in dogs that were not known to have been exposed to swine. Dog to dog transmission of TGE virus has not been proven, but it should be tried.

Starlings have been given what appears to this author a disproportionate share of credit for the spread of TGE. In the only report on experimental transmission of TGE by starlings, Pilchard (16) was able to infect pigs with the drop-
pings of these birds for up to 30 hours after feeding them massive doses of TGE virus in the form of intestines of infected pigs. Again there is need for further study of the role of starlings in the epizootiology of TGE. They may serve as mechanical vectors of the virus during severe winter weather when they are driven into farm lots to find feed but it is very unlikely that they are involved in the persistence of virus from one epidemic period to another.

Other farm animals appear to be refractory to TGE. Thus, in contrast to such diseases as foot and mouth disease which spreads readily between a variety of ruminants as well as swine, TGE should be relatively tractable.

The highly seasonal occurrence of TGE should operate in favor of its control. Well over 90% of recognized outbreaks occur in the cold-weather-months. The peak of incidence coincides with cold temperatures and low solar radiation and not with the numbers of new susceptible pigs which are highest in September, March and April in the Midwest (6). This suggests that the virus is maintained through the summer in relatively few niduses of active infection, in frozen storage of meat or infected intestines used by a few swine raisers to immunize sows or possibly in swine which become long term carriers of virus.

There is little evidence that swine become long term carriers of TGE virus. Observations in the field by Bohl (3), Ferris (6) and by us (7) have shown that repeated outbreaks of TGE in herds are very unusual where definite breaks in farrowing are made. Bohl has reported that TGE may become endemic in herds in which continuous farrowing is practiced. In these herds, most sows are immune and their pigs do not become infected until weaning when they are deprived of lactogenic immunity. Very little death loss is experienced in such herds but they may provide a nidus of active pig-to-pig infection which maintains virus through the year.

Another situation in which TGE virus could be maintained is in feeder pig concentration points where new susceptible pigs are added continuously. TGE in pigs of this age presents as a relatively trivial diarrhea which might not be recognized as TGE but, again, this type of operation could provide for the maintenance of virus which could be disseminated widely when meteorological conditions favor its survival outside of the host.

The available evidence indicates that pigs do not shed infective TGE virus for long periods after infection. A very early report by Lee et. al. (11) indicated that virus could not be recovered from pig stools for more than 2 weeks after infection and in many pigs the stool was not infectious at one week after infection. There is evidence that virus may be maintained in the intestinal mucosa for several weeks after infection. It is probably inactivated during passage through the colon or neutralized by antibody secreted in the intestines. It is possible that such pigs could shed virus in infectious form if they developed severe diarrhea or under conditions of stress. This is a very important subject for further research. A recent report by Lehnert and Uhleman (12) of East Germany provides added evidence that the period during which pigs transmit virus is short. This work involved extensive serological testing of pigs for TGE on over 100 farms. Among their findings were that pigs introduced on farms 3 weeks after signs of TGE had disappeared remained serologically negative; that in the majority of farms pigs born 4 weeks
after mortality of pigs stopped remained negative; and in two herds of fattening pigs, serologically negative pigs introduced 7 and 14 days after an outbreak did not develop TGE antibody.

Recognizing that there are gaps in the epizootiological information on TGE, most available information appears to support the contention that almost all TGE spread is either directly or indirectly, through the feces of acutely ill or recently convalescent swine; that swine rarely if ever shed virus for more than a few weeks after infection; and that TGE spreads from the relatively few sources where virus is maintained through the summer by pig-to-pig and herd-to-herd transmission during the winter when conditions favor the survival of the virus. If these assumptions are valid, an organized program involving identification and quarantine of TGE infected herds and premises for rather brief periods, perhaps as short as three weeks after the last evidence of active infection, could markedly reduce the prevalence of TGE in the swine population. Further, while it is much too early to consider an eradication program for TGE, the information accumulated in a quarantine program could eventually provide a basis for the eradication of TGE.

The details of an identification and quarantine program would have to be worked out through considerable discussion between representatives of the swine industry, state and federal regulatory agencies, and veterinarians with expertise of the subject. A start could be made with a pilot program involving an area such as one of the midwestern states. Such a program might include:

1) Investigation of natural TGE outbreaks as they occur on farms to determine the circumstances leading to introduction of virus and the persistence of infection in herds under various systems of husbandry and management.

2) Periodic checks on enterprises such as swine concentration points which could be involved in the persistence and spread of virus.

3) A study of the impact of quarantine on farms and other enterprises to determine what the cost of maintaining quarantine would be in various types of enterprises.

4) Refinements of diagnostic procedures. The diagnosis of TGE in newborn pigs based on history, lesions and the fluorescent antibody technique is relatively rapid and accurate. Diagnosis where only older pigs are involved is more difficult since pigs are usually not available for necropsy. A test capable of identifying virus in the feces of acutely ill pigs would meet the need for identification of infected herds without newborn pigs.

5) A serological surveillance of a large number of herds to serve as an index of the prevalence of TGE in the area. This would serve as a baseline to assess the results of future control program and could provide important information on unrecognized TGE infection such as may occur in herds that are infected at times when no newborn pigs are present.

6) A warning system such as has been used in England since 1970 (1). Under this system farmers and veterinarians are notified by media of the existence of active TGE infection within townships. The specific premise involved is not identified in this system but the warning serves to alert farmers to the presence of the disease in their area and advises them on precautions to prevent infection.
of their herds.

One of the problems in establishing a disease control program is the need for trained personnel to carry out the necessary investigations. The state and federal officials organized and trained for the hog cholera eradication program have done and are doing a monumental job of controlling this most important disease threat to the swine industry. Surveillance of hog cholera must be continued and even intensified during this hopefully final stage of its eradication. Eventually, however, the urgency for hog cholera investigations should decrease and more of the expertise in epidemiology developed in the hog cholera program may be diverted to TGE and other infectious diseases of swine.

REFERENCES

eases of Young Swine.


REPORT OF TRANSMISSIBLE DISEASES OF SWINE COMMITTEE

Chairman: E. A. Butler, Des Moines, Iowa
Co-Chairman: R. D. Ledgerwood, West Plains, Mo.


The Transmissible Diseases of Swine Committee was attended by some 30 people including 11 Committee Members.

Reports were made concerning Erysipelas vaccines and Assay Methods reviewed as to determination of potency from both regulatory and commercial aspects.

The Committee recommends that the increased emphasis be placed on study of the Epizootiology of TGE to serve as a basis for control by sanitary measures. A report by the TGE Sub-Committee is attached to this report.

It is recognized that swine dysentery is a major disease of swine. There has been a break-through in the etiology study and some break-throughs in treatment; however, much more work remains. Control programs in some states have been reasonably successful.

The methods employed in feeder pig movement are contrary to the principles of good health management, and perhaps a study should be made toward better marketing practices to prevent disease dissemination.

The Committee recognizes the importance of the principle of isolation and would encourage all producers to employ isolation and sanitation as an aid to disease prevention.

REPORT OF THE SUB-COMMITTEE ON TRANSMISSION OF GASTROENTERITIS OF SWINE

TGE continues to be a major threat to the health of the swine industry. Although a safe vaccine is currently available, its efficacy is limited. The sub-committee encourages the development of a more effective vaccine.

The sub-committee recommends that increased emphasis be placed on study of the epizootiology of TGE to serve as a basis for its control by sanitary measures.
Among the points deserving particular attention are:

1. A study of the carrier state in TGE-infected swine to determine its duration and whether stress or diarrheic conditions may result in the shedding of virus in infective form.

2. Virus neutralization tests appear to accurately reflect past experience with TGE. There is a need to determine whether there is a relationship between serological titers and a carrier state. Until such a relationship is established, it is recommended the current proposal to limit the export of serologically positive swine not be implemented.

3. The diagnosis of TGE in newborn pigs by means of history, lesions and fluorescent antibody has proven to be accurate and rapid, but diagnosis in older pigs is more difficult. An accurate test based on detecting TGE virus in the feces of older swine is needed.

4. More information is needed on the prevalence of TGE in the swine population. The sub-committee recommends a serological survey to determine the prevalence of TGE in the swine population.

Attempts to treat and prevent TGE with Antiviral drugs have been unsuccessful but efforts to develop and test such compounds are encouraged.

G. Lambert
M. Ristic
T. W. Tomoglia
E. O. Haelterman, Chairman
SEROLOGIC CHARACTERIZATION OF SELECTED BLUETONGUE VIRUS STRAINS FROM THE UNITED STATES

T. L. Barber and M. M. Jochim*

The plurality of antigenic types of bluetongue (BT) virus has been well established. Workers at the Veterinary Research Institute, Onderstepoort, South Africa, have described 16 immunological groups (4,5). In the United States, 6 antigenic types were described (8). These were not compared to the 16 immunological groups described elsewhere in the world except that the California BT-8 strain was determined to be the same as South African Type 10 (3).

The first evidence of antigenic differences among BT virus strains in the United States was reported in 1964 (7). In that study, strain 100, isolated from sheep in Texas in 1953, was compared with Station strain, isolated from sheep in Texas in 1962. They stated that strain 100 was the same antigenically as the California BT-8 strain which was isolated from sheep in 1953. Strain 100 and Station strain were antigenically distinct in a neutralization test in cell culture monolayer tubes. Varying virus dilutions and constant-serum (alpha procedure) were used in the test; cross protection studies in sheep were not conducted.

Six United States BT virus strains, 4 from sheep and 2 from cattle, were characterized in comparative studies reported in 1968 (8). A neutralization procedure in cell-culture monolayer tubes with the alpha procedure for virus-antibody dilutions was also used in this study. Sheep immunized with BT-8 vaccine virus were tested for susceptibility to 6 strains of ovine-virulent BT virus. Based on these results, the existence of 6 immunologic groups in the United States was postulated. It was further reported, however, that these 6 BT virus strains were serologically related in the neutralization test; the 6 strains were placed into 3 groups based on these relationships (Table 1).

Thomas and Trainer (11) reported on relationships among North American BT virus strains and compared them with the epizootic hemorrhagic disease (EHD) virus. Plaque reduction (PR) using the beta procedure (constant virus-varying serum) for virus-antibody mixtures were used. No antigenic groups were found due to extensive cross reactions among the 7 strains of BT virus; EHD virus was antigenically distinct from the BT viruses. The 7 BT virus strains included 4 of the 6 described by Luedke and Jochim (8).

Antigenic relatedness between certain strains of BT and EHD viruses has been detected by the complement-fixation test (1,9). We have also ob-

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The authors acknowledge the excellent technical assistance of Ms. Ann Hoyt, Suzanne Jones, and Phyllis Harris.
served cross relatedness by using BT and EHD viral antigens in the agar gel precipitin test.*

The purpose of this study was to compare some recent United States BT virus strains with earlier ones using a PR neutralization test and the beta-dilution procedure. One strain of EHD virus was included in the study to confirm that it was distinguishable from BT virus by the PR test as was previously reported (11).

MATERIALS AND METHODS

Virus.—The lowest available sheep passage levels of BT virus strains were used (Table 2). Ovine-virulent BT virus was inoculated intravenously into 10-day chicken embryos; virus was sometimes sub-passed 1 or 2 times until a consistent death pattern was evident in 4-7 days. Rolling bottles of lamb kidney (LK) cell monolayers were inoculated with virus in supernatant fluid taken from whole embryo slurry. Seed virus stocks were prepared from cellular debris and spent fluids if cytopathic effects were observed in 5 days or less; 1 or 2 sub-passages were occasionally necessary. From these virus seed stocks, 3 serial terminal dilutions were made in LK monolayer tubes and subsequently subpassed in rolling bottles of LK cells for plaque pools. Virus seed stocks or plaque pools were combined with equal volumes of pH 7.2 buffered lactose-peptone (6) and stored at -80°C in flame-sealed ampules.

The EHD virus, New Jersey strain, was obtained as first mouse brain passage.** It was treated the same as BT virus except it was grown in VERO (African green monkey kidney) cells without prior passage in embryonated chicken eggs.

Antiserum.—Antisera were produced by inoculation of sheep and rabbits. Virus for animal inoculation was produced in LK cells. Cells from infectious suspensions were removed by low-speed centrifugation, insonated, added back to the suspension, and titrated in tubed LK cell monolayers. Sheep were given $10^6$ to $10^7$ median tissue culture infectious doses (TCID 50) of this virus; they were bled after 28 days and given $10^4$ to $10^6$ median embryo lethal doses (ELD 50) of virulent BT virus in sheep blood. Serum was again collected 10 to 14 days later.

Viral antigen for immunization of rabbits was concentrated 20-fold from 1 liter of infectious cell-culture fluids by continuous flow ultrafiltration.***Rabbits were initially given $10^6$ to $10^8$ TCID 50 of BT virus in complete Freund adjuvant intramuscularly (IM) in the shoulders, intradermally in 6 sites on the back, and in each rear foot pad. After 3 weeks, $10^8$ to $10^9$ TCID 50 was distributed among 4 IM sites in the rear

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**American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852.

***Amicon XM 300, Amicon Corporation, Lexington, Massachusetts 02173.
limbs. Blood for serum was taken 3 weeks later (10). Antiserum was produced for each of 10 strains of BT virus and for 1 strain of EHD virus. Two sheep and 5 rabbits were used for each virus strain; antisera from individual animals were never pooled.

Animals.—Warhill and Warhill-Columbia crossbred sheep, 1 to 2 years old and raised at this laboratory, were used. Young adult New Zealand white rabbits weighing 5 to 8 lbs were purchased locally. Guinea pigs were young adults raised at this laboratory.

Serology.—Plaque-reduction neutralization tests were done in monolayer cultures of VERO cells in 1-oz prescription bottles. Bottles were tightly stoppered during both growth of cells and the plaque assay procedure. All virus dilutions were made in Hank's balanced salt solution with 20% fresh (unheated) guinea pig serum. The overlay previously described (2) contained 100 micrograms/ml DEAE dextran and Difco Noble-Agar as the solidifying agent. Tests were read in 6 (BT virus) or 7 (EHD virus) days. Serum end points were reported as the reciprocal of the serum dilution giving 80% reduction of 75 to 180 plaque forming units (PFU). All results were from at least 2 replicate tests. All serums were heated to 56°C for 30 min and tested in twofold dilutions beginning at 1:10. Rabbit and sheep antisera were first screened with the homologous virus strain; the highest titering antiserum was selected for subsequent heterologous PR neutralization tests among the 10 BT strains and EHD virus.

RESULTS

All sheep and rabbits converted from serum PR neutralization titers of 10 prior to inoculation to 20 or higher with the homologous virus after immunization. Serum dilution end points among serums within groups of 5 rabbits receiving the same inoculum ranged from no variation to as much as 32-fold variation. Homologous serum end points varied less between pairs of sheep given the same inoculum; maximum differences were 8-fold.

Table 3 summarizes PR neutralization end points with sheep antiserum to 10 BT virus strains. Homologous end points ranged from 40 to 160. Serum dilution end points with homologous virus were equal to or greater than those end points obtained with heterologous virus strains with 9 of 10 antisera. All preinoculation sheep serums were negative for plaque inhibition at the 1:10 dilution. Based on these data, the 10 BT strains were placed into 4 antigenic groups.

The PR end points for rabbit antiserum with 10 BT virus strains are shown in Table 4. Homologous serum dilution end points ranged from 40 to 2560. Serum dilution end points with homologous virus were greater than those end points obtained with heterologous virus strains with 8 of 10 antisera. When heterologous strains of BT virus were neutralized by a higher serum dilution than the homologous strain of BT virus, differences were never more than a single twofold dilution. Cross reactions among BT virus strains were more numerous with rabbit than with sheep hyperimmune antisera. These extensive cross reactions largely obscured
antigenic groupings such as those that were found when sheep antisera were used.

The EHD virus was not neutralized by rabbit or sheep antisera to any of the 10 BT strains. Likewise, sheep and rabbit antisera to EHD virus did not neutralize any of the 10 BT virus strains. Antiserum to EHD from both sheep and rabbits had serum dilution end points of 160 in the PR neutralization test with homologous EHD virus.

DISCUSSION

We believe that the 4 antigenic types of BT virus described in this study are representative of BT serotypes throughout the United States. The 10 BT strains studied were isolated from sheep, cattle, or deer. They were isolated during 6 different years spanning the period 1953-1971. They were from 8 states including 2 on the eastern seaboard.

In a previous study (8), 6 of these strains were described as antigenic types but with some antigenic similarities among them. The tentative groups reported were based on neutralization tests in LK monolayer tubes using the alpha-procedure. The serologic groups found in our study include these 6 BT virus strains in only 3 antigenic groups; strains previously reported to fit 3 groups based on antigenic similarities fit into 3 of the 4 antigenic groupings of this study.

In another study (11), 7 strains of BT virus were compared with each other and with 1 EHD virus strain. The PR neutralization test was used in that study with the beta-procedure. Antisera to BT viruses were produced in calves. Convalescent (3 weeks after infection) antiserum cross reacted with heterologous BT virus strains but less than hyperimmune antiserum. Nevertheless, antigenic classification was not possible; representatives of 4 of the 7 BT virus strains were included in the present study.

Although antigenic relatedness of BT and EHD viruses detectable by the complement-fixation test, has been reported (1,9) no cross reactions were found by PR neutralization in this study. Cross reactions were not seen even at dilutions as low as 1:10 with hyperimmune sheep and rabbit antisera. This finding is in general agreement with the previous report (11) that no cross reactions (at 1:50) were found between BT and EHD virus by PR neutralization using convalescent or hyperimmune calf antisera. It appears that the PR test is of definite value in differentiating BT and EHD viruses.

Whether the PR neutralization test for BT virus is type specific or disease specific apparently depends on the character of the antibody; results were also apparently affected by whether the alpha or beta dilution scheme is used for virus and antiserum. The sheep-source antibody in this study taken 10 days after challenge-of-immunity was more type specific than hyperimmune rabbit antiserum and was more type specific than calf antiserum was reported to be (11).

Serological typing in South Africa (4,5) was done with hyperimmune antiserum produced in young adult guinea pigs. Guinea pigs were given
infective tissue culture fluid at a 10-day interval and bled 30 days after the second inoculation. Further studies are planned to establish the most specific source of antiserum for typing and to compare the 4 United States BT virus serotypes to the 16 immunological groups reported by the Veterinary Research Institute, Onderstepoort, South Africa. The 4 United States BT virus serotypes described may also be used in cross-protection tests and in vaccine development studies.

**SUMMARY**

Serologic relationships among 10 strains of bluetongue (BT) virus and 1 strain of epizootic hemorrhagic disease (EHD) virus were determined by using the plaque reduction neutralization test. These North American BT virus strains were isolated from 8 different states between 1953 and 1971 from sheep, cattle, or deer. The BT virus strains fit into 4 serotypic groups and no cross reactions were found by the plaque reduction test between BT and EHD viruses. Using hyperimmune rabbit antisera, cross reactions were seen among all 10 strains of BT virus, but cross reactions did not occur with EHD virus.

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**Table 1. Six Antigenic Types of Bluetongue Virus from the United States as Reported in 1968 (Luedke and Jochim)**

<table>
<thead>
<tr>
<th>Current strain Designation</th>
<th>Former Designation</th>
<th>State of Origin</th>
<th>Animal Isolated From</th>
<th>Year of Isolation</th>
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<td>sheep</td>
<td>1953</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62-45S</td>
<td>BT-262</td>
<td>WY</td>
<td>sheep</td>
<td>1962</td>
</tr>
<tr>
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<td>BT (Ox)-183</td>
<td>ID</td>
<td>cattle</td>
<td>1963</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Station strain</td>
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<td>TX</td>
<td>sheep</td>
<td>1962</td>
</tr>
<tr>
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<td>BT-310</td>
<td>CO</td>
<td>sheep</td>
<td>1963</td>
</tr>
<tr>
<td>63-83B</td>
<td>BT (Ox)-193</td>
<td>CO</td>
<td>cattle</td>
<td>1963</td>
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</tbody>
</table>

*Three groups based on reported serologic relationships are separated by the dashed lines.*
Table 2. Isolation and Passage Histories of Bluetongue Viruses

<table>
<thead>
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<th>Current Strain Designation</th>
<th>Isolation History</th>
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<td>cattle</td>
</tr>
<tr>
<td>62-45S</td>
<td>WY</td>
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<tr>
<td>63-66B</td>
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<td>cattle</td>
</tr>
<tr>
<td>67-94B</td>
<td>FL</td>
<td>cattle</td>
</tr>
<tr>
<td>67-41B</td>
<td>ID</td>
<td>cattle</td>
</tr>
<tr>
<td>71-1455WR</td>
<td>NC</td>
<td>deer</td>
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*Unknown

Table 3. Results of Plaque Reduction Tests with Sheep Antiserum to Bluetongue Virus Strains

<table>
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<th>Station</th>
<th>63-75S</th>
<th>63-83B</th>
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<th>67-41B</th>
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<td>&gt;1/10</td>
<td>&lt;1/10</td>
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*Homologous serum dilution and points underlined.
Table 4. Results of Plaque Reduction Tests with Rabbit Antiserum to Bluetongue Virus Strains

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*Homologous serum dilution end points underlined.

LITERATURE CITED

V. FETUS VACCINE EVALUATION
Richard F. Hall, Extension Veterinarian
Veterinary Research Laboratory
University of Idaho
Caldwell, Idaho
September 13, 1973
(For presentation at the 1973 USAHA Meeting of the Sheep & Goat Committee)

INTRODUCTION

Since about 1966 we have had several outbreaks of Vibriosis in ewes vaccinated with commercial bacterins in Idaho. Because of this it was believed that methods should be developed to evaluate ovine Vibriosis vaccines for efficacy using laboratory methods rather than sheep inoculation.

Ristic, et al. reported in the Am. J. Vet. Res., January, 1955, that V. fetus strains of ovine origin produced testicular infection following intra-peritoneal inoculation. A preliminary experiment conducted at the University of Idaho Caldwell Veterinary Research Laboratory, suggested that further research on the male hamster as a test animal was justified. In these preliminary tests, 10 out of 15 unvaccinated inoculated hamsters had testicular atrophy. A vaccine was prepared by this laboratory in two different dilutions and was given 7 days before live V. fetus intra-peritoneal inoculation. Both groups of vaccinated hamsters had a lower incidence of testicular atrophy than the nonvaccinated group.

Because of the results of this preliminary work with hamsters, a plan of work was submitted to the Biologics Division of the NADL. It was proposed to compare immunity in male hamsters and pregnant ewes by vaccinating them with commercial and experimental bacterins and challenging them with live V. fetus organisms. The proposal was accepted, a contract was issued, and work continued under this agreement in July, 1972. The first studies were to evaluate immunity to serotype I Vibrio fetus var. intestinalis. The vaccine produced in our laboratory contained only serotype I V. fetus. Commercial vaccines contained both serotypes I & V. fetus (now known as serotypes C & A2, respectively.)

The experimental bacterin was prepared at the Caldwell Laboratory from formalin killed V. fetus intestinalis (ovine origin) serotype C. Aluminum hydroxide adjuvant was added. The experimental bacterin contained 1 mg. of vibrio cells (dry weight basis) per ml. of bacterin. The diluted experimental bacterin contained 0.2 mg. of vibrio cells per ml. All bacterins were administered subcutaneously in 5 ml. doses. Two doses were given: the first at the beginning of breeding and the second approximately 2½ months later.

A total of 107 yearling ewes were divided into groups and vaccinated as shown in Table I. All lots except lot 6 were challenged by oral inoculation with serotype IV. fetus at the rate of 15 ml. of suspension adjusted
to 18% light transmittance at 525 mu in approximately the 4th month of gestation.

TABLE I. Vaccination and Challenge of Ewes for Comparison of Potency of *V. fetus* Bacterins.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Ewes</th>
<th>Bacterin</th>
<th>Challenge</th>
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<tr>
<td>1</td>
<td>18</td>
<td>Exp. Bacterin</td>
<td><em>V. fetus intestinalis</em> (C)</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>Exp. Bacterin (diluted)</td>
<td><em>V. fetus intestinalis</em> (C)</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>Commercial Bacterin A</td>
<td><em>V. fetus intestinalis</em> (C)</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>Commercial Bacterin B</td>
<td><em>V. fetus intestinalis</em> (C)</td>
</tr>
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<td>5</td>
<td>19</td>
<td>None</td>
<td><em>V. fetus intestinalis</em> (C)</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>None</td>
<td>None</td>
</tr>
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</table>

Aborted fetuses were cultured for pathogenic microorganisms and examined microscopically for Chalmydia. No evidence of Chalmydia was found. *V. fetus* was found in all aborted lambs and most lambs which died shortly after birth. The results are in Table II.

TABLE II. Results in Ewes of Vaccination and Challenge in Test of Potency of *V. fetus* Bacterins in Ewes.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Ewes</th>
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<th>Ewes With</th>
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<td>1 Dead</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>2</td>
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<tr>
<td>3</td>
<td>20</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>2</td>
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<tr>
<td>4</td>
<td>18</td>
<td>7</td>
<td>7</td>
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<td>6</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Commercial Bacterin B (group 4) apparently afforded little or no protection.

The work in male hamsters has been very disappointing. On challenge with live organisms we have not been able to reproduce testicular infection with nearly the degree of repeatability that was earlier suggested. The reasons are not known. A growth inhibition method involving culturing of the live organism in presence of dilutions of serum of vaccinated animals is currently being investigated.

The results of this work illustrates the need for tests to be developed to properly evaluate bacterins and other biological products in the laboratory to assure protection of the host animal. If a product is not effective, it can do the producer a great deal of harm.
REPORT OF THE COMMITTEE OF
DISEASES OF SHEEP AND GOATS

Chairman: F. James Schoenfeld, Salt Lake City, Utah
Co-Chairman: Blaine McGowan, Jr., Davis, Calif.

W. A. Hickman, Pierre, S. Dak.; R. E. Simmons, Boise, Idaho; W. W.
Hawkins, Bozeman, Mont.; Ward Van Horn, Buffalo, S. Dak.; A.L.
Klingsporn, Bowie, Md.; J. H. Wommack, Citrus Heights, Calif.; L. R.
Barnes, Indianapolis, Ind.; O. H. Timm, Dixon, Calif.; H. E. Metcalf,
Lakewood, Colo.; W. W. Clark, McAllen, Tex.; T. A. Kincaid, Jr., La
Vernia, Tex.; R. F. Hall, Caldwell, Idaho; D. Osguthorpe, Salt Lake
City, Utah; C. E. Terrill, Beltsville, Md.; T. B. Snodgrass, Dallas, Tex.;
G. E. Reynolds, Corvallis, Oreg.

The committee met as instructed with six members present and ten
visitors. The agenda as to suggested subjects was presented to the com-
mittee and the proceedings of the committee are now reported.

I. "Serological Characterization of Selected Blue Tongue Virus
Strains," was discussed by Dr. H. E. Metcalf and Dr. T. L. Barber. It was
pointed out that isolates from United States yielded 4 antigenic groups.
South Africa has 16 antigenic groups. Outbreaks in the United States this
year indicated that the isolates were more virulent, and that the com-
mercial vaccine did not offer protection in some flocks. It was also shown
that the E.H.D. (Enzootic Hem. Disease) virus produces in white tailed
deer and cattle identical lesions as blue tongue virus in sheep.

II. The paper by Dr. Richard F. Hall concerning Vibrio fetas vaccine
evaluation, pointed out that laboratory methods (animal model) should be
developed for determining efficacy rather than sheep inoculation. The
committee recommends that more study be put into this disease and its
biological control.

III. Foot rot vaccine is under development and field trials are in
progress at Davis by Dr. Blaine McGowan, Jr. The use of Australian vac-
cine has not held up against challenges under U. S. conditions.

IV. Epididymitis vaccine has had label recommendations changed to
meet efficacy requirements.

V. Dr. A. L. Klingsporn reviewed the history of scrapie and bringing
up-to-date the data necessary to keep us aware of this disease. There
were three outbreaks each reported in fiscal years 1971 and 1972, and
two in 1973, these are the smallest number of outbreaks reported since
the fiscal year 1954. The number of flocks under surveillance has dropped
from 246 in fiscal year 1972 to 230 in fiscal year 1973.

The scrapie field trials at Mission, Texas have been underway since
November, 1964. The scrapie field trial has demonstrated that bloodline
animals exposed on infected and source flock premises will continue to
develop scrapie and provide foci of infection for continuing spread of the
disease unless slaughtered. The losses in blood line animals continue to
occur in succeeding generations of such animals reared on infected
premises.

Natural scrapie has now been spread by contact to every breed of
previously nonexposed, nonbloodline sheep and goats that have been used in the Mission study. Successful passage of scrapie from affected sheep and goat tissue to sheep, goats, and mice have been demonstrated.

The committee recommends that this surveillance and eradication program continue. The relationship of this disease to public health problems was explored.

VI. Concern was shown that no specific epidemiological procedures have been written, or designated persons in Veterinary Services of APHIS for sheep and goat diseases. The committee recommends that the Department of Agriculture explore this area, that training and support be given to the sheep industry as to other phases of livestock disease control with emphasis of public health significance.

VII. Concern was shown that Selenium has been approved for use in poultry and swine but not for sheep and cattle. It is the recommendation of the committee that factions of these two livestock industries get interested parties in proceeding to receive approval for its use. It is also recommended that more interest be stimulated in nutritional aspects of sheep and goat diseases.

VIII. The committee wishes to bring to the attention of USAHA the public health aspect of Hydatid Disease (Echinococcosis) in which sheep play an important part of the cycle of the dog tapeworm (Echinococcus granulosis). In our sheep communities where sheep carcasses are being fed to dogs there is an increasing amount of this disease in families associated with sheep and their dogs. The committee recommends that the industry, public health officials, and USAHA become more interested and promote surveillance programs in these sheep raising communities.

This report is submitted with attached papers on V. fetus and scrapie to the executive committee for approval by the committee of diseases of sheep and goats as appointed by the president of this association.

Dr. F. James Schoenfeld, Chairman
Dr. Blaine McGowan, Jr., Co-Chairman

Dr. C. C. Beck
Dr. Donald W. Baker
Mr. W. A. Hickman
Dr. R. E. Simmons
Dr. W. W. Hawkins
Mr. Ward Van Horn
Dr. A. L. Klingsporn
Dr. J. H. Wommack
Dr. L. R. Barnes

Mr. O. H. Timm
Dr. H. E. Metcalf
Dr. Wilbur W. Clark
Mr. T. A. Kincaid
Dr. Richard F. Hall
Dr. Delbert Osguthorpe
Dr. Claire E. Terrill
Dr. Thomas B. Snodgrass
Dr. Gary E. Reynolds

SCRAPIE

Scrapie was reported in two flocks in Illinois and Michigan during fiscal year 1973. The Illinois outbreak occurred in a Suffolk flock in Woodford County and the Michigan outbreak in a Cheviot flock in Ingham County. There were three outbreaks each reported in fiscal years 1971 and 1972 and two in 1973, these are the smallest number of outbreaks reported since fiscal year 1954. The number of flocks under surveillance has dropped from 246 in fiscal year 1972 to 230 in fiscal year 1973.
The Woodford County Illinois outbreak was reported to a veterinary practitioner by the owner, and the Ingham County, Michigan, outbreak was discovered when the owner took the affected sheep to a veterinary college for diagnosis.

The Illinois and Michigan infected flocks have been slaughtered. A second Illinois, Woodford County, flock source of the Illinois outbreak has been slaughtered. All bloodline animals in a Wabash County, Indiana flock, source of the Michigan outbreak have been slaughtered. All bloodline sales from the two source flocks and two infected flocks have been slaughtered. Nonbloodline exposed sales from the source flocks have been located and slaughtered in some States and placed under surveillance in other States. A Somerset County, New Jersey flock infected in fiscal year 1972 has not been slaughtered. The owner objects to slaughtering his flocks on the grounds that he does not trade in breeding sheep and does not therefore spread disease. The flock is being maintained under quarantine.

**SCRAPIE FIELD TRIAL, MISSION, TEXAS**

The scrapie field trial has been underway since November 1964 (107 months) during this period scrapie has been confirmed by histopathological examination or by mouse inoculation in 240 animals. These 240 cases have occurred on infected premises No. 3 in the following category of animals either taken to Mission or born on the premises.

- a. Scrapie bloodline exposed sheep 178
- b. Field suspects held for observation 12
- c. Nonbloodline exposed sheep 24
- d. Nonbloodline exposed goats 26

The scrapie field trial has demonstrated that bloodline animals exposed on infected and source flock premises will continue to develop scrapie and provide foci of infection for continuing spread of the disease unless slaughtered. The losses in bloodline animals continue to occur in succeeding generations of such animals reared on infected premises.

From October 1969 to June 1973 scrapie has been confirmed by histopathological examination in 24 nonbloodline blue (not previously exposed) sheep and 26 nonbloodline blue goats born or taken to Mission and reared in contact with affected Cheviot, Montadale, and Suffolk sheep. The 26 affected blue goats were all born at Mission and are of the following breeds: Angora or Nubian or Nubian X Toggenburg or Angora X Nubian X Toggenburg ranging in the age of 35 to 60 months. Of the 24 affected blue sheep, 21 were born and reared on the infected premises at Mission and were of the following breeds: Hampshire, Rambouillet, Suffolk, and Targhee ranging in the ages of 32 to 51 months. The additional blue affected sheep have the following history: One Hampshire was taken to Mission and placed in exposure at 8 months of age and showed signs of scrapie in June 1972 at 88 months of age, died at 89 months of age and was confirmed scrapie by histopathological examination. One Rambouillet ewe was taken to Mission and placed in exposure at 3 months of age and showed signs in August 1972 at 88 months of age, was destroyed.
15 days later and scrapie confirmed by histopathology. One Targhee ewe was taken to Mission and placed in exposure at 6 months of age and showed signs in August 1972 at 88 months of age, was destroyed one month later and scrapie confirmed by histopathology. These findings provide further evidence that scrapie can and does spread laterally when healthy animals are held in contact with animals developing scrapie, further that pre-natal or birth exposure is not required for the spread of scrapie; however, the incubation period appeared to be lengthened when first exposure occurs at 3 to 8 months of age.

Natural scrapie has now been spread by contact to every breed of previously nonexposed, nonbloodline sheep and goats that have been used in the Mission study. Successful passage of scrapie from affected sheep and goat tissue to sheep, goats, and mice have been demonstrated. These studies are being continued and are used to assist in diagnosis and for checking susceptibility of animals. Various other studies are being continued to determine the route, age, and methods by which scrapie transmission occurs naturally.

**BLUETONGUE**

During fiscal year 1973 bluetongue was confirmed by virus isolation in four cattle herds, one in California, two in Colorado, and one in North Carolina; and two sheep flocks in California and Colorado.

In addition, 29,766 modified complement fixation (MCF) tests were run on cattle, sheep, goats, deer, and zoo animals with the following results:

<table>
<thead>
<tr>
<th>Total Tested</th>
<th>Negative</th>
<th>Suspicious</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>26,329</td>
<td>25,701</td>
<td>521</td>
</tr>
<tr>
<td>Sheep</td>
<td>853</td>
<td>774</td>
<td>33</td>
</tr>
<tr>
<td>Goats</td>
<td>2,488</td>
<td>2,331</td>
<td>126</td>
</tr>
<tr>
<td>Deer</td>
<td>15</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Zoo Animals</td>
<td>81</td>
<td>73</td>
<td>8</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>29,766</strong></td>
<td><strong>28,893</strong></td>
<td><strong>689</strong></td>
</tr>
</tbody>
</table>

Positive MCF samples were collected from the States of Arizona, California, Colorado, Florida, Georgia, Idaho, Kansas, Kentucky, Michigan, Mississippi, Missouri, Montana, New York, Ohio, Oklahoma, Pennsylvania, Texas, U. S. Virgin Islands, Virginia, Washington, and Wyoming.

Further, 168 agar gel precipitin (AGP) tests were run on cattle, sheep, goats, deer, and zoo animals with the following results:

<table>
<thead>
<tr>
<th>Total Tested</th>
<th>Negative</th>
<th>Suspicious</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>125</td>
<td>96</td>
<td>3</td>
</tr>
<tr>
<td>Sheep</td>
<td>26</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Goats</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Deer</td>
<td>15</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Zoo Animals</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>168</strong></td>
<td><strong>131</strong></td>
<td><strong>3</strong></td>
</tr>
</tbody>
</table>
Positive samples were collected from the States of California, Colorado, Idaho, Oklahoma, Oregon, Tennessee, and Virginia.

Prepared By: Veterinary Research Laboratory
Dr. Richard F. Hall,
University of Idaho
Veterinary Research Laboratory
For presentation at the 1973 U.S.A.H.A. meeting of the Sheep & Goat Committee

V. FETUS VACCINE EVALUATION (revised Copy)

Introduction—
Since about 1966 we have had several outbreaks of vibriosis in ewes vaccinated with commercial bacterins in Idaho. Because of this it was believed that methods should be developed to evaluate ovine vibriosis vaccines for efficacy using laboratory methods rather than sheep inoculation.

Ristic, et al. reported in the Am. J. Vet. Res., January, 1955, that V. fetus strains of ovine origin produced testicular infection following intra-peritoneal inoculation. A preliminary experiment conducted at the University of Idaho Caldwell Veterinary Research Laboratory, suggested that further research on the male hamster as a test animal was justified. In these preliminary tests, 10 out of 15 unvaccinated inoculated hamsters had testicular atrophy. A vaccine was prepared by this laboratory in two different dilutions and was given 7 days before live V. fetus intra-peritoneal inoculation. Both groups of vaccinated hamsters had a lower incidence of testicular atrophy than the nonvaccinated group.

Because of the results of this preliminary work with hamsters, a plan of work was submitted to the Biologics Division of the NADL. It was proposed to compare immunity in male hamsters and pregnant ewes by vaccinating them with commercial and experimental bacterins and challenging them with live V. fetus organisms. The proposal was accepted, a contract was issued, and work continued under this agreement in July, 1972. The first studies were to evaluate immunity to serotype I Vibrio fetus var. intestinalis. The vaccine produced in our laboratory contained only serotype I V. fetus. Commercial vaccines contained both serotypes I & V, V. fetus (now known as serotypes C & A, respectively.)

The experimental bacterin was prepared at the Caldwell Laboratory from formalin killed V. fetus intestinalis (ovine origin) serotype C. Aluminum hydroxide adjuvant was added. The experimental bacterin contained 1 mg. of vibrio cells (dry weight basis) per ml. of bacterin. The diluted experimental bacterin contained 0.2 mg. of vibrio cells per ml. All bacterins were administered subcutaneously in 5 ml. doses. Two doses were given: the first at the beginning of breeding and the second approximately 2½ months later.

A total of 107 yearling ewes were divided into groups and vaccinated as shown in Table I. All lots except lot six were challenged by oral
innoculation with serotype I \( V.\ fetus \) at the rate of 15 ml. of suspension adjusted to 18% light transmittance at 525 \( \mu \) in approximately the 4th month of gestation.

**TABLE I.** Vaccination and Challenge of Ewes for comparison of \( V.\ fetus \) Bacterins.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Ewes</th>
<th>Bacterin</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>Exp. Bacterin</td>
<td>( V.\ fetus ) intestinalis (C)</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>Exp. Bacterin (diluted)</td>
<td>( V.\ fetus ) intestinalis (C)</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>Commercial Bacterin A</td>
<td>( V.\ fetus ) intestinalis (C)</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>Commercial Bacterin B</td>
<td>( V.\ fetus ) intestinalis (C)</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>None</td>
<td>( V.\ fetus ) intestinalis (C)</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Aborted fetuses were cultured for pathogenic microorganisms and examined microscopically for Chlamydia. No evidence of Chlamydia was found. \( V.\ fetus \) was found in all aborted lambs and most lambs which died shortly after birth. The results are in Table II.

**TABLE II.** Results in Ewes of Vaccination and Challenge in Test of Potency of \( V.\ fetus \) Bacterins in Ewes.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Ewes</th>
<th>Ewes with 1 Live Lamb &amp; 1 Dead</th>
<th>Ewes With Weak Lambs</th>
<th>Ewes With Non-Pregnant Ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ewes</td>
<td>Normal Lambs</td>
<td>Aborted</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>15</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>16</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>7</td>
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<td>1</td>
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<tr>
<td>4</td>
<td>18</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>19</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Commercial bacterin B (group 4) apparently afforded little or no protection.

The work in male hamsters has been very disappointing. On challenge with live organisms we have not been able to reproduce testicular infection with nearly the degree of repeatability that was earlier suggested. The reasons are not known. A growth inhibition method involving culturing of the live organism in presence of dilutions of serum of vaccinated animals is currently being investigated.

The results of this work illustrates the need for tests to be developed to properly evaluate bacterins and other biological products in the laboratory to assure protection of the host animal. If a product is not effective, it can do the producer a great deal of harm.
THE COMPARATIVE-CERVICAL TUBERCULIN TEST
AS AN AID TO DIAGNOSING
BOVINE TUBERCULOSIS


INTRODUCTION

The tuberculin test has proven its value as the principal tool for the detection of bovine tuberculosis in cattle throughout the world. Since the early 1920's the intradermal tuberculin test, applied in the skin of the caudal fold of cattle, has detected over 4 million tuberculin reactors in the United States and provided a means of eliminating this disease from all but a few hundred tuberculous cattle herds.

In spite of the remarkable record achieved through the use of the caudal test, it is well-known that no skin test procedure is perfect. Two types of errors occur: (1) Some cattle which are infected with Mycobacterium bovis fail to respond to the test. The "sensitivity" of the test is the proportion of the animals which are infected and do respond. Although the true sensitivity of the caudal test is not known, it has been estimated that on a single test only 85 percent of the M. bovis infected cattle may give positive test results;* (2) The second error involves cattle which are not infected with M. bovis but do respond to the test. The "specificity" of the test is the proportion of animals which are not infected with M. bovis and do not respond. The results of various studies in the United States*-* indicate the specificity to be about 95-98 percent, or a false-positive response rate of 2-5 percent.

† It is generally agreed that most false-positive responses in cattle are a result of the animal having been infected by microorganisms that contain some antigenic characteristics similar to M. bovis, which cause the host to show some degree of heterospecific response. ‡ The mycobacteria, other than M. bovis, most often isolated from cattle in the United States are, in general, more closely related antigenically to M. avium than to M. bovis. One notable exception, M. tuberculosis, which has been recovered from cattle infrequently, is antigenically very similar to M. bovis. As the prevalence of M. bovis infected herds decreases, the relative importance of false-positive tuberculin responses increases.

Since the early 1940's, procedures employing more than one type of tuberculin have been used to help differentiate M. bovis infected cattle from cattle showing heterospecific sensitivity due to other microorganisms. The procedure which has given the most reliable results to date involves the use of avian and mammalian purified protein derivative tuberculins injected simultaneously at different sites on the body.

* Former Chief Staff Veterinarian, Tuberculosis Eradication, Cattle Diseases, Veterinary Services, APHIS, USDA, Hyattsville, Maryland, now on leave of absence as Tuberculosis Consultant to the PanAmerican Health Organization.
** Acting Chief Staff Veterinarian, Tuberculosis Eradication, Cattle Diseases, Veterinary Services, APHIS, USDA, Hyattsville, Maryland.
neck. Animals which show a greater response to the mammalian tuberculin are considered possibly infected with *M. bovis*. In most European countries, the comparative-cervical test employs the use of avian PPD at a dose of one-fourth the dose of the bovine PPD. Huitema has presented data from the Netherlands to suggest that when PPD's of equal protein content are used, more specific results are achieved. This principle was used in our study by using PPD's which were adjusted to equal biologic potency in guinea pigs.

The objectives in studying such a comparative-cervical tuberculin test as an aid to diagnosing tuberculosis were to: (1) Determine if such a test could be used to reclassify caudal tuberculin test "suspects" within 7 days after the caudal test rather than by the present procedure of waiting 60-90 days; (2) Estimate the specificity of such a test in a group of cattle free of *M. bovis* infection; (3) Estimate the sensitivity of such a test in a group of cattle infected with *M. bovis*; (4) Determine if a comparative tuberculin test would be an efficient diagnostic test for clarification of the status of caudal tuberculin test "suspects". Field trials on specificity and sensitivity were conducted separately.

**SPECIFICITY STUDY**

Objectives 1, 2 and 4 were studied jointly as a separate part of a larger field trial in which we studied the relative specificity of two types of old tuberculin (OT) and two types of bovine PPD's as caudal test sensitins. Animals for this phase of the study were located in 27 publicly owned dairy herds in 15 different States. Each herd had at least a 7-year history of freedom from bovine tuberculosis and also had a history of caudal fold "suspects," none of which had been shown to be infected with *M. bovis*. Each of the 27 herds was divided into blocks (four animals per block) and tuberculins for use in the caudal test were assigned (one to each animal within each block) in a random sequence. Of the 4,120 animals tested (2 years and older) 243 were classed as "suspects" according to the subjective interpretation of the caudal fold response at 72±6 hours post-injection. Of the 243 caudal suspects, 225 were retested with the comparative-cervical (C-C) tuberculin test within 7 days following the caudal test observation, and 204 of the 225 received a second C-C test at least 60 days after the caudal test. The tuberculins use for the C-C test were a trichloroacetic acid (TCA) precipitated purified protein derivative (PPD) of *M. avium* Strain D4 (avian PPD tuberculin) and a TCA-PPD of *M. bovis* Strain AN5 (bovine PPD tuberculin), which were produced at Veterinary Services Diagnostic Laboratory, Ames, Iowa, according to the method of Green as modified by Richards & Pemberton. These PPD tuberculins were biologically balanced so they were of equal potency with the bovine PPD containing 0.996 mg. of protein per cc. and the avian PPD containing 1.128 mg. of protein per cc.

Table 1 shows the results of the C-C test conducted within 7 days following the caudal test observation. The mean increase in skin thickness in millimeters and the standard deviation at the avian and bovine PPD sites is shown for each group of suspects according to the
tuberculin used for the initial caudal test.

Since animals with different types of sensitivity may have been selected by the different tuberculins used for the caudal fold test, a paired comparison was made by analysis of variance to determine if the C-C test behaved differently in the different caudal tuberculin groups. The results of this analysis, Table 2, show that differences due to avian PPD vs. bovine PPD were statistically significant and the effects due to the type of tuberculin used on the caudal test were also significant. However, the interaction between these two factors is not statistically significant. These results are interpreted to mean that while animals (suspects) with different levels of sensitivity may have been selected by the different tuberculins, the C-C test behaved similarly in each group with respect to the relationship between avian PPD and bovine PPD. It is concluded, therefore, that the four groups of suspects can be viewed as a single homogeneous population of cattle with delayed hypersensitivity which was, based on the method of herd selection, not due to infection with *M. bovis*.

Figure 1 shows the results of the 225 individual C-C tests, conducted within 7 days following the caudal test observation, recorded on a scattergram. The increase in skin thickness in millimeters at the avian PPD site can be read on the perpendicular scale, and the increase in skin thickness in millimeters at the bovine PPD site can be read on the horizontal scale. Each dot represents one animal. All points above the diagonal represent animals which gave larger responses to avian PPD than to bovine PPD and points below the diagonal represent the converse. One hundred fifty-four (68.4 percent) of the animals had larger responses to avian PPD than to bovine PPD; 11 (4.9 percent) had equal responses; and 60 (26.7 percent) had larger responses to bovine PPD than to avian PPD.

In like manner, the results of the second C-C test, conducted at least 60 days after the caudal test, were analyzed. Table 3 shows the number of caudal suspects, the mean increase in skin thickness in millimeters, and the standard deviation at the avian and bovine PPD sites for each group of suspects. The mean response for each group and each PPD is slightly larger, with one exception, than the same data for the 7-day test, but the differences between avian and bovine PPD responses at each test period are similar. Table 4 shows the results of the paired comparison made by analysis of variance for the 204 caudal suspects. The results of this analysis are very similar to the results on Table 2. The effects of avian PPD vs. bovine PPD and of the grouping by caudal tuberculin groups are again statistically significant but the interaction is not. Therefore, it was again assumed that the four groups could properly be combined into one homogeneous population with delayed hypersensitivity not due to *M. bovis*.

Figure 2 shows the results of the 204 individual C-C tests, conducted at least 60 days after the caudal test, recorded on a scattergram. The distribution of the test results is very similar to the 7-day test results. One hundred forty-nine (73.0 percent) of the animals had larger respon-
ses to avian PPD than to bovine PPD; 9 (4.4 percent) had equal responses; and 46 (22.5 percent) had larger responses to bovine PPD than to avian PPD.

SENSITIVITY STUDY (OBJECTIVES 3 and 4)

Herds selected for study were herds known to be affected with bovine tuberculosis which had a high probability of being depopulated. It was impossible to maintain exactly the same criteria in each herd because of program restrictions, owner options, and various other factors. In order for an animal to be included in the study, it had to come from a herd from which *M. bovis* had been recently cultured and have either (1) gross pathologic lesions typical of bovine tuberculosis; or (2) histopathologic evidence of mycobacterial infection; or (3) have *M. bovis* cultured from infected body tissues. In three of the four herds studied, the C-C test was preceded by a caudal tuberculin test. The length of time between the caudal test and the C-C test was not constant from herd to herd. In no case had extensive testing or removal of reactors preceded the C-C test, although five reactors had been removed from one herd. The avian PPD and bovine PPD used were from the same production lot and of the same potency as that used in the specificity study.

A total of four herds containing approximately 700 cattle were studied. Of these cattle, 433 were tested with the C-C test. Test results and slaughter records were received for 346 animals. Of the 87 remaining animals, 66 from herd D have not been reported slaughtered, and 21 from herds, B, C, and D had incomplete test reports. Details of the history for each herd are as follows:

**HERD A:** This was a small dairy herd of 18 mature and 10 immature Holstein cattle. Bovine tuberculosis was suspected when it was learned that the owner had purchased a cow from his brother’s tuberculous herd. The caudal tuberculin test revealed responses in 13 of the 18 mature cows but no responses in the calves. The C-C test was applied 14 days after the caudal test.

Figure 3 shows the scattergram distribution of the C-C test results on this herd. Twenty-five of the animals show results which form a group along and mostly above the diagonal line. This group contained one animal with a gross lesion (square 3.5-1.5) in the mesenteric lymph nodes only. No culture attempt was made. Three animals showed test results with larger responses to bovine PPD which are quite different from the remainder of the herd. Two of the three animals (squares) were found to have lesions resembling tuberculosis in the thoracic lymph nodes. The animal which gave a 21.3 mm. response to bovine PPD is the cow which had been purchased from a known tuberculous herd, and *M. bovis* was isolated from this animal. No gross lesions of tuberculosis were found in the third animal of this group. The criteria for classifying this as an infected herd was gross pathology resembling tuberculosis.

**HERD B:** This was a beef herd of 134 Angus cattle. Bovine tuberculosis was suspected because an animal from this herd had revealed lesions of tuberculosis at routine slaughter and a standard caudal tuber-
culin test revealed 52 reactors out of 125 tested. The C-C test was applied 14 days after the caudal test to 71 adult cattle and three calves. The entire herd was slaughtered promptly. Only a few tissue specimens were collected. *M. bovis* was isolated.

Figure 4 shows the scattergram distribution of the C-C test results of the 74 animals tested. Forty-one of these animals were found to have gross lesions resembling tuberculosis (squares), and all 41 had larger responses to bovine PPD than to avian PPD. Nineteen of the 33 no gross lesion animals (circles) also had larger bovine PPD responses, while 2 responded equally and 12 had larger avian PPD responses. The criteria for classifying this as an infected herd was gross lesions of tuberculosis found at postmortem examination.

**HERD C:** This herd was comprised of 255 cattle in a feedlot. Infection with *M. bovis* was discovered when extensive lesions of tuberculosis were found at slaughter in swine that were commingled in the feedlot with these cattle. One shipment of feeders was also sent to slaughter and shown to be affected. No caudal test was conducted on these animals. The C-C test was applied to the pen of cattle known to be affected, the adjacent pens, and to one pen removed from known exposure—129 cattle in all. Tissue specimens were collected and submitted for histopathologic examination from all cattle tested.

Figure 5 shows the scattergram distribution of the C-C results of the 129 animals tested. Sixty-three of these animals (circles) either had no gross lesions or had gross lesions which did not contain acidfast organisms upon histopathologic examination. Of these 63, 34 had larger responses to avian PPD, one responded equally to both, and the remaining 28 had larger responses to bovine PPD. Sixty-six of the animals (squares) were found to have lesions containing acidfast organisms, and 65 of these 66 had larger responses to bovine PPD. The criteria for classifying this as an infected herd was gross lesions resembling tuberculosis which were compatible for tuberculosis by histopathologic examination.

**HERD D:** This herd consisted of over 300 Holstein dairy cattle which had been kept adjacent to a known bovine tuberculosis-affected herd. A cow from this herd was found at slaughter to have generalized bovine tuberculosis. The C-C test was applied 45 days after a caudal tuberculin test had disclosed responses in 55 of 168 animals tested. At this writing, 115 of the original 168 animals tested have been slaughtered and tissue samples were collected from all cattle with gross lesions resembling tuberculosis.

Figure 6 shows the scattergram distribution of the C-C results of the 115 animals tested and slaughtered. Sixty-nine of these 115 animals (circles) showed no evidence of tuberculosis on postmortem examination and 19 of these 69 were negative on histopathologic and mycobacteriologic examination. Of these 69, 46 had larger responses to avian PPD, 21 had larger responses to bovine PPD, and 2 responded equally to both. Forty-six of the 115 animals tested (squares) were found to have evidence of bovine tuberculosis. Thirty-seven of these 46 (circles around squares)
were culture positive for *M. bovis*. The criteria for classifying this as an infected herd were: (1) Gross lesions suggestive or compatible for tuberculosis on histopathologic examination, or (2) culture of *M. bovis*.

**DEVELOPMENT OF A SCATTERGRAM**

From the data presented, a scattergram was developed as a standard form for plotting the results of the C-C test. Two lines were added to divide the graph into three zones: (1) Negative for *M. bovis* infection; (2) Suspect; and (3) Reactor. The zone limits were set at the discretion of the investigators and were established to allow all responses to bovine PPD less than 2.5 mm. to be classified negative. The suspect zone was established to cover the area of greatest overlap of test results from affected and non-affected cattle, with an increased margin of safety at the higher levels of sensitivity.

Figure 7 shows a scattergram, with the respective zones identified (negative, suspect, reactor), of the combined C-C test results for 156 mature cattle with evidence of bovine tuberculosis from herds A through D. One hundred sixteen (74.36 percent) fall in the reactor zone, 22 (14.10 percent) in the suspect zone, and 18 (11.54 percent) in the negative zone. Unfortunately, we were not able to conduct a second C-C test of these suspect animals.

The classification, according to the scattergram, of animals found to have evidence of bovine tuberculosis by individual herd is shown on Table 5. The false-negative rate by herd varied from a low of 2.4 percent in Herd B to a high of 33 percent in Herd A.

Figure 8 shows a scattergram, with the respective zones identified (negative, suspect, reactor), of the 7-day C-C test results from the 225 caudal test suspects from bovine tuberculosis-free animals. Two hundred seven (92 percent) of the 225 animals were classed negative, and 16 (7.11 percent) as suspects. Fifteen of the 16 C-C suspects were retested after 60 days and 12 were classed as negative. Therefore, specificity of the combined tests is

\[
\frac{3877}{4120} + \frac{207}{225} \left(1 - \frac{3877}{4120}\right) + \frac{12}{15} \left[1 - \frac{207}{225} \left(1 - \frac{3877}{4120}\right)\right] = 99.91 \text{ percent.}
\]

The C-C test specificity for classifying caudal suspects was 97.3 percent. When the 7-day and the 60-day tests are compared independently, according to the percentage of animals falling into each zone on the graph, the 60-day test showed slightly higher percentages of suspects and reactors. This comparison is tabulated in Table 6. A comparison of the 60-day test viewed in serial with the 7-day test is shown in Table 7.

Table 8 gives a comparison of the number and percentage of cattle that were classed as "negative", "suspect", and "reactor" for the cattle with evidence of bovine tuberculosis and the cattle from herds free of bovine tuberculosis.
SUMMARY

In summarization, let us examine how well we accomplished our objectives.

1. Determine if such a test could be used to reclassify caudal tuberculin test "suspects" within 7 days after the caudal test rather than by the present procedure of waiting 60-90 days.

Tables 1 and 3 showed that all groups averaged slightly larger response to both PPD's at 60 days. The slight reduction in response size at 7 days tended to reduce the false-positive rate. The variation between the avian and bovine PPD responses at 7 days and 60 days was not significantly different. It appears that the 7-day test can be used successfully in unaffected herds and a great saving of time, money, and inconvenience will be realized.

2. Estimate the specificity of such a test in a group of cattle free of M. bovis infection.

Table 7 shows that at 7 days, 207 animals were C-C test negative, and 12 of 15 suspects retested were reclassified negative, for a combined total of 219 out of 225 tested (97.3 percent) negative. We believe a C-C test specificity of 97.3 percent for classifying caudal suspects is an acceptable level.

3. Estimate the sensitivity of such a test in a group of cattle infected with M. bovis.

Table 8 shows that 116 of the 156 M bovis infected group were reactors, for a test sensitivity of 74.36 percent. This does not reflect how efficient the procedure would be as a diagnostic test on a herd basis. If a sensitivity of 80 percent is assumed for the caudal test and the probability of getting at least one lesioned reactor on the first retest are calculated from the study figures, then the chances of positive herd diagnosis are approximately 64 percent when only one lesioned animal is present in the herd and rises to about 97 percent when four lesioned animals are present. The probability can be expected to rise with the use of a second retest or with more lesioned cases in the herd.

4. Determine if a comparative tuberculin test would be an efficient diagnostic test for clarification of the status of caudal tuberculin test "suspects."

On the basis of data presented in this study, the 7-day C-C test is recommended for use in the United States as a retest procedure on all cattle which give suspicious responses to the caudal test, followed by a 60-day C-C test on all animals that remain "suspect" on the first test and on those animals unable to be tested within 7 days.

The purpose of its use is to establish whether or not a herd is likely to contain animals infected with M. bovis. It is not required that the test give positive results on every M. bovis infected animal. It is sufficient that the test minimize false-positive results so that the slaughter of cattle in herds not affected with bovine tuberculosis can be held to an acceptable level and at the same time detect bovine tuberculosis at an earlier stage when it is present and thus accelerate our bovine tuberculosis eradication program.
ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of all Veterinary Services field personnel who participated in this study, and all Veterinary Services Diagnostic Laboratory personnel who conducted the histopathologic and bacteriologic examinations and produced the test antigens. We also express our thanks to Dr. Victor Beal, Jr. and Dr. Larry Miller for providing the statistical analyses. Last but not least we thank the management and owners of the herds used in the study, for without them it would not have been possible.

REFERENCES

5. World Health Org. MHO/PA/35.60, (4/60), Results from Preliminary Field Trials on Tuberculin Testing of Cattle with Various Doses of PPD Tuberculin (1, 4, 16, 64 TU), and a High Dose (approx. 2500 TU) of Czechoslovak OT.
Table 1—Results of the Comparative-Cervical Tuberculin Test Applied to 225 Caudal Tuberculin Test Suspects Within 7 Days After the Caudal Test on Tuberculosis-Free Dairy Cattle

<table>
<thead>
<tr>
<th>Caudal Test Tuberculin</th>
<th>Number Retested</th>
<th>Millimeters of Increased Skin Thickness</th>
<th>Avian PPD</th>
<th>Bovine PPD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>St. Dev.</td>
</tr>
<tr>
<td>HCSM Tuberculin</td>
<td>72</td>
<td>3.45</td>
<td>2.79</td>
<td>2.19</td>
</tr>
<tr>
<td>Beef Broth OT</td>
<td>53</td>
<td>3.41</td>
<td>2.35</td>
<td>2.82</td>
</tr>
<tr>
<td>PPD 5</td>
<td>44</td>
<td>5.22</td>
<td>4.46</td>
<td>3.85</td>
</tr>
<tr>
<td>PPD 7</td>
<td>56</td>
<td>3.78</td>
<td>2.58</td>
<td>2.13</td>
</tr>
<tr>
<td>TOTAL</td>
<td>225</td>
<td>3.87</td>
<td>3.11</td>
<td>2.64</td>
</tr>
</tbody>
</table>

TABLE 2—Paired Comparison Analysis of Variance of the Comparative-Cervical Tuberculin Test Results of 225 Caudal Test Suspects

Statistic of Fit for Dependent Variable

<table>
<thead>
<tr>
<th>Source of Error</th>
<th>Degrees of Freedom</th>
<th>Partial Sum of Squares</th>
<th>F. Value</th>
<th>Probability &gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avian-Bovine PPD’s</td>
<td>1</td>
<td>162.5152</td>
<td>19.84931</td>
<td>0.0001</td>
</tr>
<tr>
<td>Caudal Tuberculins</td>
<td>3</td>
<td>183.2480</td>
<td>7.46053</td>
<td>0.0002</td>
</tr>
<tr>
<td>Interaction</td>
<td>3</td>
<td>17.2486</td>
<td>0.70224</td>
<td>0.5545</td>
</tr>
</tbody>
</table>
Figure 1--Comparative-Cervical Tuberculin Test Results from 225 Caudal Test "Suspects" from Bovin Tuberculosis Free Herds Tested within Seven Days after Caudal Test.
TABLE 3--Results of the Comparative-Cervical Tuberculin Test Applied to 204 Caudal Tuberculin Test Suspects at Least 60 Days After the Caudal Test on Tuberculosis-Free Dairy Cattle

<table>
<thead>
<tr>
<th>Caudal Test Tuberculin</th>
<th>Number Tested</th>
<th>Millimeters of Increased Skin Thickness</th>
<th>Avian PPD</th>
<th>Bovine PPD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\bar{X}$</td>
<td>St. Dev.</td>
</tr>
<tr>
<td>HCSM Tuberculin</td>
<td>65</td>
<td></td>
<td>3.88</td>
<td>2.86</td>
</tr>
<tr>
<td>Beef Broth OT</td>
<td>50</td>
<td></td>
<td>4.03</td>
<td>2.30</td>
</tr>
<tr>
<td>PPD 5</td>
<td>41</td>
<td></td>
<td>5.32</td>
<td>4.92</td>
</tr>
<tr>
<td>PPD 7</td>
<td>48</td>
<td></td>
<td>3.92</td>
<td>2.44</td>
</tr>
<tr>
<td>TOTAL</td>
<td>204</td>
<td></td>
<td>4.22</td>
<td>3.21</td>
</tr>
</tbody>
</table>

TABLE 4--Paired Comparison Analysis of Variance of the Comparative-Cervical Tuberculin Test Results of 204 Caudal Test Suspects

Statistic of Fit for Dependent Variable

<table>
<thead>
<tr>
<th>Source of Error</th>
<th>Degrees of Freedom</th>
<th>Partial Sum of Squares</th>
<th>F. Value</th>
<th>Probability $&gt; F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avian-Bovine PPD's</td>
<td>1</td>
<td>149.6621</td>
<td>15.57950</td>
<td>0.0007</td>
</tr>
<tr>
<td>Caudal Tuberculins</td>
<td>3</td>
<td>178.4178</td>
<td>6.19097</td>
<td>0.0003</td>
</tr>
<tr>
<td>Interaction</td>
<td>3</td>
<td>4.5982</td>
<td>0.15955</td>
<td>0.9230</td>
</tr>
</tbody>
</table>
Figure 2--Comparative-Cervical Tuberculin Test Results from 204 Caudal Test "Suspects" from Bovine Tuberculosis Free Herds Tested at Least 60 Days after Caudal Test.
Figure 3--Herd A: Comparative-Cervical Tuberculin Test Results of 28 Dairy Cattle by Post-mortem Examination Results.

- No Gross Lesions (25)
- Gross Lesions Resembling Tuberculosis (3)
Figure 4--Herd B: Comparative-Cervical Tuberculin Test Results of 74 Beef Cattle by Post-mortem Examination Results

- No Gross Lesions [33]
- Gross Lesions Resembling Tuberculosis [41]
Figure 5--Herd C: Comparative-Cervical Tuberculin Test
Results of 129 Beef Cattle by Presence or Absence of Acid-Fast Bacteria

- No Lesions with Acid-Fast Bacteria (63)
- Lesions with Acid-Fast Bacteria (66)
Figure 6--Herd D: Comparative-Cervical Tuberculin Test Results of 115 Mature Dairy Cattle by the Presence of Evidence of Tuberculosis as Determined by Histologic and Bacterologic Examination or the Absence of such Evidence as Determined by Post-mortem Examination and/or Laboratory Examination.

- No Evidence of Tuberculosis [69]
- Lesions Suggestive or Compatable on Histologic Exam. [46]
- Culture Positive for M. bovis [37]
Figure 7--Comparative-Cervical Tuberculin Test Results from 156 Mature Cattle with Evidence of Bovine Tuberculosis
TABLE 5--The Classification by Individual Herds of 156 Animals Found to Have Evidence of Bovine Tuberculosis

<table>
<thead>
<tr>
<th>Classification</th>
<th>HERD A</th>
<th>HERD B</th>
<th>HERD C</th>
<th>HERD D</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Suspect</td>
<td>0</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>Reactor</td>
<td>2</td>
<td>31</td>
<td>58</td>
<td>25</td>
<td>116</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>3</td>
<td>41</td>
<td>66</td>
<td>46</td>
<td>156</td>
</tr>
</tbody>
</table>
Figure 8--Comparative-Cervical Tuberculin Test Results from 225 Caudal Test Suspects from Bovine Tuberculosis Free Herds—Seven Day Test
TABLE 6--Comparison of the Results of Comparative-Cervical Tuberculin Tests Conducted Within 7 Days and at Least 60 Days After the Caudal Fold Test on Tuberculosis-Free Dairy Cattle

<table>
<thead>
<tr>
<th>Test</th>
<th>Total Tested</th>
<th>Negative</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
</tr>
<tr>
<td>7 Days</td>
<td>225</td>
<td>207</td>
<td>92.0</td>
<td>16</td>
<td>7.1</td>
<td>2</td>
</tr>
<tr>
<td>60 Days</td>
<td>204</td>
<td>182</td>
<td>89.2</td>
<td>17</td>
<td>8.3</td>
<td>5</td>
</tr>
</tbody>
</table>
TABLE 7--Comparison of the 60-Day Test Viewed in Serial with the 7-Day Test

<table>
<thead>
<tr>
<th>7-Day Test</th>
<th>60-Day Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td>Negatives</td>
</tr>
<tr>
<td></td>
<td>Suspects</td>
</tr>
<tr>
<td></td>
<td>Reactors</td>
</tr>
<tr>
<td></td>
<td>Not Tested</td>
</tr>
<tr>
<td>Suspects</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Negatives</td>
</tr>
<tr>
<td></td>
<td>Suspects</td>
</tr>
<tr>
<td></td>
<td>Reactor</td>
</tr>
<tr>
<td></td>
<td>Not Tested</td>
</tr>
<tr>
<td>Reactors</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Suspect</td>
</tr>
<tr>
<td>TOTAL TESTED</td>
<td>225</td>
</tr>
</tbody>
</table>

TABLE 8--Comparison of Scattergram Classification of Tuberculous Cattle with Cattle From Herds Free of Tuberculosis

<table>
<thead>
<tr>
<th>Test</th>
<th>Total Tested</th>
<th>Scattergram Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>Percent</td>
</tr>
<tr>
<td>Tuberculous Cattle</td>
<td>156</td>
<td>18</td>
</tr>
<tr>
<td>Tuberculosis-Free Cattle</td>
<td>225</td>
<td>207</td>
</tr>
</tbody>
</table>
Ten years ago Dr. A. F. Ranney, former Chief Staff Veterinarian, stood before this same group and reported "The need for continued vigilance in our Tuberculosis Eradication Program was never more obvious than it is today. While the incidence of bovine tuberculosis is relatively low, we must remember that tuberculosis is still dispersed countrywide." I believe you will see from the data presented in this report that although the incidence is much lower than in 1963, tuberculosis is still dispersed countrywide and our need for continued vigilance has never been greater.

Much progress has been made in our Tuberculosis Eradication Program as we attempt to qualify the entire United States as accredited-free. On March 7, 1973, the State of New Mexico became the only State not in the northeast portion of the United States to be officially declared Tuberculosis Accredited-Free. Figure 1 shows five States in the United States and the Virgin Islands to be accredited-free. States with asterisks have not discovered a tuberculous cattle herd in more than 5 years; thus, they meet the 5-year freedom from *M. bovis* infection requirement for Tuberculosis Accredited-Free status. However, most are lacking in some other requirement set forth by the *Uniform Methods and Rules—Tuberculosis Eradication*, such as proper cattle identification regulations or cattle dealer regulations.

We expect that a careful review in each of these States would reveal many of them could qualify for Tuberculosis Accredited-Free status by one or two regulation changes. One of these States has discovered an *M. bovis* herd since the close of the fiscal year, after an apparent absence of the disease for 8 years.

During fiscal year 1973, there were 33 *M. bovis* confirmed herds and five *M. bovis* suspicious herds detected, for a total of 38 lesion herds in the United States. Figure 2 shows the location of these 38 lesion herds in 15 States and Puerto Rico. As was mentioned previously, tuberculosis is still dispersed countrywide. An outbreak in Wisconsin was the first known tuberculous cattle herd there in over 2 years. This affected herd was located by tracing a cow found to have well-marked mediastinal lesions on routine postmortem examination. A tuberculin test of the 32 dairy cattle on the farm of origin revealed 25 reactors. The only possible source of infection was fence line contact with a neighboring infected herd in 1963. An unsolved report of an *M. bovis* calf on regular kill in October 1971 has also been traced to this same herd. An isolation of *M. bovis* was made from a cat destroyed as a part of the depopulation.

* Acting Chief Staff Veterinarian, Tuberculosis Eradication, Cattle Diseases, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture.
Figure 3 shows the incidence of lesion herds for the past 10 years, with an all-time low of 38 herds for FY 1973. Twenty-nine of these lesion herds were found as a result of traceback testing and nine as a result of testing for other purposes. You will note that the number of herds found annually by traceback testing has not improved, even though this is our major tuberculosis surveillance system.

In FY 1963, over 8 million cattle were tested to locate 213 lesion herds as opposed to 3 million cattle tested in FY 1973 to locate 38 lesion herds. How many additional lesion herds would have been located if more tests had been conducted is only a guess.

Of the 29 lesion herds located as a result of traceback testing in FY 1973, 11 of them were the direct result of an *M. bovis* lesion case in a regular kill animal. Unfortunately, we were unable to find a herd of origin for 23 other *M. bovis* lesion cases on regular kill. Figure 4 shows the location where these 23 cases were slaughtered and a possible source or sources for each case. You will note that four cases have been closed as having originated from Mexico. The disturbing fact is that all steers entering the country from Mexico are tuberculin negative and identified by an ear-tag at the border. We have yet to recover our first Mexican ear-tag in one of these regular kill lesion animals which supposedly originated in Mexico.

Our motto used to be “Find it, confine it, and eradicate it.” It is more accurate today to say “Find it, confine it, and depopulate it.” Figure 5 shows that 17 of the 38 lesion herds in FY 1973 were depopulated. This is the only absolute way to eliminate the infection and prevent further spread. Significant factors in depopulation were: (1) Completion of the depopulation of a beef herd of 6,506 head in Louisiana; (2) Depopulation of a beef herd of 979 head in Oregon—its first *M. bovis* herd in 12 years; (3) Depopulation of a beef herd of 1,417 head in Texas—a herd that had been under quarantine since 1969, the original infection dating back to 1951. Seven States depopulated all known affected herds. Since the close of the fiscal year, three more of these herds (Florida, Tennessee, and Massachusetts) have been depopulated. Unfortunately, indemnity rates and funds were not sufficient to depopulate all lesion herds.

The lower line in Figure 6 shows the number of affected herds that were completely depopulated in the past 10 years. The 17 herds shown for fiscal year 1973 represents 44.7 percent of the 38 lesion herds, as compared to 40.4 percent for FY 1972. This is a new high, but still a long way from our goal of 95 percent depopulation necessary for an accelerated eradication program.

Our major tuberculosis surveillance system is investigating suspicious lesions reported to us by meat inspection personnel. Of the 1,044 investigations completed in FY 1973, 234 were suspicious of tuberculosis. The herd of origin was located in 101 cases, or 43.2 percent of the time. Tracing success is directly related to identification.

Figure 7 shows that the herd of origin was located in 67 percent of the 105 cases which had identification and only 24 percent of the 129 cases that had no identification. The identification and tracing of the tuber-
culous animals has been recognized as a valuable program aid for more than 60 years as evidenced by recommendations made in 1910 by the International Commission on the Control of Bovine Tuberculosis from which I quote: "This Commission recognizes that the discovery of tuberculosis in animals slaughtered for food purposes furnishes one of the best possible means of locating the disease on the farm, and therefore recommends the adaption of some system of marking, for purposes of identification, all cattle 3 years old and over shipped to slaughter." Sixty-three years later, we are only able to recover identification devices from 44.8 percent of our suspicious lesion cases.

When we look at these same 234 suspicious lesion cases by slaughter class, we find in Figure 8 that 70 percent of the investigations were successful when adult animals, generally identified, are involved and only 19 percent successful when feeder-type animals are involved. Until such time as we have uniform identification for all classes of animals, we must concentrate our efforts on identifying every adult animal going to slaughter back to the farm of origin.

If we are to locate herds at a faster rate before spread of infection has occurred, we need to increase the number of investigations of tuberculosis traceback cases from regular kill. Figure 9 shows that this number has not varied greatly in the last 4 years. An explanation for this, of course, is that Veterinary Services can only investigate as many cases as meat inspection personnel submit to them.

Figure 10 shows that 41 federally inspected establishments (nearly 50 percent) slaughtering over 100,000 cattle annually did not submit any granulomas. Only 10 establishments submitted three or more granulomas per 100,000 cattle slaughtered.

Figure 11 shows that 30 federally inspected establishments (over 30 percent) slaughtering over 20,000 adult cows annually did not submit any granulomas, and another 35 establishments only submitted 0.1-2.9 cases per 20,000 animals slaughtered.

The Tuberculosis and Johne's disease Committee of the U.S. Animal Health Association in their 1972 report recommended the use of the comparative-cervical tuberculin test as an official retest procedure. You have just heard a report on the use of the comparative-cervical test and its potential as a diagnostic aid in detecting early infection that might otherwise be missed. Figure 12 shows the location of the 124 veterinarians in 28 different States that were approved as of August 1, 1973, to conduct the comparative-cervical test. Since then, additional personnel have been trained in Montana, Indiana, and Minnesota.

Our goal is the eradication of \( M. \) *bovis* from domestic livestock in the United States. It seems inconceivable that we can reach this goal as long as we continue to isolate \( M. \) *bovis* from other animal species which generally have no restrictions regarding interstate movements. During FY 1973 \( M. \) *bovis* was isolated at Veterinary Services Diagnostic Laboratory, Ames, Iowa, from bison, kudu, monkey, swine, tapir, fallow deer, aoudad, axis deer, and a cat.

The new meat inspection regulations regarding the disposition of
tuberculous swine carcasses has forced the swine industry to face squarely the unsolved problem of swine tuberculosis. A mandatory swine identification program is the first step toward initiating a swine tuberculosis program during the last half of FY 1974 so we can enter Phase II (eradication with indemnification) in FY 1975. We currently have special projects in South Dakota and North Carolina studing some of the problems associated with a swine tuberculosis eradication program.

REFERENCES
2. Rutherford, J. G. et al: The Control of Bovine Tuberculosis, Resolu-
tion 5, Rep. of the International Commission of the AVMA, USDA, BAI, Circ. 175, 10-11, 1910.
3. Roswurm, J. D. and Konyha, L. D.: The Comparative-Cervical Tuber-
culin Test as an Aid to Diagnosing Bovine Tuberculosis, Proceedings Seventy-Seventh Annual Meeting of USAHA 1973.
Tuberculosis Eradication

Lesion Herds Confirmed and Suspicious

Fiscal Year 1973

Detected Herds with TB Infection
Tuberculosis Eradication

**23 LESION CASES* CONFIRMED M. BOVIS NOT TRACED TO ADDITIONAL INFECTION**

Fiscal Year 1973

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**Tuberculosis Eradication**

**PROPORTION OF LESION HERDS DEPOPULATED**

Fiscal Year 1973
Tuberculosis Eradication

**LESION HERDS-FY 1963 THROUGH 1973 AND THOSE DEPOPULATED**

![Graph showing lesion herds and depopulated numbers from 1963 to 1973.]

FISCAL YEAR

**234 SUSPICIOUS LESION CASES (Regular Kill)**

**ANIMALS IDENTIFIED AND UNIDENTIFIED**

*Fiscal Year 1973*

- **105 WITH IDENTIFICATION**
  - **33%** Unsuccessful or Inconclusive
  - **67%** Successful

- **129 NO IDENTIFICATION**
  - **76%** Unsuccessful or Inconclusive
  - **24%** Successful

*U.S. Department of Agriculture Veterinary Services Animal and Plant Health Inspection Service*
TUBERCULOSIS ERADICATION PROGRAM

Tuberculosis Eradication

234 SUSPICIOUS LESION CASES (Regular Kill)
BY SLAUGHTER CLASS
Fiscal Year 1973

- Feeder Total: 118
  - 81% unsuccessful or inconclusive
  - 19% successful

- Adult Total: 91
  - 30% unsuccessful or inconclusive
  - 70% successful

- Unknown Total: 25
  - 40% unsuccessful or inconclusive
  - 60% successful

U.S. DEPARTMENT OF AGRICULTURE
VETERINARY SERVICES
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

Tuberculosis Eradication

TUBERCULOSIS TRACEBACK CASES
(VS 6-35)

- Total cases closed: 1,025
  - 240 in 1970
  - 150 in 1971
  - 152 in 1972

- Cases confirmed or suspicious of tuberculosis: 1,044
  - 234 in 1973

U.S. DEPARTMENT OF AGRICULTURE
VETERINARY SERVICES
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
Tuberculosis Eradication

FEDERAL ESTABLISHMENTS SLAUGHTERING OVER 20,000 COWS BY GRANULOMA SUBMISSION RATE

30 EST. (NONE)
19 EST. 3.0-39.0 CASES PER 20,000 SLAUGHTERED
35 EST. 0.1-2.9 CASES PER 20,000 SLAUGHTERED

U.S. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE

FEDERAL ESTABLISHMENTS SLAUGHTERING OVER 100,000 CATTLE* BY GRANULOMA SUBMISSION RATE

41 EST. (NONE)
10 EST. 3.0-21.4 CASES PER 100,000 SLAUGHTERED
33 EST. 0.1-2.9 CASES PER 100,000 SLAUGHTERED

* EXCLUDING CALVES

U.S. DEPARTMENT OF AGRICULTURE VETERINARY SERVICES ANIMAL AND PLANT HEALTH INSPECTION SERVICE
TUBERCULOSIS ERADICATION PROGRAM

Tuberculosis Eradication

VETERINARIANS APPROVED*
TO CONDUCT COMPARATIVE-CERVICAL TEST
As of August 1, 1973

*TOTAL APPROVED - 124

Figure 12
1973 REPORT OF THE COMMITTEE ON TUBERCULOSIS AND PARATUBERCULOSIS

Chairman: Dr. R. M. Scott, East Lansing, Michigan
Co-Chairman: Dr. A. R. McLaughlin, Madison, Wisconsin

Dr. Joseph L. Blair, Washington, D.C.; Dr. C. E. Boyd, Columbia, South Carolina; Dr. John Dick, Harrisburg, Pa.; Mr. Burton Eller, Denver, Colorado; Dr. J. G. Flint, St. Paul, Minn.; Dr. Irwin H. Huff, Pierre, South Dakota; Dr. Hudson Jones, Austin, Texas; Dr. A. F. Kaufmann, Atlanta, Ga.; Dr. L. D. Konyha, Hyattsville, Md.; Dr. A. B. Larson, Ames, Iowa; Mr. Rodney Larson, Fruitdale, South Dakota; Dr. W. L. Mallmann, East Lansing, Mich.; Dr. G. R. McKeown, Ottawa, Ontario, Canada; Dr. A. P. Schneider, Boise, Idaho; Dr. P. L. Smith, Sacramento, Calif.; Dr. G. R. Snyder, Washington, D.C.; Dr. G. W. Spangler, Des Moines, Iowa; Dr. R. J. Stadler, Hartford, Conn.; Dr. Charles Thoen, Ames, Iowa.

The committee on Tuberculosis and Paratuberculosis met on Monday and Tuesday afternoons. The following individuals gave presentations before the committee:

1. Dr. Billy Perryman, APHIS, Veterinary Services, North Carolina, reported on epidemiologic studies currently in progress on tuberculosis infected swine herds in North Carolina.

2. Dr. Irwin H. Huff, APHIS, Veterinary Services, South Dakota, reported on special studies he is conducting on tuberculosis infected swine herds in South Dakota.

Committee discussions during the two days of meetings were concerned with a number of topics related to tuberculosis in cattle, swine, poultry, and zoo or exhibition animals as well as Johne’s Disease.

TUBERCULOSIS IN CATTLE

Dr. Lloyd Konyha, Tuberculosis Staff, APHIS, Veterinary Services, Hyattsville, Maryland reported to the committee that a special lot of *M. bovis* PPD tuberculin has been prepared and is currently under evaluation. It is anticipated that this *M. bovis* PPD tuberculin may be available for field use by July 1975. It is intended that this *M. bovis* PPD tuberculin will be submitted for consideration as the International Standard. The committee recommends that reports of the evaluations of this *M. bovis* PPD tuberculin be distributed to all state and federal animal health officials.

The committee was informed that regulatory veterinarians in 29 states have been approved to conduct the comparative cervical tuberculin test. The committee recommends that the comparative cervical tuberculin test be adopted on a nation-wide basis and that the Uniform Methods and Rules-Tuberculosis Eradication be amended as follows:

Part I: Definitions—paragraph 8—to read “Comparative-Cervical Tuberculin Test.”
Part II: Official Test Procedures—paragraph F 3 (B) to read “Negative—‘N—Animals showing no response to tuberculin or those animals with response which have been classified negative for *M. bovis* by the comparative cervical tuberculin test.

Part III: Disposition of Tuberculin Response Animals—paragraph B (1) to read “Suspects to the tuberculin test shall be quarantined to the premises where found until: (a) Retested by the comparative cervical tuberculin test within 10 days of the caudal fold injection or (b) Retested by the comparative cervical tuberculin test or caudal tuberculin test after 60 days or (c) shipped under permit direct to slaughter in accordance with State and Federal Laws and regulations.

Part III: paragraph C.—add sub-paragraph 2 to read “Movement of deviators classified negative for *M. bovis* by the comparative cervical tuberculin test should not be restricted.”

Part IV: Quarantine Procedures—paragraph 5 to read “Suspects in herds where only suspect animals are disclosed shall be quarantined to the premises until retested and classified negative or shipped direct to slaughter under permit.

The committee reviewed problems involving the slaughter and disposition of tuberculin reactors. In this regard, the committee recommends that Part III—paragraph A(1) of the Uniform Methods and Rules be amended to read “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 in maintained within 15 days of classification or otherwise be destroyed under the direct supervision of a regulatory veterinarian to assure that the carcass is either cooked or condemned.”

A representative of the American Meat Institute was asked to contact the Pet Food Institute to determine if it could assist in disposing of “Passed for Cooking” Carcasses.

Tuberculin testing of livestock by government employed paramedical personnel was discussed. The chairman appointed a sub-committee to make contacts, explore complications, and investigate the potential, for use of such personnel.

It is obvious to the committee that the 1980 projected eradication date for bovine tuberculosis will not be met unless we have an increased rate of discovery of infected herds and an increased rate of herd depopulations. The committee urges that all recommended steps be taken to achieve the goal. The U.S.D.A. should enforce all the provisions of the Uniform Methods and Rules in each and every state or suspend the accredited status.

State and Federal officials are urged to do everything in their power to increase reporting of thoracic granulomas. The committee recommends that the present reporting form be simplified.

A state official reported on steps being taken by his state to compensate packers for losses due to tuberculosis disclosed in cattle on
regular kill. After discussing the matter, the committee decided that no recommendation would be made at this time.

**TUBERCULOSIS IN SWINE**

The committee discussed the initiation of a swine tuberculosis eradication program. It is recommended that mandatory swine identification regulations be implemented by U.S.D.A. and the respective states as soon as possible.

A sub-committee was appointed by the chairman to prepare a draft of Uniform Methods and Rules for Swine Tuberculosis Eradication for committee consideration.

**TUBERCULOSIS IN ZOO OR EXHIBITION ANIMALS**

The subject of tuberculosis in zoo or exhibition animals and the potential danger of spread of disease to livestock was discussed. The committee recommends that consideration be given to the development of interstate and intrastate regulations for the control of movement of these animals. The committee was informed that laboratory support is available to all exhibitors of exotic animals from Veterinary Services Diagnostic Laboratory, Ames, Iowa.

**JOHNE'S DISEASE**

Dr. A.B. Larson, Ames, Iowa reported on the Wisconsin Vaccine trials.

The committee recommends that the official title of this committee be changed to “Committee on Tuberculosis and Johne's Disease.”

Respectfully Submitted

R. M. Scott, Chairman
Thank you, Dr. Steele.

In accepting this diploma I should like to pay tribute to all those who supported me and who in any way encouraged the application of epidemiologic principles in the eradication of bovine tuberculosis. The list of people would be too long to enumerate here but I will mention two among many who inspired me to push forward during the years that I was assigned to working on this dreaded disease.

Dr. Fred Soper, once director of the Pan American Sanitary Bureau, gave us a clear definition of eradication when he said, "Eradication has no meaning except as an absolute, without modifying phrases or limiting adjectives."

Dr. J. Arthur Myers, formerly Professor of Medicine and Preventive Medicine and Public Health, University of Minnesota Medical and Graduate Schools, made the following statement, "For numerous activities of life, it has long been observed that many persons work enthusiastically and untiringly on health and other projects until the glamour is gone and the drudgery of the final clean-up begins. The number willing to carry through to complete accomplishment is greatly limited, which is apparently why so many worthwhile projects are never completed."

There are two basic reasons for completing our task of eradication. First, we should think of the public health significance of this disease even though it is less evident than when it was estimated that 25% of tuberculosis in children and 50/6 of all tuberculosis in humans was of the bovine type. Secondly, let us consider an interesting statement: "The world can exist without lawyers, businessmen, bureaucrats or any other profession, but it cannot survive without the farmer." (Charles W. Wampler) In eradicating bovine tuberculosis we are supporting a basic industry, the farmer who is a cattle owner.

When we consider the procedures used and the years of effort in attacking bovine tuberculosis, it is time for us to settle down and make sure that we incorporate sound energetic epidemiologic procedures whenever and wherever bovine tuberculosis may be found. I have confidence in those who are continuing the attack.

I am grateful for the honor of this diploma from the American Veterinary Epidemiology Society.
Dr. Albert F. Ranney has had a long and distinguished career in preventive veterinary medicine and epidemiology. He received his veterinary medical education at the New York State Veterinary College, Cornell University graduating in 1932. Following graduation he went to work for the Bureau of Animal Industry, U.S. Department of Agriculture. The following year he completed his Master of Science at Cornell in veterinary science. In 1934 he was assigned to Vermont as a field veterinary officer where he was to concentrate on bovine tuberculosis control until he entered the U.S. Army Veterinary Corps in 1941. On the termination of his military service in 1946 he returned to Vermont where he remained until 1951, when he was assigned to North Dakota. After a short time in North Dakota he was called to Washington in 1952. In 1953 he was placed in charge of bovine tuberculosis eradication program in the United States.

From 1953 on he served with one goal in view—the total eradication of bovine tuberculosis in the United States. He was on the U.S. Livestock Sanitary Association Tuberculosis Committee, later the U.S. Animal Health Association, several committees of the National Tuberculosis Association and was a member of the International Union Against Tuberculosis. He contributed several papers on Bovine Tuberculosis, one of which I am proud to have coauthored. Dr. Ranney's persistence in devoting all his energies to the eradication of bovine tuberculosis has brought this goal to the verge of success. We are all thankful for his contribution and unaltering belief that bovine tuberculosis could be eradicated.

We are proud to present the Honorary Diploma of the American Veterinary Epidemiology Society to Dr. Ranney which reads as follows:

"The American Veterinary Epidemiology Society is proud to present the Honorary Diploma to Dr. Albert F. Ranney for his distinguished service and contribution to the progress of public health. His efforts and vision, compassion and understanding, desire and energy, have advanced Veterinary Public Health, and research, his counsel and advice have been of great value to his country.

Your colleagues are honored to have you accept this diploma."

Respectfully,

James H. Steele, President
K. F. Meyer, Honorary President
RESPONSE TO PRESENTATION OF HONORARY DIPLOMA

THE American Veterinary Epidemiology Society

is proud to present the

Honorary Diploma

to

ALBERT F. RANNEY

for his

DISTINGUISHED SERVICE and Contribution to the progress of public health. His efforts and vision, compassion and understanding, desire and energy, have advanced Veterinary Public Health, and research. His counsel and advice have been of great value to his country.

YOUR COLLEAGUES are HONORED to HAVE YOU ACCEPT THIS DIPLOMA.

respectfully,

President

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Albert F. Ranney

Cornell 1932 D.V.M.
B.A.I. 1933 M.S.
Cornell 1934 Assigned to Vermont

U.S. Army 1941-46 Returned to Vermont
1946 North Dakota
1951 Washington D.C.
1952 T.B. Chief
1953 Retired
1972

Committee on T.B., International Union Against T.B., Several Committees Nat. T.B. Assoc., U.S. A.H.A. T.B. Committee
PROPOSED AGENDA FOR SPECIAL WORK SESSION ON RNA TUMOR VIRUSES

Moderator: Dr. S. McConnell

I. Opening Remarks .................. Dr. C. J. York

II. Introduction of Speakers .......... Dr. C. J. York

III. Recent Developments:

   (a) Avian RNA Tumor Viruses ...... Dr. Lyman Crittenden
   (b) Feline RNA Tumor Viruses ..... Dr. Charles Rickard
   (c) Murine RNA Tumor Viruses ..... Dr. Padman Sarma
   (d) Bovine RNA Tumor Viruses ..... Dr. Martin Van Der Maaten
   (e) Terminology & Classification .. Dr. Ben R. Burmester

IV. Laboratory Safety ................ Dr. Victor Cabasso

V. Commentary ........................ Dr. A. J. Kniazeff

VI. General Discussion ............... Committee Members

VII. Recommendations ................. Committee Members
A REPORT OF NOMENCLATURE OF THE RNA TUMOR VIRUSES

B. R. Burmester

Dr. Peter K. Vogt, Chairman of the Leukovirus Study Group of the IC-NV Vertebrate Virus Subcommittee, has reported that no definite decisions have been made concerning the nomenclature of the RNA tumor viruses.

A poll was taken, and the majority favored “oncornaviruses”; however, no action was taken by the subcommittee because the selection of this term would be contrary to the established rules of the ICNV.

The term “oncornaviruses” is being used by some prominent scientists, and it may be difficult to avoid its adoption by the ICNV. The committee will meet at the next Virology Congress in 1975 at which time changes in the rules may be considered.
RECENT DEVELOPMENTS IN THE BIOLOGY OF AVIAN ONCORNAVIRUSES

L. B. Crittenden*
United States Department of Agriculture

INTRODUCTION

There have been many developments in the study of the avian RNA tumor viruses over the past several years. Rather than attempt to cover them all, I prefer to cover the major developments in our understanding of the contributions of the host genome to virus transmission and to the genetic makeup of the progeny virus, and to briefly discuss developments that should lead to intensive studies of virus genetics and a better understanding of the viral genome.

ENDOGENOUS SUBGROUP E VIRUSES

Nature of RSV(0): Soon after a cell culture assay system for Rous sarcoma viruses (RSV) was perfected, Rubin and Vogt found that stocks of the Bryan high-titer strain of RSV (BH-RSV) contained an associated virus (RAV) that did not transform cells in culture. This virus did produce lymphoid leukosis in chickens and therefore is considered an avian leukosis virus (ALV). Cells transformed by solitary infection with a transforming particle of BH-RSV or the Bryan standard strain (BS-RSV) failed to yield detectable progeny virus. However, these cells contained sarcoma virus genes (S) for transformation because pseudotypes of RSV; that is, new stocks of infectious transforming virus, could be produced by super-infection of these "nonproducer" (NP) cells with RAV and ALV, which were called "helper" viruses. Figure 1 outlines the production of pseudotypes that can be obtained by infecting NP cells carrying the defective viral genome with a leukosis virus. Stock preparations of RSV pseudotypes contained two types of virions. The infecting ALV having the genetic material for the ALV envelope properties (A) and for the leukotic transformation (L) occurred in high proportion (1 in Figure 1). RSV nucleoids containing genes for defective envelope properties (O), and for transformation (S) in ALV envelopes (2 in Figure 1) that is phenotypically mixed particles, occurred in a smaller proportion. It is likely that some particles represent the parental genomes (AL) and (OS) in defective envelopes (not shown in Figure 1). Significantly, phenotypic mixtures never yielded detectable genotypic mixtures that contained non-defective sarcoma viruses, with the envelope genes (A) and transforming genes (S) in the same particle (3 in Figure 1). Therefore, no recombination between envelope markers and

*Animal Physiology and Genetics Institute, Agricultural Research Service, Beltsville, Maryland 20705.
cell transformation markers was noted in these stocks. The other recombinant type (OL) is difficult to detect but is assumed not to occur. Pseudotypes were very useful for studies of virus classification and genetic resistance because they had all the viral envelope properties; namely, host range, interference patterns, and antigenicity of the superinfecting ALV. These properties were combined with the ability to transform cells, a property of the RSV genome. Avian oncornavirus subgroups A, B, C, and D have been identified by envelope criteria.

Doughtery and Di Stephano found C-type particles typical of the avian tumor viruses in cultures of NP cells, suggesting that a virus was actually produced by these cells. Robinson soon found that the fluids from NP cell cultures that have been labeled with H-ruidine contained labeled particles that banded at the same density as typical leukemia-sarcoma virus particles in sucrose gradients. Soon after, Weiss and Vogt showed that this virus was infectious for Japanese quail and certain rare chicken cell types. It was called RSV(O) because no helper virus appeared to be needed for complete infectivity. Because NP cells produced RSV-O, the name NP was discarded, and these cells were called leukosis virus negative Rous (L-R) cells. The Hanafusas and Weiss found three distinct types of virus produced by different L-R clones. The first virus is now called RSV(RAV-0), RSV(RAV-60) or RSV(chf) and is produced by cells which carry natural group-specific (gs) antigen that contributes subgroup E envelope properties to the virus through phenotypic mixing. The second virus is produced in cells that lack gs antigen and is called RSV(-). RSV(-) has the typical buoyant density and reverse transcriptase of the avian oncornaviruses and can infect cells when introduced into the cell by cell fusion with inactivated Sendai virus. The envelope properties of RSV(O) can be readily changed by growth in cells that lack gs antigen or the envelope properties of RSV(-) can be changed by growth in cells which possess gs antigen (Table 1). A third virus, RSValpha(O), is apparently a stable variant that is produced by certain L-R clones. This virus has a typical buoyant density, but it lacks reverse transcriptase that is typically found in RNA tumor viruses and cannot replicate in any cell type, even after introduction by cell fusion methods because viral reverse transcriptase is necessary for replication. Table 1 summarizes the properties of these cell clones and viruses.

Nature of Endogenous Virus Information: Dougherty et al have also found C-type particles and gs antigen in normal embryos from which no infectious virus could be isolated by methods available at that time. Some of these embryos came from flocks that had been kept free of infectious virus for several generations. Therefore, the viral information must have been transmitted vertically in the flocks previously thought to be virus free.

Payne and Chubb detected gs antigen in embryo extracts from one inbred line, but not from another. These lines were free of infectious virus when tested by available assay methods. From crosses and backcrosses
of these lines Payne and Chubb determined that gs antigen was inherited as a single dominant gene called gs+. These observations suggested that viral information may be integrated into the host genome. It was not clear, however, whether gs+ was a structural gene for viral protein or a regulator gene controlling the expression of a structural gene.

In subsequent studies, Weiss, Weiss and Payne, and Hanafusa et al. showed that this same endogenous information controlled by the gs+ gene regulated the ability of L-R clones to produce infectious RSV in some lines of chickens. As shown in Table 1, propagation in gs+ cells invariably resulted in the production of infectious RSV(RAV-O). The factor present in these cells that gave the progeny viral envelopes subgroup E characteristics was called chick cell-associated helper factor (chf) by Hanafusa et al. The gs+ gene and chf are not always associated in the same cell type, and further work is needed to clarify their interrelationships.

We now know that any virus propagated in chf+ cells acquires the unique envelope properties of RSV(RAV-O). Hanafusa et al. propagated RAV-1, belonging to subgroup A, in chf+ cells and were able to isolate a virus that they called RAV-60 mixed with the parental type RAV-1. Viruses with the unique host range, interference properties, and antigenicity of RAV-60 and RSV(RAV-O) were grouped in subgroup E.

Some embryos of RPRL line 7 spontaneously released a subgroup E leukemia virus. This virus, called RAV-O, had all the chemical and physical properties of a typical leukemia virus except that, unlike RAV-60, it did not propagate well on Japanese quail cells and was particularly susceptible to high temperatures and to freezing and thawing.

Weiss et al. used X-irradiation and chemical agents to induce virus from cells free of infectious virus before treatment. They induced subgroup E virus production in all cell types tested, but at a frequency lower than has been observed for mouse cells. These results suggest that all types of embryos have the potential for complete virus production. No striking difference in the rate of induction between gs+ and gs- cells was found, suggesting that all the viral structural genes for subgroup E virus are present in the host genome and that genes or environmental stimuli may allow them to be expressed. It has been found that all cell types tested have viral subgroup E information that can be rescued by infection with viruses of other subgroups, confirming the idea that at least some viral genetic information is present in all chicken cells.

The Hanafusas have found, as noted in Table 1, that infection of gs+ cells with the reverse transcriptase defective variant RSValpha(O) does not result in virus production. Therefore, the cellular genetic information for this essential enzyme may not be readily inducible or may be lacking at least in some host cell types.

The studies of Weiss et al. and our own showed that even cells that are resistant to subgroup E virus may release endogenous virus of the
excluded subgroup. Because the cells are resistant to reinfection, RAV-O is unlikely to be detected in the original host cell. Methods of co-cultivation with susceptible ringneck pheasant or chicken cells were devised to detect subgroup E virus production. Rescue methods of this sort must be incorporated into studies of the prevalence of endogenous viruses if confounding with genetic resistance is to be avoided.

Recently, we have found that RPRL line 7 and the related line 100 produce RAV-O at a very high frequency in cell culture and in the sera of intact birds up to the age of 17 weeks. Crosses of the virus producing (V + ) inbred lines with other chicken lines that do not produce virus (V -) are V + , suggesting that dominant genes controlling virus production may be present in line 7 related chickens or that vertical transmission from both male and female parents is very efficient. Virus production is apparently entirely dependent on the presence of genes controlling the V + phenotype and is independent of the gs + gene because RAV-O is produced in the presence or absence of natural gs antigen. The virus particles observed by Dougherty et al. in embryos presumed to be free of infectious virus may represent RAV-O. A low frequency of C-type particles has been observed in line 100 or line 7 cells that are resistant to subgroup E. RAV-O can be isolated from these cells by co-cultivation with subgroup E susceptible cells free of RAV-O (Smith et al., unpublished). If the cells observed by Dougherty et al. actually did release RAV-O, then this virus may be rather prevalent. Studies of the frequency of RAV-O production in a much larger sampling of flocks are needed.

The preceeding biological data suggest that genes in the host genome control the expression of avian oncornavirus structural virus information. The best example of this is the dominant autosomal gs + gene that controls the expression of gs antigen. A crucial question concerning the nature of these genes is posed by the provirus and viral oncogene hypotheses; that is, do all chicken cells carry viral genetic information in their genomes but express them only in the presence of certain genes, or are the segregating genes structural genes themselves?

This question has been approached by looking for DNA base sequences in the host genome which are homologous to viral RNA. All types of virus-free chicken cells tested contain DNA homologous to viral RNA and infected cells or tissues have an increased amount of viral DNA. Varmus et al. measured by the effect of host-cell DNA on the reassociation kinetics of DNA produced by reverse transcriptase. They found that viral sequences exist in the host-cell DNA in equal amounts whether the cells were gs + or gs -. However, this method did not detect an increase in viral DNA in the host cell after infection (Table 2).

From the above results, one must conclude that all types of chicken cell genomes carry some structural genes homologous to viral RNA, but one cannot conclude that all the necessary genes are present for complete virus production. Only biological experiments such as those of Weiss et al. suggest that information for complete virus is present. More
Recently Hayward and Hanafusa and Bishop et al. studied homology between cellular RNA and reverse transcriptase produced DNA to determine if endogenous viral genes are transcribed into RNA. They showed that gs+ cells had much more viral RNA than gs- cells (Table 2), suggesting that the gs+ gene controls transcription of viral sequences from DNA to RNA. These data support the idea that normal host cells contain viral structural genes for gs antigen and that the segregating genes at the gs+ locus control their expression through a regulatory system perhaps at the level of transcription. However, it must be determined if the same sort of regulatory system applies to all genes necessary for infectious virus production.

Table 3 summarizes the viral status of cells or individuals that are present in populations of chickens. Virus can be produced in the presence or absence of the gs+ gene that codes for the natural gs antigen. However, the virus cannot grow to high titers except in susceptible hosts. The small amounts of virus present in resistant cells can be detected only through co-cultivation with cells susceptible to but free of virus.

INTERACTION OF SUBGROUP E VIRUSES WITH VIRUSES OF OTHER SUBGROUPS AND HOST CELLS

The endogenous viruses have been placed in subgroup E because of their unique envelope properties. Table 4 gives the interference patterns observed among the presently known viral subgroups (A, B, C, D, E). Subgroup B, D, and E leukosis viruses strongly interfere with subgroup E RSV, whereas subgroups E leukosis viruses interfere slightly with subgroup D RSV and strongly with subgroups E RSV. Note that RAV-60 interferes with subgroup E RSV much more than does RAV-O, perhaps because the spontaneously produced RAV-O replicates less efficiently than RAV-60.

Chicken sera that neutralize subgroup E are very rare. However, Weiss and Biggs observed at least one antiserum that neutralized only subgroup E. Antisera to other subgroups rarely neutralized subgroup E virus. These observations suggest that subgroup E viruses possess unique viral envelope antigens. Perhaps birds produce antisera to subgroup E viruses only rarely because envelope antigens are commonly present during embryonic development and induce immunological tolerance.

The control of resistance to subgroup E viruses is more complex than control of resistance to other subgroups. Two loci have been reported to have dominant alleles for susceptibility. The tumor virus E (tve) locus was described by Payne et al. in lines of chickens that carried susceptibility to subgroup B virus. However, subgroup B virus resistant embryos have never been found to be susceptible to subgroup E viruses, suggesting that the tvb locus is involved. Therefore, it is not clear whether one or two loci have dominant alleles for susceptibility to subgroup E viruses. Table 5 presents alternative hypotheses for the
location of dominant genes for subgroup E virus susceptibility. Tests for allelism or independent segregation are in progress. These dominant genes for susceptibility apparently control receptors for penetration similar to the genetically controlled receptors for other viral subgroups.45

Another locus influences susceptibility to subgroup E viruses. This locus carries a dominant gene (le) that inhibits susceptibility in cells that carry the receptor for subgroup E virus penetration46 controlled by the tve and tvb loci. Existing data suggest that this gene may be the same as the gs+ gene; that is, gs+ cells are resistant to subgroup E while gs- cells are susceptible, provided they carry susceptibility alleles at the tve and tvb loci. Because gs+ cells carry subgroup E envelope information, the subgroup E viral envelope antigens possibly are concentrated in the cell membrane and interfere with viral penetration46, and thus make the cells resistant.

Another factor that influences susceptibility to subgroup E is the R' erythrocyte antigen47 associated with the tvb8 allele. The presence of this antigen enhances susceptibility to subgroup E in the presence of Ie. The mechanism of action is unknown.48

REPLICATION OF AVIAN ONCORNAVIRUSES THROUGH A DNA INTERMEDIATE

The original suggestion by Temin44 that the avian RNA tumor viruses replicate through an intracellular DNA intermediate probably associated with the host genome is now accepted by most workers. The accumulated evidence supporting this conclusion is summarized in Table 6. (See Baluda44 for review.)

The earliest evidence was the fact that actinomycin D, which specifically inhibits transcription from DNA to RNA but does not inhibit replication of viral RNA from an RNA template, blocks viral replication at any stage of the infectious cycle.49-50 This observation contrasts strikingly with the experience with non-oncogenic RNA viruses such as influenza viruses that replicate after the initial infectious stages in the presence of actinomycin D.49 A requirement for DNA synthesis early in the infectious cycle observed first by Temin44 and then by Bader45 supported the idea that virus specific DNA must be produced to establish infection.

Some of the strongest evidence for this notion came with the discovery of an RNA-directed DNA polymerase called reverse transcriptase associated with oncornavirus virions identified independently by Temin and Mizutani44 and Baltimore.45 Many other workers have confirmed these observations and found other important virion enzymes. This discovery identified an enzyme that provided the mechanism for the postulated reverse transcription. It also provided a powerful diagnostic tool for specific detection of oncornavirus infection.

The search for cellular DNA complementary to viral RNA has given further evidence for this hypothesis. Baluda44 and Rosenthal et al.37 have
shown that the number of viral DNA sequences per cell increases with RSV infection. This increase is also true of mammalian cells infected with avian oncornaviruses even though no infectious virus is produced (Table 2). These observations indicate that overt infection increases the gross amount of virus-specific DNA present in avian cells.

The most recent and striking evidence in support of the idea that these viruses can replicate through a DNA intermediate is the work of Hill and Hillova and Svoboda et al. These workers have produced productive infection of avian cells with DNA extracted for mammalian cells transformed with avian sarcoma viruses. The viruses so produced have the same phenotypic properties as the virus originally used to transform the mammalian cells.

Figure 2 illustrates the postulated steps in oncornavirus replication. After penetration of the nucleoid into the cytoplasm, the free RNA becomes a template for a minus DNA strand through the action of the viral reverse transcriptase. The DNA then becomes integrated into host DNA and replicates with cellular DNA. New viral RNA is transcribed from the host DNA by the host cell enzymes and codes for the viral proteins and structural components necessary for virus maturation.

STRUCTURE OF THE VIRAL GENOME AND VIRUS GENETICS

The avian oncornavirus genome has long been known to consist of single stranded RNA of about 10^7 molecular weight. Recent studies of viral genetics and RNA structure indicate that the 60-70S RNA probably consists of 30-40S functional subunits.

The first suggestion of a functional subunit structure came from the work of Duesberg and Vogt who found that helper independent, non-defective, avian sarcoma viruses had two RNA components called a and b, whereas the non-transforming leukemia viruses had only the smaller b component. The subunit structure was also attractive to explain the high frequency of recombination observed between host range and transformation markers when helper independent strains of RSV are studied.

Figure 3 represents a simple model of such recombination experiments. A subgroup A leukemia virus and a subgroup B sarcoma virus infect a cell susceptible to both subgroups. The progeny virus stock contains particles with phenotypically mixed subgroup A and B envelope properties. However, the genomes represent parental (1 + 2) and recombinant (3 + 4) particles, which can be differentiated by cloning. In the recombinant particles, the genes controlling the envelope (A and B) are reassorted with genes controlling transformation (L and S). As noted previously, recombination does not occur at high frequency in the defective strains of RSV. The reason biological difference between defective and non-defective avian sarcoma viruses is unknown.

Vogt has considered four models of the avian oncornavirus genome. Models I and II represent non-segmented genomes that appear to be unlikely for reasons previously stated. Models III and IV represent two
possible forms of a segmented genome that has subunit structure.

Model III supposes that the segments carry different genetic information; that is, the genome is haploid. This supposition is consistent with the high frequency of recombination observed, assuming that host-range and transformation markers occur on different, independently inherited segments. It is not compatible with the observation of heterozygosity and recent observations that cloned sarcoma viruses contain only the RNA subunit “a”. Alternatively, Model IV proposes that there are identical subunits. This model is compatible with the observation of heterozygosity and the occurrence of a single subunit structure of RNA, but high frequency recombination is more difficult to explain. To explain recombination in Model IV, Vogt proposed a copy-choice method of replication of the DNA provirus, in which one copy only is finally used as the template for progeny RNA. Alternatively, Weiss has suggested recombination among daughter strands of proviral DNA.

Accumulated temperature-sensitive mutant viruses affecting replication and transformation are providing excellent genetic material for recombination and complementation analysis of the viral genome. Analysis of these mutants should help resolve some of the remaining questions concerning the nature of the viral genome.

Recombination of Viral and Host Genes: Because many avian cells carry endogenous virus information that can be rescued by super-infecting virus, great care must be exercised in choosing the host cell for genetic studies. Recently, Weiss et al. have studied recombination between the endogenous host range marker (subgroup E) and the transformation marker of an infecting sarcoma virus of another subgroup. Table 7 summarizes the results. Note that recombination takes place only in gs+ cells which contain virus-specific RNA and not in gs- cells that do not. Since both cell types apparently carry the provirus, Weiss suggested that recombination with host elements takes place among the RNA progeny strands and not at the DNA level. These studies show that further progress in viral genetics and definition of the viral genome will depend heavily on knowledge of the genetic control of endogenous virus information. Reciprocally, definition of host genetics will depend on knowledge of the viral genome.

CONCLUSIONS

The studies reviewed here point to the key role that the host genome plays in the replication and genetic make-up of the progeny virus. The most recent studies indicating that recombination can take place between host controlled viral genomes and exogenous viral genes has far reaching implications for biology, raising the possibility that the oncornaviruses can transduce cellular genetic information that is not viral in nature. These observations also raise questions about the origin of information in the host genome that codes for complete virus. Possibly the host genes have been modified to become viral genes. In this view one would say that the virus contains host genes. On the other hand, viral
genes may have been inserted into the host genome. The host or viral origin of these genes is now lost in evolutionary history and is essentially unresolvable.

The occurrence of endogenous viruses raises vital questions about conventional concepts of transmission and control of diseases caused by exogenous viruses.

Repeated observations have been made that vertical virus transmission from dam to offspring is more important means of spreading lymphoid leukosis viruses than horizontal transmission through the environment of a chicken flock. Therefore, the notion that endogenous virus information and spontaneously produced subgroup E virus may be coded for by host genes introduces a new mechanism for vertical virus transmission.

Figure 4 illustrates congenital transmission of subgroups A and B leukosis viruses, which occurs regularly at a low frequency in most populations of chickens. Transmission of this type leads to a high frequency of immune tolerance to infection, and an increased frequency of lymphoid leukosis in mature chickens.

In genetic transmission, infectious or latent subgroup E virus information is inherited through host genes. Little is known about the role of these genes in the occurrence of lymphoid leukosis. Preliminary data indicate that spontaneously produced RAV-O has little influence on the incidence of lymphoid leukosis in lines of chickens in which it occurs.

Much more complete studies are needed to determine if the expression of gs antigen and RAV-O have a real influence on the occurrence of neoplasms, or if they interact with other leukosis viruses or stress factors to enhance the incidence of lymphoid leukosis. Under commercial conditions, chickens are kept only a small fraction of their natural lifetimes, but it has been observed that in mice the majority of neoplasms associated with endogenous viruses occur in the last third of their lifetime. Possibly, chickens must be kept for several years before the oncogenic potential of subgroup E viruses is fully realized. Therefore, we do not know whether it is important to the poultry industry to reduce or eradicate subgroup E virus infection. However, it is extremely important to understand the influence of subgroup E viruses on commonly used leukosis virus assay procedures. For example gs antigen in embryos or culture can occur either through infection with subgroup A or B viruses or through subgroup E infectious or latent virus. Differential identification is now possible through the use of appropriate genetic cell types and isolation methods.

The introduction of recessive genes for genetic cellular resistance has been suggested as a means of virus eradication. However, if subgroup E viruses, in latent or infectious forms, play an important role in the occurrence of lymphoid leukosis, genes for resistance to infection by exogenous viruses would not be useful for the eradication of inherited subgroup E information. Because genes for resistance to subgroup E viruses are inherited independently of genes controlling the spontaneous
release of RAV-0, they can be selected for in order to limit the growth of the virus by spread from cell to cell. Antiviral vaccines aimed at eradication of exogenous viruses would also be largely ineffective against inherited subgroup E virus information because most chickens appear to be immunologically tolerant to gs and subgroup E envelope antigens.

If endogenous viruses are important in the etiology of lymphoid leukemia, vaccines against virally induced tumor antigens and selection for genes that repress the viral genome or enhance the immunological defenses against new tumor antigens would be more effective than measures aimed at exogenous viruses. The key question that must be answered before rational choices among approaches to control can be made is: Do endogenous viruses influence the rate of lymphoid leukemia losses during the commercial life of the chicken?

**Figure 1.** Production of a subgroup A pseudotype of RSV by mixed infection with subgroup A ALV and defective sarcoma virus. 1. a complete progeny subgroup A ALV particle with a subgroup A envelope. 2. Represents a defective sarcoma virus genome with a subgroup A envelope that is infective. 3. Represents a recombinant genome that is not observed because only defective sarcoma virus particles are found among progeny. 4. Represents a defective ALV that cannot be assayed in cell culture. Note that each of the genomes may or may not be associated with subgroup A envelope antigens.
Figure 2. Avian and mammalian oncornaviruses are presumed to replicate through a DNA intermediate; that is, the viral RNA through the action of virion associated reverse transcriptase is transcribed to a DNA provirus that in turn becomes double stranded DNA and is integrated into the host genome. New RNA molecules are transcribed from the integrated DNA and code for viral proteins and act as the viral genome.

Figure 3. Mixed infection by subgroup A ALV and helper independent subgroup B RSV yield recombinant progeny 3 and 4. Cloning of such progeny yields recombinant clones, showing that genotypic as well as phenotypic mixing has taken place.
Figure 4. Infectious ALVs may be transmitted from infected dams through the ovum to the progeny. Recent evidence suggests that subgroup E ALVs can arise from genes transmitted by the parents. Therefore, vertical transmission of virus may act as an inherited trait.

Table 1

Properties of sarcoma viruses produced by L- retro cells of different types.

<table>
<thead>
<tr>
<th>Parental Virus Introduced at Limiting Dilution</th>
<th>Reverse Transcriptase Activity of Parental Virus</th>
<th>Host Cell</th>
<th>Progeny Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV(-) 1/</td>
<td>+</td>
<td>gs+</td>
<td>RSV(RAV-0)</td>
</tr>
<tr>
<td>RSV(RAV-0)</td>
<td>+</td>
<td>gs+</td>
<td>RSV(RAV-0)</td>
</tr>
<tr>
<td>RSV α(0) 1/</td>
<td>-</td>
<td>gs+</td>
<td>none</td>
</tr>
<tr>
<td>RSV(-) 1/</td>
<td>+</td>
<td>gs-</td>
<td>RSV(-)</td>
</tr>
<tr>
<td>RSV(RAV-0)</td>
<td>+</td>
<td>gs-</td>
<td>RSV(-)</td>
</tr>
<tr>
<td>RSV α(0) 1/</td>
<td>-</td>
<td>gs-</td>
<td>none</td>
</tr>
</tbody>
</table>

1/ Penetration of cell achieved only through cell fusion with inactivated Sendai virus.
TABLE 2

Estimated genome equivalents of viral genome homologous DNA and viral RNA in different types of chicken cells.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Virus Production</th>
<th>gs Antigen</th>
<th>Genome Equivalents of Viral Nucleic Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEF</td>
<td>-</td>
<td>-</td>
<td>10-15</td>
</tr>
<tr>
<td>CEF</td>
<td>-</td>
<td>+</td>
<td>10-15</td>
</tr>
<tr>
<td>RSV Infected</td>
<td>+</td>
<td>+</td>
<td>10-15</td>
</tr>
<tr>
<td>CEF RAV-0</td>
<td>+</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>Normal Mammalian Cells</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>RSV Transformed Mammalian Cells</td>
<td>-</td>
<td>+</td>
<td>2</td>
</tr>
</tbody>
</table>

1/ Varmus et al. (55)
2/ Bishop et al. (42)
3/ Not Done
Table 3

Status of endogenous virus information in various cell types.

<table>
<thead>
<tr>
<th>Susceptibility to Subgroup E</th>
<th>Occurrence of gs antigen</th>
<th>Viral gs antigen</th>
<th>Infectious RAV-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>-</td>
<td>+</td>
<td>v^+</td>
</tr>
<tr>
<td>S</td>
<td>-</td>
<td>-</td>
<td>v^-</td>
</tr>
<tr>
<td>R</td>
<td>-</td>
<td>-</td>
<td>(v^+) 2/</td>
</tr>
<tr>
<td>R</td>
<td>+ 3/</td>
<td>-</td>
<td>(v^+) 2/</td>
</tr>
<tr>
<td>R</td>
<td>+</td>
<td>-</td>
<td>v^-</td>
</tr>
</tbody>
</table>

1/ gs antigen due to growth of infectious virus.
2/ Small amounts of virus released can be detected only by co-cultivation with susceptible cells.
3/ Cells that carry the gs^+ gene are resistant to subgroup E. See text.

Table 4

Interference patterns between leukosis and sarcoma viruses of the five known subgroups.

<table>
<thead>
<tr>
<th>RSV Subgroup</th>
<th>Subgroup of Preinfecting Leukosis Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>A</td>
<td>R</td>
</tr>
<tr>
<td>B</td>
<td>S</td>
</tr>
<tr>
<td>C</td>
<td>S</td>
</tr>
<tr>
<td>D</td>
<td>S</td>
</tr>
<tr>
<td>E</td>
<td>S</td>
</tr>
</tbody>
</table>

R Resistant to superinfection with RSV
S Susceptible to superinfection with RSV
RS Partially resistant to superinfection with RSV
### TABLE 5

**Alternative mechanisms of genetic control of susceptibility to subgroup E virus**

<table>
<thead>
<tr>
<th>No. of Loci</th>
<th>Eloci</th>
<th>( R_1 )</th>
<th>Susceptibility to Subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tvb</td>
<td>tve</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>( p^{s1} )</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>( p^{s2} )</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>( p^{s3} )</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>( p^e )</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>( p^{s1} )</td>
<td>( e^s )</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>( p^{s2} )</td>
<td>( e^s )</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>( p^e )</td>
<td>( e^s )</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>( p^{s1} )</td>
<td>( e^r )</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>( p^{s2} )</td>
<td>( e^r )</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>( p^e )</td>
<td>( e^r )</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 6

**Evidence for a DNA Intermediate in Avian Oncornavirus Replication:**

1. Inhibition of virus production by Actinomycin D.
2. Transient early requirement for DNA replication in oncornavirus replication.
3. Detection of RNA-dependent DNA polymerase and other enzymes in virions (no need for induction of cellular enzymes).
4. Increase of avian oncornavirus DNA in cells infected with avian tumor viruses (mammalian cells).
5. Production of infectious virus by DNA extracted from RSV infected mammalian cells.
### Table 7

Percent Recombination Between Envelope Markers of RSV and the Endogenous Viral Genome

<table>
<thead>
<tr>
<th>Source of Subgroup E Genes</th>
<th>Source of Transforming Genes</th>
<th>Recombination</th>
</tr>
</thead>
<tbody>
<tr>
<td>gs+ chf+ C/E Cells</td>
<td>SR-RSV-B</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>PR-RSV-B</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>BH-RSV(RAV-7)</td>
<td>0.0</td>
</tr>
<tr>
<td>gs- chf- C/O Cells</td>
<td>SR-RSV-B</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>PR-RSV-B</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>BH-RSV(RAV-7)</td>
<td>0.0</td>
</tr>
</tbody>
</table>
REFERENCES


FELINE RNA TUMOR VIRUSES

During the past year there has been much greater acceptance of the fact that feline leukemia virus can be transmitted horizontally under both natural and experimental conditions. The evidence has been accumulating for several years. Many people have observed clusters of lymphosarcoma in groups of genetically unrelated cats. William Jarrett was stimulated to start his first transmission trial by observing 8 cases of lymphosarcoma in a household colony of unrelated cats. In 1969, we reported the spread of feline leukemia virus to uninoculated control kittens housed with inoculated animals. Essex, Hardy, the Jarretts, and others have observed that kittens are less susceptible to inoculation with feline sarcoma virus if their mothers had previous litters that had been inoculated with FeLV or FeSV. This suggested that the mothers had acquired infections, became immune, and gave protective antibody in their colostrum to kittens in subsequent litters. These observations have been confirmed by specific serological tests, namely, an indirect membrane fluorescent antibody test.

Hardy observed that leukemic cats have feline RNA tumor virus group-specific antigen in their circulating leukocytes and blood platelets which can be detected by an indirect FA test on acetone-fixed blood smears. The test was positive in 177 of 543 (33%) normal cats living in contact with FeLV infected cats. Twenty-four of these normal cats developed lymphosarcoma within 6 months later. The FA test was positive in only 2 of 1,462 (0.14%) normal cats not exposed to FeLV.

Additional experimental studies on horizontal transmission, with positive results, have been done by the Jarretts, our group, and others. In a small, but more rigorously controlled trial, we have on three occasions put an adolescent SPF cat from a colony known to be free of conventional FeLV and negative to all tests in a Horsfall isolation cage with a known carrier of FeLV. In all three trials, the exposed cat developed gs antigen and replicating virus in its hematopoietic cells within 3-4 weeks.

SUBGROUP CLASSIFICATION

The classification of feline RNA tumor viruses was summarized recently by Dr. Padman Sarma and T. Log (Virology 54:160-169, 1973). They describe three subgroups, A, B, and C which can be distinguished by interference and neutralization tests. In nature, the B and C subgroup viruses generally occur in association with subgroup A virus. The only known natural occurrence of a single subgroup (A) of feline leukemia virus is the strain we reported in 1969 and have done most of our work with since then. In table 5 of Dr. Sarma's paper and the accompanying text, he refers to two strains of subgroup A virus. In fact, this is the same isolate. Our F-161 was the original spontaneous case of feline lymphosarcoma
from which the strain was obtained, and F-422 was a third-passage experimental case of lymphosarcoma from which a suspended cell line was derived. This cell culture produces a large quantity of virus, has been sent to many other laboratories, and is used to produce virus which is available commercially. The frequent association of two or even all three subgroup viruses is a curious phenomenon. Sarma pointed out that serial passage of a mixture in cal cells maintains a similar virus subgroups population, and that all known cat cells seem equally permissive and non-selective in maintaining a mixed population. Yet, a mixture is not necessary for oncogenic activity. We have produced large numbers of neoplasms in cats and also in dogs with subgroup A virus. Tumors have been produced in cats with virus which had been purified by terminal dilution and cloning by the agar suspension culture method. However, the fact that subgroup A virus is sufficient to induce neoplasms does not exclude the possibility that viruses of two or three subgroups might exert a synergistic effect in oncogenesis. An infection with one subgroup doesn’t interfere with infection by another. Perhaps cats with mixed subgroup infections shed the mixture, and all components infect the next animal. In any event, it is clear that the natural mixtures can be separated in the laboratory by terminal dilution or by treatment with specific neutralizing antiserum.

It is generally stated that FeLV is infective for cat, dog, human, pig, marmoset, and other nonhuman primate cells but not rodent cells. As mentioned above, cat cells seem generally susceptible, and a system of subgroup classification based on resistant cat cells has not been developed. Some work has been done on testing the relative susceptibility of resistance to FeLV infection by cells of different special of animals. Oswald Jarrett reported one isolate of FeLV which didn’t grow in human cells. It has been suggested that it is possible to classify FeLV into subgroups A, B, and C by comparing the relative resistance of cells of different species. Perhaps specific, well-studied and standardized cell lines might work. Presently there is no such acceptable system. It seem doubtful that all human cells or dog cells will perform uniformly. In a book that was published this year, “The Molecular Biology of Tumour Viruses”, edited by John Tooze, there is a table on page 556 which lists is infective for cat cells, but not for human or dog cells. This cannot be supported. Hundreds of limiting titer trials of subgroups A virus in cat, human, dog, and other cells have been done. The infectivity for human and dog cells was equal, or one or two logs less than for cat cells, when low passage subgroup A virus was used. However, if virus is used that has been maintained in tissue culture for many passages, it has a lower infectivity for cat cells, and eventually little or no infectivity for human cells. The virus produced by our F-422 suspended culture maintained its infectivity quite well for a year or so in continuous culture, but after more than four years it won’t infect human cells. Inasmuch as this high passage subgroup A virus is commercially available, it seems possible it has been used by others for infectivity studies.
Dog cells are also susceptible in tissue culture to infection by subgroup A virus. In addition, newborn and fetal dogs develop fatal, generalized lymphosarcomas after they are inoculated with it.

A method for classification of subgroup A, B, and C feline leukemia virus by cell resistance will require better standardized cells and should use viruses of low-passage. On the other hand, the restricted host range of the feline endogenous virus, to be discussed later, is distinctive and useful in its recognition.

The membrane fluorescent antibody test, using unfixed cells, also identifies the type-specific viral envelope antigens. Three years ago, Riggs and his associates reported positive membrane FA tests on cats in a seroepidemiology study. The antigen was unfixed lymphoblastoid cells of Thielen's FL-74 suspended cell line, which sheds viruses of A, B, and C subgroups. Jarrett, Essex, and our group have also used these cells in similar tests for serum antibody, finding 31 to 45% of animals positive in populations associated with neoplasia. Cells infected with a single subgroups virus can also be used in a membrane FA test to conduct more selective seroepidemiology of single subgroup infections.

ENDOGENOUS VIRUS

During the last year, evidence from two kinds of observations has demonstrated the presence of a feline endogenous C-type virus. Previously the RD114 C-type virus had been isolated from human rhabdomyosarcoma cells which had been implanted in the brain of a fetal cat. It was first suspected to be a possible human candidate leukemia virus. Recently a virus was isolated from certain sublines of the Crandall continuous kidney cell line, which when compared by Todaro and others with the RD114 virus, seemed to be the same. The elements in common between the two were the feline origin, and yet the C-type viruses observed were different in several respects from the feline leukemia viruses of known subgroups.

Studies of cats have also contributed to an understanding of this. We had observed in our Caesarean-derived SPF cat colony of about 200 animals, some unusual C-type virus particles. Extensive testing by electron microscopy, COCAL tests, serial blind passage of cat cells, and various serological tests had given no evidence that conventional feline leukemia virus was present. Fetal cat cells and fresh, low-passage tissue cultures had been free of demonstrable virus. However, C-type virus was observed under two conditions. Routine electron microscopy of 19 placentas revealed C-type virus in three of them. This virus was not infectious when inoculated into feline tissue cultures. Similar virus was found in tissue culture derived from cat fetuses from this colony, when the tissues had been preserved by freezing in 5% DMSO solution. Again, they did not transmit by inoculated to other cat cells. Later it was found that similar virus could be induced in cat embryo cultures by treating them with IUDR (20 or 30 micrograms/ml).
There seems to be a genetic factor in the inducibility of the endogenous virus. Our SPF colony is relatively resistant, in that only 5 of 100 fetuses yielded the virus after IUDR treatment. Four of the 5 were kittens from three different litters of the same mother cat. In contrast, this virus was induced by IUDR treatment in about half of the fetuses from another somewhat inbred conventional colony.

**CHARACTERISTICS OF THE ENDOGENOUS VIRUS**

1. Very restricted host range. It is difficult or impossible to infect cat cells. However, the virus is infective for cells of the dog, pig, rhesus monkey, and man. We have recently infected mink and skunk cells.
2. This virus shares the group-specific ga-interspecies (gs 3) antigen with conventional feline leukemia virus and the other known mammalian RNA tumor viruses.
   
   However, it has its own distinctive gs-species (gs 1) antigen, which exhibits crossed perecipitin lines in Ouchterlony tests with the gs 1 antigen of subgroup A, B, and C feline RNA tumor viruses. These two gs 1 antigens also have a different mobility in acrylamide gel electrophoresis and different molecular weights.
3. The feline endogenous virus has a distinctive type-specific antigen. It does not exhibit viral interference or cross neutralization with subgroups A, B, and C viruses. Antisera against type-specific endogenous virus antigen can be produced by inoculating the virus into fetal or newborn dogs. We inoculated 5 letters six months ago, and have produced antibody but no tumors. Subgroup A virus under the same circumstances induced a high incidence of generalized lymphosarcomas. Direct evidence of oncogenic activity by the endogenous virus is not available at this time. Indirect evidence might be obtained in any of several ways. The finding of endogenous gs 1 antigen in tumors would be suggestive. Serum antibody to endogenous virus antigens might be correlated with neoplasms, if such antibody were demonstrable. However, cats might be expected to be tolerant to the endogenous virus antigens, unless the antigens are completely repressed before cats acquire immune competence, and not produce antibody. Endogenous virus and their distinctive antigens should be sought in spontaneous tumors, and those induced by chemical or other means.
4. Feline endogenous virus can act as a helper virus, and rescue defective mammalian sarcoma viruses.

Morphology of the endogenous virus is certainly type C, with replication by budding at cell membranes. However, there are slight differences in certain details.

**TESTS FOR VIRUS**

Test procedures for recognizing the feline RNA tumor viruses are useful in experimental work, and for monitoring cells used for vaccine production.
## FELINE RNA TUMOR VIRUSES

### Whole Particle
(without surface spikes) | Nucleoid
---|---
Subgroup A | 91 μm | 59 μm (65%)
Endogenous | 99 μm | 43 μm (43%)

### Surface spikes
Subgroup A

<table>
<thead>
<tr>
<th>Intermediate membrane on budding particles</th>
<th>Twin buds and clusters of up to 5 per stalk</th>
</tr>
</thead>
<tbody>
<tr>
<td>75%</td>
<td>rare</td>
</tr>
<tr>
<td>10%</td>
<td>common</td>
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</table>

Conventional FeLV is demonstrable by the COCAL, or similar tests. Grow in cat cells for 21 days, with appropriate media changes and at least 3 subdivisions of the cultures. Test the cells for gs antigen, using a complement fixation or fluorescent antibody test. The procedure is specific for conventional FeLV if gs 1 antiserum prepared from this virus is used.

Endogenous FeLV can be demonstrated by the following procedure:

1. Grow cat embryo tissues to a monolayer, usually 2 days.
2. Divide the primary culture, add 1UDR—30 micrograms/ml. Seed 2—250 ml Falcon flasks and 3 Leighton tubes.
3. After 72 hours, a Leighton cover slip is fixed in acetone and used in an FA test for gs antigen. If antiserum specific for the gs 1 antigen of either the endogenous for conventional viruses is available, this test can identify which virus is involved.
4. Also at 72 hours, 1 flask has the cells scraped off, and centrifuged for E.M. 1 flask is trypsinized and a similar number of NC37 cells are added for co-cultivation. NC37 are suspended human lymphatic cells which grow well. This flask is observed daily for syncytium formation in the adherent cells on the wall of the flask. Endogenous virus produces syncytia in this system. Specific identification, however, depends on serological identification. The observation of syncytia is presumptive evidence of sufficient amounts of virus to permit serological tests.
5. After the co-cultivation has proceeded 30 or 4 days, the suspended NC37 cells are poured off. An equal amount of fresh media is added to them, and they are incubated another 3 days. Then a membrane FA test with dog serum containing endogenous virus antibody provides specific identification. Electron microscopy on these cells can be done to confirm the presence of a C-type virus.

### PLACING THE ENDOGENOUS VIRUS IN THE SUBGROUP CLASSIFICATION

The Animal Virus Characterization Committee has been concerned with standardization of terminology and the classification of viruses.
Historically, when an avian endogenous RNA tumor virus (RAV-O) was identified, it was put into a new subgroup identified by the next unused letter of the alphabet—subgroup E. If this serves as precedent, the feline endogenous virus would be named subgroup D. Although the feline endogenous virus is different in certain antigenic respects from feline subgroup A, B, and C viruses, it has many striking biological similarities. The type-C morphology, shared gs-interspecies (gs 3) antigen, and capacity of exhibit helper activity in the rescue of defective mammalian RNA tumor viruses are especially significant. The feline endogenous virus appears biologically to be analogous to the avian endogenous (subgroup E) virus. Therefore, if an orderly classification of the RNA tumor viruses is to be maintained, it seems rational to assign the designation of “feline subgroup D” to the feline endogenous virus.

Charles G. Rickard
New York State Veterinary College
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BOVINE RNA TUMOR VIRUSES

M. J. Van Der Maaten*

The title of this discussion is misleading in that it suggests that there is a group of well-characterized RNA viruses of cattle that have been associated with neoplastic transformation. This is not true. In fact, only in studies of bovine lymphosarcoma have any extensive efforts been made to identify such viruses. This discussion, therefore, will be limited to a consideration of viral agents identified in studies of this disease.

The non-random distribution of the adult form of bovine lymphosarcoma has been adequately documented and reviewed. These observations led to repeated speculation that a viral agent, similar to the leukemia viruses of other species, is involved in bovine Lymphosarcoma. In spite of rather intensive investigative efforts, however, a leukemia virus has not been identified. Therefore, detailed information regarding the characteristics and classification of a bovine leukemia virus is not available, and a discussion of the virological aspects of bovine lymphosarcoma must concern itself with a consideration of candidate agents.

The early etiologic investigations consisted primarily of attempts to transmit the disease from affected animals to calves by experimental inoculation. Some successes were reported; but the extremely long incubation periods, the small numbers of animals involved, the infrequency of the development of tumors, and the lack of specific proof of the presence of an infectious agent in the inoculum, all have resulted in serious difficulties in the interpretation of the results of animal transmission experiments.

There have been reports of the isolation of infectious agents by the inoculation of materials from lymphosarcomatous cattle into mice and embryonating eggs, but the agents have not been characterized, and in some instances the experiments could not be satisfactorily repeated. Convincing proof of the association of these agents with lymphosarcoma has also been lacking.

Extensive ultrastructural studies were undertaken in efforts to identify virus particles in tumors or other materials from lymphosarcomatous cattle, but reported results were disappointing from a virological viewpoint. Only very limited numbers of virus-like particles were found, their identity as C-type viruses was open to question, and the ultrastructure studies could not provide proof of biologic activity. The ultrastructure studies of Dutcher et al., which identified virus-like particles in milk, are of historical significance because they apparently

served as a stimulus that resulted in an increased interest in the investigation of the viral etiology of bovine lymphosarcoma.

In vitro investigations have included attempts to establish cell cultures from tumors or circulating leukocytes from lymphosarcomatous cattle and attempts to inoculate or co-cultivate numerous types of cell cultures with these materials. Continuous lymphocyte suspension cultures were established in some of these investigations but virus particles were not identified in them. Cell proliferation, lymphocyte adsorption, resistance to superinfection with vesicular stomatitis virus, nuclear fragmentation, bizarre nuclei, and syncytia formation, all were observed in monolayer cell cultures from lymphosarcoma tissues and were considered to be indirect evidence of virus infection. Ultrastructural studies of these cultures have occasionally revealed C-type virus particles in very limited quantities. The biologic activity of these particles was usually not demonstrated, however, and they have not gained acceptance as representatives of bovine leukemia viruses.

In other cell culture studies, viruses were isolated but their etiologic significance was not established. Bovine syncytial virus was at one time thought to be involved in bovine lymphosarcoma, but additional investigations failed to provide proof of such an association. Subsequently, a slowly replicating herpes-like virus was isolated from bovine lymphoid tumors, but there is no direct proof of its association with tumor formation. A virus morphologically similar to Visna and Maedi viruses was also identified. This virus causes a lymphoproliferative response when inoculated into calves and may be associated with persistent lymphocytosis in some animals, but there is no evidence that it is of primary significance in lymphosarcoma.

In spite of this historical background of discouraging and disappointing results, more recent studies seem to provide reasons for a cautious optimism in virological investigations of bovine lymphosarcoma. A C-type virus particle has now been identified in short-term lymphocyte suspension cultures by Miller et al., and sheep experimentally inoculated with material from these cultures have developed lymphoid tumors.

The presence of virus particles in short-term lymphocyte suspension cultures treated with phytohemagglutinin was quickly confirmed by Kawakami et al., Dutta et al., and Wittman et al. Stock and Ferrer have found virus particles in both short-term and continuous lymphocyte suspension cultures (previously considered to be virus negative) if the continuous cultures were removed from medium containing horse serum to one containing fetal calf serum. Small quantities of similar virus particles have also been identified in non-stimulated lymphocytes from cattle with lymphosarcoma.

The phytohemagglutinin-stimulated, short-term lymphocyte culture technique made available for the first time a satisfactory, although laborious, technique for culturing and testing for the presence of C-type virus in cattle. Virus was identified in inocula prepared from these
cultures, and the examination of preinoculation and postinoculation lymphocyte cultures from experimentally inoculated animals confirmed the fact that infection had been established.

The virus particles present in phytohemagglutinin-stimulated cells were 90-120 m\(\mu\) in diameter, with a central nucleoid 60-90 m\(\mu\) in diameter. Morphologically, the virus particles were similar to the C-type viruses of other animal species. Only a few budding particles were observed in short-term lymphocyte cultures; but budding particles were more frequently encountered in continuous cell cultures, and the budding process is apparently identical with that described for other leukemia viruses.

Epidemiologic studies based on virus isolations in short-term lymphocyte suspension cultures indicated that there was an association between lymphosarcoma and C-type virus infection, but only limited numbers of animals could be evaluated with this technique. These studies have been greatly facilitated by the subsequent discovery by Miller et al. that serum from most, but not all, cattle infected with bovine C-type virus contains precipitating antibodies that can be demonstrated in an agar-gel diffusion test with ether-treated virus as an antigen. With this technique, we have determined that most lymphosarcomatous cattle have antibodies to C-type virus antigens, that antibodies are frequently found among apparently normal cattle in multiple case lymphosarcoma herds, and that they occur much less frequently or are absent in cattle from herds where lymphosarcoma has not been diagnosed. Ferrer et al. have reported similar results on limited numbers of lymphosarcomatous cattle tested by an immuno-fluorescent antibody technique. They have also identified precipitating antibodies in cattle sera tested with an antigen prepared from long-term lymphocyte suspension cultures but could not demonstrate an antigenic relationship between the bovine C-type virus and murine or feline leukemia viruses when they used either the immunofluorescent or immunodiffusion test.

In preliminary tests of materials prepared in our laboratory, we have been unable to detect an antigenic relationship between the bovine and feline or murine viruses. These findings contrast to an earlier report of preliminary studies by Shafer et al., which indicated that material from phytohemagglutinin-stimulated bovine lymphocytes showed a weak reactivity with rabbit antibodies to the GS antigens of mammalian leukemia viruses. All of these results must be regarded as somewhat tentative because large quantities of the bovine virus are not available for definitive testing.

The oncogenic activity of the bovine C-type virus is indicated by epidemiologic studies but has not been proven by cattle inoculation. Its infectivity for cattle has been amply demonstrated, but the practical limitations imposed by the long incubation period of the natural disease and the difficulties in maintaining large numbers of cattle on experiment for a long period of time have made it impossible to obtain conclusive results at this time. The observations by Wittmann et al. are
that sheep inoculated with blood from leukotic and experimentally inoculated cattle later developed lymphoid tumors have major significance in animal transmission studies. Olson et al. have subsequently infected sheep and produced tumors with virus from phytohemagglutinin-stimulated bovine lymphocytes. The virus has been reisolated from the sheep tumors and its identity with bovine C-type virus demonstrated by gel-diffusion tests. Therefore, the bovine virus probably did not activate an endogenous sheep leukemia virus that, in turn, caused the neoplasms. These experiments provide the most convincing proof, to date, of the in vivo oncogenicity of the bovine C-type virus.

C-type virus particles have recently been found in monolayer cell cultures established from bovine lymphoid neoplasms and in fetal lamb spleen or bovine embryonic spleen cell cultures inoculated with tumor material. Virus replication in these cultures apparently varies greatly from passage to passage, but at times considerable numbers of particles are found that are morphologically and antigenically identical with particles in short-term lymphocyte suspension cultures. Filtrates of fluids from these cultures have provided additional proof of the biologic activity of the bovine C-type virus in cell cultures and experimentally inoculated sheep and calves. Some syncytia formation occurs in infected monolayers but degenerative cytopathic effects are absent, so fluids from these cultures may be suitable for use in large-scale virus concentration and purification techniques because the medium does not contain large quantities of cell debris.

In summary, a viral agent with morphologic characteristics of a C-type virus has been isolated and identified from lymphocytes and tumor tissues of cattle with lymphosarcoma. Epidemiologic studies, although not extensive, indicate that infection with the virus may be associated with the occurrence of the neoplasms. Reliable information regarding the oncogenic potential in experimentally inoculated calves is not yet available, but an oncogenic activity has been demonstrated in experimentally inoculated sheep. These are characteristics that the bovine C-type virus shares with the murine and feline viruses (Table 1) and support the designation of the agent as a candidate bovine leukemia virus. There are, however, several aspects in which the virus differs from the leukemia viruses of other species. The ease with which sheep, a heterologous host, are infected and the rather marked immunologic response of the natural bovine host are characteristics that are not typical of the responses of other species to their endogenous leukemia viruses. The apparent absence of mammalian group specific (GS-3) antigen will also require further investigation.

Information regarding characteristics such as the type and approximate size of the nucleic acid contained in the bovine C-type virion is not available, nor have attempts to demonstrate reverse transcriptase been reported. Obviously, therefore, any attempt to definitively classify the virus at this time would be premature.
REFERENCES


55. Wittmann, W., Urban, D., Seils, H., and Beyer, J.: Unter-

**Table 1. Comparison of bovine feline and murine leukemia viruses**

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<th>Species of origin</th>
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<th>Antigenic determinants</th>
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<th>Oncogenicity host</th>
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<td></td>
<td></td>
<td>Mammalian specific</td>
<td>Natural host</td>
<td>Natural host</td>
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<td></td>
<td></td>
<td>group specific</td>
<td>Heterologous host</td>
<td>Heterologous host</td>
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<tr>
<td></td>
<td></td>
<td>antigen (CS-3)</td>
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<td></td>
<td></td>
<td>antigen (CS-1)</td>
<td></td>
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<td></td>
<td></td>
<td>Reverse transcriptase</td>
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<td></td>
</tr>
<tr>
<td>Bovine</td>
<td>C type</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Feline</td>
<td>C type</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Murine</td>
<td>C type</td>
<td>+</td>
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*Preliminary studies indicate that the bovine virus does not contain the mammalian CS-3 antigen.

The bovine virus contains a species specific antigen but biochemical and biophysical characterizations of the antigen have not been reported so it may not be analogous with the CS-1 antigen of viruses from other species.
UNITED STATES ANIMAL HEALTH ASSOCIATION COMMITTEE ON ANIMAL VIRUS CHARACTERIZATION

Chairman—S. McConnell
Co-Chairman—C. J. York

The committee on Animal Virus Characterization continued to be active in a broad spectrum of activity. The members serve this organization through their interplay with the many committees of the USAHA, State Agencies, National Agencies and International Organizations.

The committee members advise the American Type Culture Collection with the accessioning of viruses of importance to the USAHA so they will continue to be available for diagnostic purposes and vaccine production. The committee interacts with and assists the National Institutes of Health and the WHO/FAO Board of Comparative Virology with their programs in evaluation, collection and storage of reference reagents. These associated activities are essential to this organization's program since they relate to diseases of food animals and companion animals.

The committee sponsored a seminar on RNA Tumor virus to provide current information on these tumor viruses. The oncogenic characteristics of this virus group were reviewed, followed by an active discussion. Of particular interest to this group is the results of studies concerning the horizontal transmission of the feline leukemia viruses. The "B" type feline leukemia virus has been shown experimentally to infect dogs, mice and other animals; the implications of this transmission are evident.

We would also like to call to your attention the isolation and identification of a virus from Sea Lions which closely resembles morphologically the vesicular exanthema virus of swine and some respiratory viruses of felines. This information has also been presented by other committees of the USAHA.

The revision of the publication "Animal Reference Virus Recommendations" by this committee is in the final stages of preparation for submission to the American Journal of Veterinary Research. During committee deliberations, the members agreed on maintaining the term "THYLAXOVIRUS" as a genus name for the RNA tumor viruses. We feel this terminology is more in keeping with the ICNV guidelines and unlike "leukovirus" or "oncornavirus" would allow for inclusion of viruses which may be identical except for proven oncogenicity.
APPENDICIES

1. Agenda for Work Session on RNA Tumor Viruses.

2. Papers presented by:
   (a) Dr. L. Crittenden
   (b) Dr. C. Richard
   (c) Dr. P. Sarma
   (d) Dr. M. Vandermaaten
   (e) Dr. B. R. Burmester

Finally as a word of caution to individuals using human blood for diagnostic and research purposes, the dangers of becoming infected with hepatitis virus should be emphasized. The committee suggests that the guidelines to Laboratory Safety recommended by the WHO (Who Technical Report Series #512, Annex 2, Geneva, Switzerland) be examined and followed.

This committee submits this report and appendicies for your consideration and submission to the Executive Committee for approval.
AMERICAN ASSOCIATION OF
VETERINARY LABORATORY
DIAGNOSTICIANS

SIXTEENTH ANNUAL MEETING
OF THE AMERICAN ASSOCIATION OF
VETERINARY LABORATORY
DIAGNOSTICIANS

(Formerly
Conference of Veterinary Laboratory
Diagnosticians)
# AMERICAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS

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(formerly Conference of Veterinary Laboratory Diagnosticians)

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CONSTITUTION AND BY-LAWS
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AMERICAN ASSOCIATION OF VETERINARY LABORATORY
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Constitution

Article I—NAME
The name of the Association shall be the American Association of Veterinary Laboratory Diagnosticians.

Article II—UNITED STATES ANIMAL HEALTH ASSOCIATION AFFILIATION
The American Association of Veterinary Laboratory Diagnosticians shall be an affiliate of the United States Animal Health Association in accordance with a memorandum of agreement mutually agreed upon between the two parties and approved by the Executive Board.

Article III—PURPOSE
The purpose of this Association shall be the dissemination of information relating to the diagnosis of animal diseases, the coordination of the diagnostic activities of regulatory, research and service laboratories, the establishment of uniform diagnostic techniques, the improvement of existing diagnostic techniques, the development of new diagnostic techniques, the establishment of accepted guides for the improvement of diagnostic laboratory organizations relative to personnel qualifications and facilities, and to act in a consultant capacity to the United States Animal Health Association on uniform diagnostic criteria involved in regulatory animal disease programs.

Article IV—MEMBERSHIP
Any laboratory worker engaged in the field of disease diagnosis in animals is eligible for membership.

Article V—MEETINGS
The meetings of the Association shall be annual and special.

Article VI—OFFICERS
The officers of the Association shall be: President, President-elect, and Secretary-Treasurer.

Article VII—BOARD OF GOVERNORS
The Board of Governors shall be composed of the President, President-elect, Secretary-Treasurer, Past-President and one Board member from each of the four designated regions, two members-at-large and the Direc-
The President of the Association shall be Chairman of the Board of Governors.

Article VIII—PROGRAM COMMITTEE

The Program Committee shall consist of the President-elect and four other members, one each, respectively, from the four districts of the United States, appointed by the Association President. Said districts shall be known as the "Northeast," consisting of the states of Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island and Vermont; The "North Central," consisting of the states of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin; The "Southern," consisting of the states of Alabama, Arkansas, Georgia, Florida, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia, Puerto Rico and the Virgin Islands; and the "Western" district, consisting of the states of Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington and Wyoming.

Article IX—DUTIES OF THE OFFICERS

1. President: It shall be the duty of the President to preside at all meetings of this Association and to appoint all committees. The President shall be an ex officio member of all committees.

2. President-elect: In the absence of the President, the President-elect shall preside at the meetings of the Association. In the event of the absence, disability or resignation of the President, he shall perform all duties of the President. He shall be a member of the Board of Governors and Chairman of the Program Committee.

3. Secretary-Treasurer: The Secretary-Treasurer shall keep an accurate record of the meetings of the Association. Whenever authorized by the Board of Governors, he shall publish newsletters and distribute them to the members of the Association. The Secretary-Treasurer shall also keep an accurate record of the meetings of the Board of Governors and shall furnish a copy to each member of said Board of Governors.

He shall keep an accurate account of all Association moneys received and disbursed. He shall also present to the President a list giving the name and address of each member and an annual financial report. He shall perform such other duties as may be authorized and prescribed by the Board of Governors. He shall be the Secretary of the Board of Governors, also an ex officio member of the Program Committee.
Article X—AMENDMENTS

The Constitution of the Association may be amended by the Executive Board, provided that the specific amendment to be acted upon shall have been presented in writing at a previous executive Board meeting.

Article XI—EXECUTIVE BOARD

The Executive Board shall constitute the legislative body of the Association. Membership of the Executive Board shall consist of one delegate from each state, Canada and Mexico. A member of the Executive Board must be a veterinarian who is a supervisor of a veterinary laboratory actively engaged in diagnostic medicine and who has been duly designated by the veterinary diagnostic personnel of the state he represents. Alternates may be designated to serve in the absence of regular delegates.

A quorum shall consist of 12 members.

The Board shall report its activities annually to the Association’s membership, and establish its own rules of conduct.

The President, President-Elect, and Secretary-Treasurer of the Association shall be ex officio members. The Association’s President shall act as the presiding officer of the Executive Board and shall cast a ballot in tie votes.

The Director of the Veterinary Services Diagnostic Laboratory or equivalent title of the United States Department of Agriculture and the United States Animal Health Association Liaison officer to the American Association of Veterinary Laboratory Diagnosticians shall be members of the Executive Board with full voting powers.

BY-LAWS

Article I—ORDER OF BUSINESS

Registration.
Call to order.
Report of Secretary-Treasurer.
President’s Address.
Committee Reports.
Discussion.
Unfinished Business.
New Business.
Nomination and Election of Officers.
Adjournment.

A suspension of the By-laws may be made by the Executive Board for the purpose of changing the order of business or to facilitate important business.

Article II—APPLICATIONS FOR MEMBERSHIP

Applications for individual membership shall be made in writing to the Secretary-Treasurer.

An individual member may be expelled for cause upon recommenda-
tion of the Board of Governors and/or a majority vote of the Executive Board.

Article III—MEETINGS

The annual meeting of the Association will be held the two days preceding the dates of the annual meeting of the United States Animal Health Association and at the same location.

Article IV

Six members of the Board of Governors shall constitute a quorum.

Article V—PROCEEDINGS


(The articles for publication shall be assembled by the Secretary-Treasurer of the Association and forwarded by him to the Secretary of the United States Animal Health Association immediately following each annual meeting.

All articles for publication in the United States Animal Health Association Proceedings shall conform to the style requirements of the Journal of the American Veterinary Medical Association.)

Abstracts of the articles to be presented at the annual scientific meeting shall be assembled by the Secretary of the Association at least 30 days prior to the annual meeting date.

Article VI—AMENDMENTS

The By-laws may be amended by the Executive Board.

Article VII—ELECTION OF OFFICERS AND BOARD OF GOVERNORS

The terms of office for President, President-elect and Secretary-Treasurer shall be for one year following their election by the Executive Board at the annual meeting.

The Board of Governors, other than President, President-elect, Secretary-Treasurer and Past-President shall be elected for four-year terms. The elections shall be arranged to allow one new regional Board of Governors member to be elected annually. (See Article VII, Constitution for Regions). (This first year, 1973-1974, the duration of terms in office will be as follows: Western Region, 1 year, Northeastern Region, 2 years, Southern Region, 3 years, North Central Region, 4 years).

Each region shall elect its member of the Board of Governors from the constituency of the Executive Board from that region.

The two Board of Governors members-at-large shall be elected by the Executive Board. The candidates shall be presented by the nominating committee. Each Board member-at-large shall be elected for a four year term. Vacancies in the office of Board members-at-large shall be filled by election at the next Executive Board meeting. The elected member(s)-
at-large shall complete the normal term of office of the member he replaces.

Article VII—DUES
The amount of registration fees and/or dues shall be determined by the Executive Board.

Article IX—STANDING COMMITTEES
The President shall appoint committees when instructed to do so by the Executive Board or, if it is not in session, by the Board of Governors.

Article X—MINIMUM STANDARDS
FOR VETERINARY DIAGNOSTIC LABORATORIES
The Association shall develop minimum standards for the accreditation of veterinary diagnostic laboratories.
Requests from industry and government sources to the American Association of Veterinary Laboratory Diagnosticians for the development of recommended laboratory protocol for the diagnosis of certain selected diseases of the male bovine used for the production of semen for artificial insemination resulted in the activation of this committee. The committee, in deliberation, has decided to confine this discussion to specific disease entities now covered by regulation or proposed regulations for the production, sale, movement, or use of bovine semen. These diseases are tuberculosis, brucellosis, leptospirosis, vibriosis, trichomoniasis, and Johne's Disease (paratuberculosis). The committee recognizes the possibility of several other endemic and exotic diseases being transmitted through semen and urges regulatory officials and the bovine A.I. industry to give this problem a high priority for future consideration and action.

The committee recommends that Veterinary Services of the Animal and Plant Health Inspection Service, United States Department of Agriculture, consider and implement laboratory training at the bench level for the conduct of the various technical procedures described in this manual.

The committee gives special thanks for assistance to Dr. David Bartlett and associates of Madison, Wisconsin and Dr. Ed Ellis of the National Animal Disease Laboratory, Ames, Iowa.

**TUBERCULOSIS**

All tests for the detection of tuberculosis shall be conducted in accordance with Uniform Methods and Rules—Tuberculosis Eradication as adopted by the United States Animal Health Association on October 27, 1971, and approved by the Animal and Plant Health Inspection Service, Veterinary Services, United States Department of Agriculture, effective March 3, 1972.
BRUCELLOSIS

All tests for brucellosis shall be conducted according to Uniform Method and Rules ARS 91-79. In addition to the tests delineated in ARS 91-79, some regulations require a semen plasma agglutination test as follows:

Semen Plasma Test Procedure

Raw, unprocessed, undiluted semen is centrifuged at 2,000 R.P.M. for 10 minutes to sediment the sperm and other particulate matter. A minimum of 1/2 to 1 ml of semen should be utilized. The supernatant material (plasma) is used for conducting the standard plate and/or tube test as described for blood serum in ARS 91-79. The standard tube test is the preferred method. Interpretation of test results is similar to that for blood serum. A complete reaction at the 1:25 dilution or greater is cause for concern. Evidence indicates that early infection may be detected by a semen plasma test reaction prior to detectable serological titers.

LEPTOSPIROSIS

PLATE AGGLUTINATION TEST FOR LEPTOSPIRA

Antigen: Only licensed plate antigens should be used for the test. As the reactions are specific, *L. Pomona, L. hardjo, L. Grippotyphosa, L. canicola* and *L. icterohaemorrhagiae* are needed for complete testing. With serum, it is suggested that the plate test be used only for screening and positive reactions be confirmed with the microscopic agglutination test.

Test procedure: Add 0.01 ml of serum or semen to each glass square for each antigen to be used.

Place a drop of antigen on each drop of serum or semen. Mix each antigen and serum or semen drop with a clean applicator stick making a drop of about 1/2 inch diameter. Rotate the plate 5 to 6 times. Incubate at room temperature. After 6 minutes, remove plate from box, rotate plate and tilt for reading. Read reactions against a dark background with an oblique light source.

Readings: Positive agglutination is recognized by accumulation of clumps along periphery of the drop.

Negative samples will have a homogenous appearance throughout the drop. Artifacts such as fibrin are recognized by scattered debris distributed throughout the drop.

THE MICROSCOPIC AGGLUTINATION TEST FOR THE DIAGNOSIS OF LEPTOSPIROSIS

(AGGLUTINATION-LYSIS TEST)

Specific antibodies can be demonstrated in the blood serum of the affected animal usually about the eighth day after infection. The maximum titer is generally obtained during the third or fourth week after the onset of the disease. The titer may remain at a high level (1:10,000 or higher) for some weeks or months and then gradually decreases.

With the microscopic agglutination technique, serial dilutions of serum are kept in contact for a certain period with an equal volume of a suspen-
sion of leptospires. When the test is performed with living micro-organisms two phenomena, agglutination and lysis, occur simultaneously. Only agglutination occurs when formalin-killed leptospires are used for antigen, as the organisms have become resistant to the action of the lysins. The lysis differs from the well-known bacteriolysis in that it takes place without the presence of complement.

The leptospirosis microscopic agglutination test is species-specific. It is recommended that serums be tested against the leptospiral species known to be present in the country or locality. Bovine serums should be tested against *L. pomona*, *L. hardjo*, *L. grippotyphosa*, *L. canicola* and *L. icterohaemorrhagiae*.

Procedure:

1. Place 0.9 ml. of physiological saline or Sorenson's phosphate buffer in each of four test tubes. To the first tube add 0.1 ml. of a 1/5 dilution of the unknown serum, and mix. Make serial 10-fold dilutions by transferring 0.1 ml. of this dilution to tube 2, and mix. In turn transfer 0.1 ml. of this dilution to tube 3, etc. See Table 1. Do the same for each unknown serum.

2. At the same time that you are making serial 10-fold dilutions, transfer 0.1 ml. of each dilution into a properly identified agglutination tube.

3. To each agglutination tube containing the 0.1 ml. of diluted serum, add an equal volume (0.1 ml.) of antigen which consists of a 5- to 7-day-old motile culture of the leptospira grown in Stuart's medium enriched with 7-10% rabbit serum. This will give final dilutions of 1/100, 1/1000, 1/10,000 1/100,000. Positive and negative serum and saline controls should be included in each test run.

4. Allow the serum-antigen mixtures to incubate at 37°C. for approximately 2 hours.

5. Examine the individual tubes by viewing small drops of the serum-antigen mixture by darkfield illumination at a magnification of about 120-150x. A serum is judged to be negative when the leptospires remain as freely moving individuals, uniformly spread throughout the field in all dilutions, similar to the antigen control. Serums are designated as positive when agglutination and/or lysis of approximately one-half or more of the leptospires has occurred in one or more of the serum dilutions. End titers are determined by observation of a reaction in the highest serum dilution.

NOTE: In general, the first traces of agglutination and lysis do not occur in the same dilution; usually agglutination sets in first and is super-
seded by lysis at a higher titer. If a serum is strongly positive, the leptospires can be found agglutinated in the weaker serum dilutions. Some free-moving leptospires may also be found. Agglutination is detected by the occurrence of small or large networks of entangled leptospires in which movements of parts of the free ends of the leptospires may still be perceptible.

In the higher dilutions of strongly positive serum, one observes, next to the agglutination structures, the signs of lysis. A disintegration of the leptospires which are entangled in the agglutinated masses occur, so that the network is gradually transformed into clumps and bright white masses with round contours, from the periphery of which parts of leptospiral bodies will be seen to protrude, moving sluggishly. In some cases the masses may be entirely lysed, so that the field of vision is practically empty, with the exception of a few white specks and a few single leptospires moving about freely.

A zoning reaction may occur in strongly positive sera.

**SEmen AGGLUTINATION TEST**

Semen can be used in the microscopic test instead of serum. Although a semen agglutination test has been used for a number of years, the reliability of the test has not been well established.

**ISOLATION TECHNIQUE FOR LEPTOSPIRES**

Urine provides the most reliable source for isolation of leptospires following an acute illness. Leptospires are usually present in the urine at the time of an acute illness and for several weeks following.

During acute leptospirosis, indicated by temperature elevation and hematuria, organisms may be present in the blood and semen.

**Collection of Urine**

Use a sterile container for collection of urine from midstream flow.

Urination can be induced by a diuretic such as "Lasix" if urine is not obtainable naturally. Inoculate media as soon as possible as organisms may be inactivated within several hours in undiluted urine.

**Collection of Blood or Semen**

Bleed animal with sterile syringe and inoculate liquid and semisolid media before clotting. Semen should be inoculated into liquid and semisolid media before addition of extender.

**Inoculation of Media**

Inoculate 0.1 ml of urine, blood or semen into screw cap tubes of liquid and semisolid media. Incubate tubes at 30°.

If an incubator is not available, maintain the tubes at room temperature out of direct sunlight.

**Submission of Samples to a Laboratory**

Label each inoculated tube with the animal number, inoculum and the date. Wrap tape around screw to prevent leakage and pack in a shipping container.

Mail cultures to laboratory as soon as convenient without incubation.

Complete a history sheet and submit it along with the cultures.
QUALIFYING BULLS FOR THE PRODUCTION OF SEMEN 459

Use airmail if distance is more than 200 miles.

**LEPTOSPIRAL MEDIA**

*Fletcher's Medium*

Nutrient broth .8 grams  
Bacto agar 2.5 grams  
Sodium chloride .8 grams  
Distilled water 100 cc  

Heat the above to dissolve.  
Then add 60 cc of the above solution to 860 cc of distilled water.  
Sterilize at 15 lbs. for 20 minutes.  
Cool to 56°C, then add 80 cc (8%) of sterile inactivated (56°C for 30 min.) rabbit serum.  
Distribute 5-6 ml per sterile screw cap tube. Check sterility before using. Store at room temperature.

*Modified Stuart's Medium for Leptospires*

Sodium chloride 1.43 grams  
Ammonium chloride 0.27 grams  
Asparagine 0.13 grams  
Magnesium chloride 0.19 grams  
Sodium phosphate (Dib) 0.67 grams  
Potassium phosphate (Mono) .087 grams  
Thiamine hydrochloride 2.0 mg  
Distilled water 1000 cc  

Sterilize at 15 pounds pressure for 15 minutes.  
Cool to 56°C and add 100 cc (10%) of sterile inactivated rabbit serum.  
Distribute 5-6 ml per sterile screw cap tube. Check sterility before using. Store at room temperature.

**BOVINE ALBUMIN POLYSORBATE MEDIUM**

*25X buffer solution*

Mix in this order:  
700 cc sterile distilled water  
16.6 grams Sodium phosphate  
2.172 grams Potassium phosphate  
Then bring up to 1000 ml.  

*Concentrated basil salts*  
*20X Concentrated solution*

700 cc sterile distilled water  
38.5 grams Sodium chloride  
5.35 grams Ammonium chloride  
3.81 grams Magnesium chloride  
Then bring up to 1000 ml.  

*Trace Metal Solution*  

Cupric sulfate:  
30 mg. to 100 ml. sterile distilled water (1 ml. to 1000 cc)
Zinc sulfate:
80 mg. to 200 ml. sterile distilled water (10 ml. to 1000 cc)

Ferrous sulfate:
500 mg. to 200 ml. sterile distilled water (20 ml to 1000 cc)

Vitamin B-12 crystalline:
10 mg. (0.01 gm.) to 100 cc sterile distilled water (concentrated)
Dilute—90 cc sterile distilled water and 10 cc concentrated solution (20 cc to 1000 cc)

Thiamine B1:
200 mg. to 100 cc sterile distilled water (1 cc to 1000 cc)

*Tween 80 10% solution*

10 cc tween 80 in 70 cc sterile distilled water 56°
Bring up to 100 cc
Then make a 1% solution
Add 900 cc sterile distilled water to the 100 cc of the 10% solution
Freeze 65 cc or 125 cc amounts of 1% tween 80 solution.

**Phosphate buffer (25X)**

40 ml. of 25X buffer solution — bring up to 1000 cc.
200 cc per flask sterilize 15 minutes 15 lbs.
Flake 5 grams of bovine albumin on 100 cc of the above solution (5%)
Filter the bovine albumin solution through a seitz filter.
Check sterility — incubate at 37°

*Completed media made with stock solutions*

40 ml. of 25X buffer
50 ml. of 20X basil salts
700 ml. of sterile distilled water
1 ml. of cupric sulfate
10 ml. of zinc sulfate
20 ml. of ferrous sulfate
    shake well
200 mg. L. cystine
    shake
Filter through 2 sheets of No. 1 filter paper
    (refilter if necessary)
Put in flask
20 ml. of diluted B12 solution
.1 ml. of Thiamine B1 solution
Then add filtered solution
Layer on 120 cc of the 1% tween 80 solution
Bring to volume (1000 cc) with sterile distilled water (layer)
Tube and autoclave 15 lbs. for 15 minutes
For Semi solid flake on 2 grams -.2% agar per 1000 cc.

*Amounts*

8 ml. of media
2 ml. of 5% bovine albumin
QUALIFYING BULLS FOR THE PRODUCTION OF SEMEN 461

80 ml. of media
20 ml. of 5% bovine albumin

400 ml. of media
100 ml. of 5% bovine albumin


VIBRIOSIS

A. SPECIMIN COLLECTION AND PRELIMINARY EXAMINATION

1. Specimen Collection

Vibrio fetus var. venerealis can be isolated from bovine semen. Samples should be collected in such a manner as to minimize fecal contamination. In addition, the specimens should be protected from the oxygen in air since this is toxic to V. fetus. The specimens therefore should be cultured on blood agar soon after collection, and must be incubated under microaerophilic atmospheric conditions. If the samples cannot be cultured within about two hours, they should be protected from air by sealing and preserved at about 0°C.

a. Semen is collected in an artificial vagina from bulls that are to be tested. Numerous bacterial species contaminate semen, so the samples must be spread over the surface of blood agar to obtain colony separation. About 0.1 ml. of semen can be cultured on each plate. However, swarming bacteria are occasionally found in semen from certain bulls and the samples must be diluted to obtain adequate colony separation on the plate. Blood agar with inhibitory antibiotics enhances V. fetus isolation.

b. Perputial samples suitable for bacteriological culture and immunofluorescent staining can be collected readily with a plastic pipette and attached rubber bulb as described by Bartlett for procuring samples for Trichomonas foetus examination. One must keep in mind the importance of the precise source from which smegma is obtained. The greatest numbers of V. fetus (and T. foetus) are obtained from the glans penis and immediately adjacent preputial membrane. Samples are collected by passing the plastic pipette into the preputial cavity, scraping the surface of the glans penis, and then flushing the material into a test tube containing 5 ml of nutrient broth. No fluid is introduced into the preputial cavity, as introduction of fluid or swabbing will collect extraneous debris from areas with low V. fetus populations, thereby increasing the difficulty in cultural isolation of the pathogen.

Perputial samples are processed as follows:
RECOMMENDED UNIFORM DIAGNOSTIC PROCEDURES

5-ml broth suspension of preputial smegma

4 ml

1 ml cultured for T. foetus

Centrifuge 600 g/15 min.

Supernatant fluid
a. 0.02 ml cultured on inhibitory medium
b. 1.0 ml passed through
0.65 μ membrane filter;
0.1 ml of filtrate cultured on blood agar plate.

Sediment resuspended in unused portion of supernatant fluid; passed through Whatman no. 1 filter paper using slight vacuum

filtrate centrifuged at 12,000 g/20 min/4° C.

resuspend sediment in
0.2 ml of distilled water
discard supernatant fluid

Smear 0.3 ml aliquot on marked area of alcohol-cleaned microscope slide; fix; stain with FITC conjugated antibody.

BACTERIOLOGY

The causative agent of vibriosis is a Gram-negative comma or S-shaped rod.

Classification (Bergey's Manual):
Order I : Pseudomonadales
Family VII : Spirillaceae
Genus I : Vibrio

Among the vibrios isolated from genitalia of cattle and sheep there are many serotypes. Clinically they are divided into three main groups based on biochemical typing:

Vibrio fetus venerealis: Natural pathogen of the bovine genital tract, causing infertility and occasionally abortion.

Vibrio fetus intestinalis: Natural inhabitant of intestines of cattle, sheep and pigs. Sometimes invades the pregnant uterus thereby causing abortion.

Vibrio bubulus: Non pathogen.

Serological tests with CFT based on phenol extracted antigens (Mitchellich) distinguished six different types of vibrio isolated from cattle. According to their biochemical features determined in culture, strains with antigen factors 1, 2 and 7 appear to be species of Vibrio fetus and strains with antigen factors 3, 4 and 5 to be Vibrio bubulus.

Whether V. jejuni could be identical with one of these vibrio types remains unexplained. (The organism associated with winter diarrhoea).

B. ISOLATION—VIBRIO FETUS

1. Cultural

Blood agar in petri plates is the preferred culture medium for primary isolation of V. fetus from moderately contaminated specimens such as genital secretions, semen, or aborted fetuses. Blood agar containing antibiotics is used for culturing highly contaminated
QUALIFYING BULLS FOR THE PRODUCTION OF SEMEN 463

materials such as preputial samples and feces. Filtration of samples through membrane filters (av. pore size, 0.65 μ) prior to culture essentially separates vibrios from most contaminating bacteria. The vibrios in the filtrates may be concentrated by centrifugation and cultured on inhibitory media. Blood agar cultures are incubated 3 to 5 days at 37°C in desiccator jars, using a gaseous mixture consisting of 5% oxygen, 10% carbon dioxide and 85% nitrogen.

Hemocultures from cases of vibrionic septicemia in animals and man are made by inoculating febrile phase blood into semisolid brain-heart infusion (BHI) broth. These cultures are incubated up to 10 days at 37°C., and examined daily for vibrios by phase contrast microscopy. The hemocultures are subcultured onto blood agar plates for isolation of vibrios.

MEDIA

Vibrio fetus is a micro-aerophilic organism, some strains of which will grow aerobically when adapted to laboratory conditions. Solid and semisolid media can be used. Semisolid media are of less use for obtaining primary cultures from contaminated material as they do not separate V. fetus from other organisms due to its slow growth. (V. fetus may be quickly overgrown by contaminants even on solid media). Vibrio fetus is very fastidious and grows slowly. Blood is required for primary isolation, and growth is stimulated by the addition of thiol, Vitamin B, etc. Often the organisms have to be isolated from contaminated material (semen, preputial washings, vaginal mucus, aborted fetuses, etc.). Dyes and sometimes antibiotics are, therefore, used in the solid media to prevent the growth of contaminants.

Bacteriostatics used are Bile, Crystal violet, Alkyl-aryl sulphate and Brilliant green. The latter has been shown to be the best.

Many investigations have been carried out to determine the sensitivity of V. fetus to antibiotics. Some of the antibiotics will inhibit growth of fecal streptococci, diphtheriods, etc., but they will not prevent the growth of cardinal contaminants in the material, namely, Proteus vulgaris and Pseudomonas aeruginosa. Noviobiocin has been used to inhibit the swarming of Proteus in Salmonella identification and has also been shown to be useful in vibrio isolation.

Several media have been introduced by different workers. For the sake of simplicity, only a few of the most common media are described here.

1) Semisolid media:
   a) Brucella Broth (Albimi) Pfizer No. 153A
      Pfizer Peptone “M” ........................ 20.0 g.
      Dextrose .................................. 1.0 g.
      Yeast Autolysate ........................... 2.0 g.
      Sodium Chloride ............................ 5.0 g.
      Sodium Bisulfite ........................... 0.1 g.
      Suspend 29 g in 1000 mls distilled water. Steam in steamer or autoclave
until completely dissolved. Pour 10 mls into each tube. Sterilize in the autoclave for 15 minutes at 15 lbs. pressure (120°C.).

b) Fluid Thioglycollate Medium—BBL 01-140

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypticase</td>
<td>15.0 g.</td>
</tr>
<tr>
<td>1-Cystine</td>
<td>0.5 g.</td>
</tr>
<tr>
<td>Dextrose</td>
<td>5.0 g.</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>5.0 g.</td>
</tr>
<tr>
<td>Sodium Choloride</td>
<td>2.5 g.</td>
</tr>
<tr>
<td>Sodium Thioglycollate</td>
<td>0.5 g.</td>
</tr>
<tr>
<td>Resazurin</td>
<td>0.001 g.</td>
</tr>
<tr>
<td>Agar</td>
<td>0.75 g.</td>
</tr>
</tbody>
</table>

Suspend 30 g in 1000 mls distilled water. Steam in steamer or autoclave until completely dissolved. Pour 8 mls into each tube. Sterilize in the autoclave for 15 minutes at 15 lbs. pressure (120°C.).

c) The medium of Reed and Orr

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose Peptone No. 3. Difco (B112)</td>
<td>20 g.</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5 g.</td>
</tr>
<tr>
<td>Sodium Thioglycollate</td>
<td>1 g.</td>
</tr>
<tr>
<td>Agar</td>
<td>1 g.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 mls.</td>
</tr>
</tbody>
</table>

Steam in steamer or autoclave until completely dissolved. Adjust pH to 7.4. Pour 8 mls into each tube. Sterilize in the autoclave for 15 minutes at 15 lbs. pressure (120°C.).

d) Liver broth. Oxoid (Granules CM77)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion from fresh liver</td>
<td>23 g.</td>
</tr>
<tr>
<td>Peptone</td>
<td>10 g.</td>
</tr>
<tr>
<td>Potassium phosphate</td>
<td>1 g.</td>
</tr>
<tr>
<td>Extracted liver tissue</td>
<td>30 g.</td>
</tr>
</tbody>
</table>

Add 64 g to 1000 mls distilled water. Soak for 15 minutes with occasional stirring. Distribute 8 mls into each tube so that bottom of tube is filled with liver particles. (Agitate frequently during distribution to keep the liver particles in suspension). Sterilize in autoclave for 20 minutes at 10 lbs. pressure (115°C.).

2) Solid media:

a) Florent agar:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>5 grams</td>
</tr>
<tr>
<td>Peptone</td>
<td>10 grams</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5 grams</td>
</tr>
<tr>
<td>Agar</td>
<td>20 grams</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 mls.</td>
</tr>
</tbody>
</table>

Steam in steamer or autoclave until completely dissolved. Adjust pH to 7.6. Distribute in 300 mls. aliquots. Sterilize in the autoclave for 15 minutes at 15 lbs. pressure (120°C.). Keep in stock. Immediately before use add 0.3% Thiol (Difco). Resterilize as above. Remove from autoclave and place in water bath at 63°C. After half hour, add 10% defibrinated cattle blood.
Add brilliant green solution to give a concentration of 1:50,000. (Stock solution of brilliant green 0.2%). Add 3.3 mls. to 300 mls medium to give 1:50,000.  

b) Modified Florent agar with Thioglycollate:  
Suspend 24 g. Thioglycollate broth (Difco) and 20 g. agar in 1000 mls. distilled water. Steam in steamer or autoclave until completely dissolved. Distribute in 300 mls. aliquots. Sterilize in the autoclave for 15 minutes at 15 lbs. pressure (120°C.). Keep in stock. Prepare before use as Florent agar described above without adding 0.3% Thiol.  
c) Chocolate agar:  
Use 10% cattle blood.  

Colony Types  
Considerable variation in colony types occurs on solid media. The basic types are shiny, pale-grey, semi-translucent, and conical. Small and large colonies appear on the same plate. Very often the only evidence of growth is a light “frosting” of the surface of the medium. The variation in the colonies makes extensive microscopic examination important.  

With pure cultures on semisolid thioglycollate medium, the growth is seen as a ring 2-3 mm. below the surface.  

Microscopy  
Three methods advised: stained smears for bright field microscopy, examination by phase contrast, or dark field microscopy.  

1) Stained preparations: Vibrios are Gram negative. Gram’s stain or 10% carbolfuchsirn (Ziehl-Nielsen) solution can be used. On initial isolation, the vibrios are comma and S-shaped. Flying seagulls shape is claimed to be characteristic. In older cultures long, filamentous forms may appear. Sizes range from 0.2 to 0.5 by 1.5 to 5.0 microns.  

2) Phase contrast microscopy: As with all microscopes, the phase contrast microscope must be handled very carefully. The phase rings must be carefully centered, or true phase will not occur, causing poor definition. A magnification of 1000 X to 1250 X, under oil, is advantageous. One loopful is transferred from semisolid media to the slide. A cover glass must be used. From solid media, one portion of a selected colony is transferred by loop to a slide and suspended in one loopful of sterile isotonic saline. Rapid forward and backward movement is typical under phase contast. (Vibrare in Latin means quick forward and backward movement).  
The vibrio has a single polar flagellum on the comma forms and a single flagellum at each pole on the S-forms. In old cultures, the motility is often lost and coccoid forms with one or more flagella often occur. (For demonstration on the flagella, a special staining technique is needed).  

3) Darkfield microscopy: With the dark field microscope, the typical movements of the vibrios can be observed, but the shape of the organism is clearer when phase contast is used.  

Preparation of Cultures  
Due to the variation in colony types, intensive microscopy of all inocu-
lated media is necessary. Occasionally, a pure culture can be obtained on the primary inoculated media, but this is very rare. Usually, the vibrios are seen in between the contaminants.

C. CHARACTERIZATION AND IDENTIFICATION

1. Growth Characteristics

Colonies of \textit{V. fetus} appear on blood agar after incubation for 2 to 5 days. They are 1 to 3 mm in diameter, round, smooth, entire, raised, translucent, butyrous, and have a pink cast, but are non-hemolytic. In reflected light, the colonies appear to have an internal pattern of white filamentous projections extending radially to the periphery. Viewed by transmitted light, \textit{V. fetus} colonies grown on Albimi Brucella Agar (ABA) are honey-colored, variably granular, and exhibit a swarming activity when touched with a needle.

2. Cellular Morphology and Staining Characteristics

\textit{V. fetus} cells are curved rods, comma or spiral shaped, 0.3 u diameter by 2 to 10 u long, motile with a single polar flagellum, Gram-negative, and nonsporeforming. Long spiral forms and coccoid cells appear in aging cultures, but certain strains exhibit these characters in early growth stages.

3. Differential Reactions on Media

An isolated colony of vibrio is inoculated into FTG semisolid broth, incubated 3 days at 37°C, examined microscopically for contamination, and used as inoculum for differential metabolic tests.

a. Catalase test. Inoculate 1 ml of vibrio culture into a tube (18 X 150 mm with metal cap) containing fresh FTG semisolid broth. After 3 days incubation, add 10 ml of 3% \textit{H}_2\textit{O}_2 solution; place a rubber stopper with glass capillary tube insert into opening of culture tube, invert over sink and mark fluid level. After 20 minutes, measure the amount of fluid displaced by gas. \textit{V. fetus} gives an average catalase reaction of 50 mm with a range of 20 to 130 mm.

b. Hydrogen sulfide test. Inoculate 1 ml of vibrio culture into a tube (16 X 150 mm with cotton plug) containing CBI semisolid broth. Insert a strip of lead acetate impregnated filter paper into tube and replace cotton plug. Record \textit{H}_2\textit{S} production (−), (T), (+), (+ +), or (+ + +) - positive at 5 days.

c. Salt tolerance test. Inoculate 1 ml of vibrio culture into tubes (18 X 150 mm, with metal cap) containing FTG semisolid broth with 4% NaCl. Incubate 5 days at 37°C and examine for growth. Very few strains of \textit{V. fetus} grow at the 2% NaCl level, and only to slight degree, whereas \textit{V. bubulus} and some vibrio isolates from fecal samples grow well at the 3% and 4% levels.

d. Glycine tolerance test. Inoculate 1 ml of vibrio culture into a tube (18 X 150 mm, with metal cap) containing FTG semisolid broth with 1% glycine. Examine for growth after 5 days. \textit{V. fetus Venerealis} is inhibited by 1% glycine, whereas intestinalis grows well in this medium.
e. Heat tolerance test. Inoculate 1 ml. of vibrio culture into each of 3 tubes (18 X 150 mm. with metal cap) containing FTG semisolid broth; incubate one at each temperature: 25° C., 37° C., 42° C., for 5 days, and examine cultures for growth. *V. fetus venerealisis* grows at 25° C. to 42° C.

Most vibrios commonly isolated from the genital and intestinal tracts of animals are nonproteolytic and nonfermentative, although they reduce nitrates to nitrites.

The following table compiled from various sources outlines the key metabolic characteristics used to distinguish the vibrio species under consideration. *Vibrio facelis, V. coli* and similar vibrios from the bovine intestines are tabulated, although their identity in respect to habitat, pathogenicity and antigenic structure, has not been determined.

<table>
<thead>
<tr>
<th>Species</th>
<th>Natural Habitat</th>
<th>Pathogenicity</th>
<th>Catalase</th>
<th>NaCl</th>
<th>Growth Tolerance 1% glycine</th>
<th>Growth Tolerance 4° NaCl</th>
<th>Growth Tolerance 42° C sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Vibrio fetus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. <em>venerealisis</em> (1)</td>
<td>bovine genital tract</td>
<td>infertility and abortion</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>b. <em>venerealisis</em> (sub-1)</td>
<td>bovine genital tract</td>
<td>infertility and abortion</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>c. <em>intestinalis</em></td>
<td>bovine and ovine intestinal tract</td>
<td>abortion</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>+/−</td>
</tr>
<tr>
<td>2. <em>Vibrio bubulus</em></td>
<td>bovine and ovine genital tract</td>
<td>none</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>3. <em>Vibrio coli</em></td>
<td>bovine intestinal tract</td>
<td>dysentery</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4. <em>Vibrio fecalis</em></td>
<td>bovine and ovine intestinal tract</td>
<td>unknown</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+/-</td>
<td>+++</td>
</tr>
</tbody>
</table>

4. Animal Pathogenicity

Rabbits, guinea pigs, hamsters, and mice may be experimentally infected with *V. fetus* but pathogenicity is limited at best. *Vibrio fetus var. intestinalis* can be differentiated from venerealisis by its predilection for the gallbladder and intestines of experimentally inoculated animals.

5. Immunofluorescent Procedures

Adequate knowledge of the serological classification of *V. fetus* will be essential for the widespread application of this test. It is known that the heat-stable O-antigen is the major component in the reaction, in contrast to the stereo interference by the K-antigen which occurs in the serum-agglutination reaction. Thus, a suitable conjugate should include antibody directed toward each of the O sero-
types.

The use of the fluorescent antibody (FA) reaction is useful in diagnosing bovine genital vibriosis in bulls. Its use on cervico-vaginal mucus has not yet received universal acceptance due to difficulties involving nonspecific reactions. A practical FA test using vaginal mucus requires separation of *V. fetus* from contaminating mucus and cellular debris.

a. Serum fractionation. Rabbits are hyperimmunized with *V. fetus* var. *venerealis* as described by Winter. The antibody fraction is separated by repeated ammonium sulfate precipitation and dialyzed in saline at 4°C until free of (NH₄)₂SO₄. The protein content (anti-(NH₄)₂SO₄ body fraction) is adjusted to 2.0 percent.

b. Conjugation. The 2 percent protein solution is conjugated with fluorescein isothiocyanate (FITC) using 50 ug of the dye per mg of protein as described. The mixture is held at 25°C for 18 hours, then passed rapidly through a column of G-25 Sephadex, dialyzed, and the F/P ratio is determined. The conjugated antibody can be stored at 4°C for up to 3 weeks, but should be lyophilized or frozen for longer periods.

c. Fixing, staining, and examination of smears.

1) *Fixation.* Dip slides into ethyl alcohol, 95%, for 15 minutes, then rinse with phosphate buffered saline (PBS: 0.01 M phosphate, pH 7.5) and air dry.

2) *Staining.* Add 0.05 ml. conjugate and incubate 30 minutes in a moist chamber at 37°C. Rinse thoroughly in several changes of PBS, counterstain, wash thoroughly with distilled water, dry and apply mounting fluid and coverslip. (Fluormount: Edward Gurr, Ltd., 42 Upper Richmond Rd., West London, S.W. 14 England; or Difco FA Mounting Fluid: Difco Laboratories, Detroit Michigan). Counterstaining is recommended in preparing smears for *V. fetus* examination, preferably with Erichrome Black A (Nutritional Biochemicals Corp., Cleveland, Ohio). Stock solutions, when refrigerated, retain activity for several months with little diminution. Counterstaining requires only a few seconds (5-10); excessive exposure will obliterate even specifically stained *V. fetus* cells. The optimal time will depend upon the concentration of protein, fluoresceine, and specific antibody, as well as potency of the counterstain. Both counterstaining and the conjugate dilution contribute to a decrease of nonspecific staining. Normally, one should utilize the lowest concentration of conjugate with which specific staining is undiminished. When counterstain is used, 4 to 8 times the minimal concentration of conjugate must be applied.

b. *Examination of FA-stained smears.* A microscope equipped with a source of ultraviolet light and proper light filtering system is
utilized. In examining smears where autofluorescence is not a consideration, the use of primary filters permitting the passage of blue light greatly increases the brilliance of FA staining. The BG-12 primary filter with a blue-absorbing secondary filter are used in the above system. Smears should be scanned carefully at a high power with oil immersion. It is well to examine a smear carefully for 10 to 15 minutes before concluding a negative diagnosis. The inclusion of positive and negative control samples is essential.

REFERENCES


V. TRICHOMONIASIS (Trichomonas foetus)

A. Collection of Samples

Collection of adequate samples of preputial smegma and prompt submission to the laboratory is necessary for demonstration of Trichomonas foetus. Attempts should be made to collect the sample from the area of the glans penis as this is shown to be the area of highest concentration of trichomonads (Bartlett, 1949) in infected bulls. Aspirate 2 to 3 ml of preputial secretion by use of an inseminating tube and syringe and express into a tube containing 5 ml of physiological saline if the sample can be presented to the examining laboratory within 12-18 hours. If the delay from collection to laboratory examination will exceed 18 hours, 5 ml of a thioglycollate broth with bovine serum and antibiotics added should be used as a carrier media in place of the saline solution. An alternative method is collection of preputial smegma by use of a gauze or cotton swab and immersing the swab in either saline or carrier media as described above. In either method, the samples should be kept under normal refrigeration temperatures 34-38°F during transport to the
To thioglycollate broth (Difco or BBL) add 10% bovine serum (inactivated at 56°C for 30 minutes) and penicillin at the rate of 1000 units per 1 ml of medium and streptomycin 1 mg per 1 ml of medium. Dispense in sterile screw cap tubes in 5 ml aliquots and refrigerate until used. The shelf life of this medium should be considered as not exceeding two weeks, but can be increased by not adding the antibiotics until shortly before use.

**B. Examination of Samples**

Centrifuge at 2500 R.P.M. for 10 minutes. Remove one or two drops of sediment by sterile pipette from the bottom of the tube and place on slide without cover slip. Examine under low power. If protozoa are observed, put cover slip in place and examine under high power to determine morphology (Levine, 1961). If this direct examination is negative, remove another two or three drops of sediment and inoculate into a tube containing 5 ml of the thioglycollate broth media as described above and incubate at 37°C for five days. The inoculum should be pipetted to the top of the medium in such a manner as to minimize mixing. Examinations should be repeated on the 2nd, 4th and 5th days during incubation and should always be conducted on a drop of sediment removed from the bottom of the tube. It is recommended that a cover slip not be used for the initial low power examination each time as the drop does not spread out and can be examined more rapidly.

Collection and handling of the sample is of primary importance in the diagnosis of *Trichomonas foetus* as the diagnosis in the laboratory can only be as good as the sample obtained.

**VI. Johne's Disease**

**A. Isolation and Identification of Mycobacterium Paratuberculosis from Fecal Specimens**

1. Processing Fecal Specimens

   a. Collection, shipment and identification: The fecal specimen is taken from the rectum using a dry single service glove. Approximately one-half ounce of feces is placed in a one-ounce ointment tin. The tin is identified, sealed with masking tape and shipped in an insulated container to the laboratory in the fastest way. No refrigerant or chemical preservative used.

   b. Suspension and decontamination of feces in the laboratory: Approximately one gram of feces is transferred with a sterile wooden tongue depressor to a 50 ml centrifuge tube containing 40 ml of sterile distilled water. The mixture is shaken for 30 minutes at room temperature. The larger particles are allowed to settle for 30 minutes. The uppermost 5.0 ml of feces suspension is transferred to a 50 ml centrifuge tube containing...
35 ml of 0.3% Zephiran. The tube is inverted several times to assure uniform distribution and allowed to stand undisturbed for 24 hours at room temperature.

c. Inoculation of culture mediums: A sterile 1 ml pipette is used to transfer 0.1 ml of the undisturbed sediment to each of four slants of Herrold's Egg Yolk Agar (three with ethyl alcohol-mycobactin and one without ethyl alcohol-mycobactin). The inoculum is distributed evenly over the surface of the slants. The tubes are allowed to remain in a slanted position at 37°C for approximately one week with screw caps loose. The tubes are returned to a vertical position when the free moisture has evaporated from the slants. The lids are tightened and the tubes are placed in baskets in an incubator operated at 37°C. The egg in Herrold's Medium contributes sufficient phospholipids to neutralize the bacteriocidal activity of residual Zephiran in the inoculum. The slants are observed biweekly for 14 weeks. Contaminated slants are discarded and all colonies resembling those of mycobacteria are selected for identification. All uncontaminated slants are incubated for the entire period even though mycobacterial colonies may have been isolated earlier.

d. Identification:

1) Appearance time—Primary colonies of *M. paratuberculosis* may be expected to appear at any time from the fifth to the fourteenth week after inoculation. Most colonies appear during the seventh week, but many appear during the fifth and sixth weeks.

2) Colonial morphology—Primary colonies on Herrold's Egg Yolk Agar Medium with mycobactin are very small (1 mm diameter), colorless, translucent, and hemispherical. Their margins are round and even. Their surfaces are smooth and glistening. The colonies become more opaque and increase in size as incubation is continued. Pigmentation is never observed in primary colonies. Isolated colonies may increase in diameter to 4 or 5 mm. Roughness increases with age, but the round and even margin is maintained regardless of incubation time.

3) Staining characteristics—The cells of primary colonies of *M. paratuberculosis* are highly acid-fast and gram positive. The cells average 0.5 micron in diameter and 1.0 micron in length. Spores and capsules are absent.

4) Mycobactin dependency—Primary colonies of *M. paratuberculosis* exhibit a strict dependency upon mycobactin (an extract of *M. phlei*) for growth. Therefore, if typical colonies are observed on the 3 slants containing mycobactin and are absent on the slant without mycobactin, the results are reported as positive for *M. paratuberculosis*. If typical colo-
nies are observed on only one or two of the slants containing mycobactin and are absent on the remaining slants, a single typical colony is subcultured on two slants of Herrold's medium containing mycobactin and two slants of Herrold's medium without mycobactin. If the bacillus grows equally well on all four of the slants, it is not *M. paratuberculosis*. If it grows only on the slants containing mycobactin, it can be reported as *M. paratuberculosis*. Colonies of organisms other than *M. paratuberculosis* are sometimes isolated, however, their growth characteristics and medium requirements are such that they will be rejected.

SUGGESTED READING

1. U. S. Department of Agriculture, APHS, Diagnostic Services, National Animal Disease Laboratory, Box 70, Ames, Iowa 50010; Laboratory Methods in Veterinary Mycobacteriology.

HERROLD'S EGG YOLK MEDIUM WITH MYCOBACTIN

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>For 1020 mls Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone (Difco 0118)</td>
<td>9.0 gm</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>4.5 gm</td>
</tr>
<tr>
<td>Agar (Special Noble-Difco 0142)</td>
<td>15.3 gm</td>
</tr>
<tr>
<td>Beef Extract (Difco 0126)</td>
<td>2.7 gm</td>
</tr>
<tr>
<td>Glycerol</td>
<td>27.0 ml</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>870.0 ml</td>
</tr>
<tr>
<td>*Mycobactin</td>
<td>2.0 mg</td>
</tr>
<tr>
<td>Ethyl Alcohol</td>
<td>4.0 ml</td>
</tr>
<tr>
<td>Egg Yolks</td>
<td>6</td>
</tr>
</tbody>
</table>

Aseptically prepared from strictly fresh eggs.

Eggs must be from hens that have had no antibiotics in feed or medicine.

2% Sterile Malachite Green Dye Solution (aqueous) (Oxalate) 5.0 ml

Equipment

Prepare and sterilize:

One 2 liter aspirator bottle with cotton stopper and small bell filling attachment for dispensing. A magnetic stirring bar may be included for later use in mixing the contents.

Rat tooth forceps for each egg.

Test tubes - 20 x 125 mm screw cap tubes.

*Mycobactin is obtained in 2 mg quantities from Diagnostic Services, NADL, P. O. Box 70, Ames, Iowa 50010.*
Method of Preparation

Mix and heat all ingredients (except egg yolks, mycobactin, ethyl alcohol, and dye solution) until agar is melted. Cool to 60°C. and adjust pH to 7.5 with 1 N. Sodium Hydroxide.

Pour into aspirator bottle, add a magnetic stirrer and while mixing on the magnetic stirrer, add the mycobactin dissolved in the 4 ml of ethyl alcohol. The mycobactin solution should be added with a pipette, dropping it directly into the medium rather than allowing it to come in contact with the inside surface of the aspirator.

Autoclave at 120°C. for 20 minutes, cool to 56°C. and add 6 sterile egg yolks prepared as follows:

Scrub eggs with a brush in warm detergent water and rinse with tap water. Air dry on a towel. Place in 75% isopropyl alcohol for 30 minutes. Dry between sterile towels. Crack shell at one end with sterile forceps, making a 10 mm hole and remove egg white with forceps and gravity. Make the hole larger and break yolk. Twirl forceps to mix yolk, pick out yolk sac with forceps and pour mixed yolk into sterile medium (in the aspirator bottle). Repeat process for each egg.

Mix the medium gently on the magnetic stirrer. Add sterile Malachite Green dye solution with sterile pipette.

When blending is complete, dispense aseptically into 20 x 125 mm sterile screw cap tubes in 8-10 ml aliquots. Allow medium to harden in a slanted position. Check for sterility at 37°C. for 48 hours.
Veterinary diagnostic laboratories have a history with which most older veterinarians are familiar. Commercial companies supplied various facilities and personnel (usually free of charge) to their accounts. At the same time, there was a rather feeble attempt at offering veterinary diagnostic assistance in most land grant colleges. These laboratories were often given a secondary role and as a result, the commercially supported laboratories were most often utilized.

Commercial veterinary diagnostic services were gradually phased out leaving a void in this essential area of disease control. There are several reasons for the elimination of this service by commercial companies. These include:

1. Purchase of the smaller companies by larger corporations, many of which were already in production of human products and did not offer diagnostic service.

2. Veterinary Biologics regulation that diagnostic services should be maintained completely separate from the biologic and other production areas. The reason for this is obviously to keep from contaminating products produced for sale.

3. Increased demand by the veterinarian for this service along with a better understanding of the use of laboratories and a better capability of utilization of diagnostic service. Owners are also more demanding in recent years because they have a better understanding of livestock diseases.

4. Increased cost of diagnostic services. Labor is higher, equipment is more expensive and more refined equipment is available.

Because of the elimination of commercially supported diagnostic laboratories, there has been an acute demand upon established institutional diagnostic laboratories. New or revised versions of laboratories have been developed. Among those are the so-called regional or "out state" laboratories. The Veterinary Science Laboratory at the North Platte Experiment Station of the University of Nebraska is an example of the regional laboratory.

The need for the services offered is indicated by the fact that accessions increased nearly five times in four years (see table 1). The number of services also increased but because of limited space and personnel...
the latter has not reached the potential. Neither has the number of accessions.

Increased utilization of diagnostic laboratories and especially the North Platte Veterinary Science Laboratory can be attributed to the following:

1. Elimination of commercial diagnostic laboratories.
2. Increased ability and willingness of veterinarians to use a diagnostic laboratory.
3. Pressure of the owner for a more refined diagnosis.
4. Identity of diseases previously not recognized and the development of corresponding diagnostic techniques.
5. Increased concentration of livestock making an early confirmed diagnosis imperative. We have cow herds ranging to 4000 head and several large feedlots in Western and West Central Nebraska.
6. Increased movement of livestock creating the possibility of the spread of disease and establishing diseases in an area where they were not originally endemic. Cattle are brought to feedlots along the Platte River valley from Texas, Mississippi, Missouri, Kansas, Colorado, Wyoming, Utah, Idaho, Oklahoma, New Mexico, Mexico, and so on.
7. Increased value of livestock.
8. Regional laboratories have the additional advantage of accessibility. For example, our laboratory is one and one-half miles south of North Platte 1-80 interchange, so the owner does not have to drive through city traffic and hunt through a University campus for a building located in some hidden corner.

Regional laboratories are probably more the exception than the rule in the United States. Those which are operating are usually developed as a result of concentration of livestock in an area away from the University or Capitol of the state where the original laboratory was developed.

Distance involved in shipping specimens to Lincoln prompted early discussion of the development of the Veterinary Science Laboratory in North Platte by the Chairman and other personnel at the University of Nebraska Department of Veterinary Science, Lincoln, Nebraska. Subsequent to this the livestock owners and veterinarians in Western Nebraska also became interested in the development of the laboratory in that area. These three groups not only saw the current need but also the future need for diagnostic services in Western Nebraska. Nebraska extends approximately 450 miles west from Omaha. The original diagnostic laboratory was located at Lincoln, which is 414 miles from Scottsbluff and 237 miles from North Platte. Needless to say, the distance of shipping specimens was a problem. North Platte was selected because of location, the North Platte Experiment Station, and the fact that this is a transportation center of West Central Nebraska. It is adjacent to the Sandhills where there are large numbers of cattle and to the Platte River Valley where feeding operations are prevalent.

The original laboratory building was completed early in 1969. This was a much smaller building than originally planned; however, the 1973
Nebraska Legislature appropriated $100,000 for physical expansion. This is currently being processed.

The laboratory has facilities for complete necropsy, microbiology, fluorescent antibody, limited serology, histopathology, clinical pathology, and animal inoculation. Many other procedures are done at other laboratories such as the local medical pathology laboratory, and veterinary diagnostic laboratories at Lincoln; Brookings, South Dakota; Ames, Iowa; and Manhattan, Kansas. The Diagnostic Services, National Animal Disease Laboratory at Ames is also utilized extensively.

Professional personnel includes myself and Dr. R. Gene White, D.V.M., M.S., who is 75% extension. Technical personnel includes Douglas Johnson, B.S., M.S., microbiologist; Leonard Current, animal health technician; Mrs. Yolanda Coker, B.S., ASCP (part time); Ellen Pinion, secretary. Junior college students are utilized on a part time basis in the secretarial office and in the laboratory.

Utilization of para-medical trained people is necessary because of the case load of professional personnel. Responsibilities are delegated to each member of our organization. The microbiologist is responsible for identifying bacteria as well as preparing and reading fluorescent antibody slides, entering specimens, and labeling specimens. The animal health technician is responsible for opening carcasses (this is a great time saver for professional personnel), caring for the laboratory animal colony, trimming tissues for histopathology, and other incidentals such as setting up fecals. This is not ideal but facilitates getting a preliminary report. If tissues are not satisfactory we simply retrim. Conscientious para-medical personnel soon learn to do these properly. The medical technologist is responsible for clinical pathology, preparing histologic slides, and serology. She also goes through each day’s entries at 1:00 p.m. to see that work is initiated and to eliminate errors. The secretary is responsible for typing letters, filing, and other things related to the laboratory. Mimeograph work, making up annual reports, preparing manuscripts, and acetates are also part of her responsibilities.

Because of the lack of personnel, we purchase much of our media already prepared. We currently have a contract with Central Biologics at Omaha. All types of prepared supplies are used which are economically warranted. For example, limited use of the Enterotube® Roche Diagnostics, Nutley, New Jersey) was initiated this summer to speed identification of enteric bacterial isolates. The laboratory selectively uses disposable material and prepackaged material as much as possible. These include such things as sterile swabs, vacutainers for collecting blood, sterile pipettes, sterile disposable tubes, disposable specimen cups, and so on.

Because the secretarial staff is currently located in another building, all answers are dictated and delivered to that office for typing and filing copies. We send the original copy of the case report to the veterinarian and a copy to the owner. Although not all practitioners agree, it is my opinion that this is an equitable procedure. After all, the owner deserves to know about his animal. Letters are typed and corrected by the secre-
tential staff. They use a stamped signature. Window envelopes are used to the veterinarian. This saves typing one address per letter dictated. Attempts are in process of developing form letters for such things as aborted fetuses and calf scour cases because we do have a routine which is followed at the laboratory for these types of cases.

The Veterinary Science Laboratory at North Platte has an auxiliary function which is field training second year veterinary Vocational Technical students from the University of Nebraska School of Technical Agriculture. Each student spends at least two days in our laboratory. Two veterinary preceptors from Colorado State University have trained in our laboratory. In addition, two students from Kansas State University have been employed for summer work. High school students from the advanced science class have worked in our laboratory. In other words, we try to utilize this unit to its fullest extent. We are currently trying to arrange a part of the graduate program with a graduate student who is interested in concentrated diagnostic training.

Our laboratory also forwards research material to the interested people. Some of the transmissible gastroenteritis material which has been reported by Professor Norman Underdahl at the Veterinary Science Department at Lincoln was collected in the North Platte Laboratory. Most of the diagnostic work for Dr. Gene White's field studies of a reo-like vaccine and corona-like vaccine was done at North Platte. Cooperative studies of anomalies with Dr. Horst Leipold, Kansas State University, have been functioning for the last three years.

From the above information, it is evident that regional laboratories can serve a useful purpose in the community, state, and nation. It is also apparent that para-medical personnel can serve a useful function in a laboratory. Without para-medical assistance, our laboratory would not be capable of processing the volume which it does.

<table>
<thead>
<tr>
<th>Year</th>
<th>Accession Numbers</th>
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<tbody>
<tr>
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<td>868</td>
<td>335</td>
</tr>
<tr>
<td>1970</td>
<td>2,000</td>
<td>936</td>
</tr>
<tr>
<td>1971</td>
<td>2,773</td>
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<td>1972</td>
<td>3,966</td>
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</table>
An Indirect Fluorescent Antibody Test for Antibodies to *Nosema cuniculi* (Encephalitozoon) in Rabbits

S. J. Jackson, D.V.M., M.S.;
R.F. Solorzano, B.S., M.S., Ph.D.;
C. C. Middleton, D.V.M., M.S.

**SUMMARY**

Inoculation of mice, cortisone treated and untreated, failed to yield large enough numbers of cell free parasites in peritoneal fluids or tissues for use in serological procedures. Treatment of cell cultures with antibiotics or Ethylene Diamine Tetra-Acetate had no effect on parasite multiplication in the cultures. Large numbers of purified *Nosema cuniculi* parasites were prepared in primary baby rabbit kidney cell cultures after three passages and were purified easily by differential centrifugation. An indirect fluorescent antibody test (IFAT) was used successfully to detect antibodies to *Nosema cuniculi* in rabbits exposed experimentally and naturally to the parasite.

**INTRODUCTION**

Nosematosis is a disease which in rabbits is characterized by focal interstitial scarring and pitting of the kidneys, and granulomatous lesions of the brain. The disease is caused by an intracellular protozoon parasite similar in size to *Toxoplasma gondii* and produces a subclinical infection which is difficult to diagnose in many species of animals, including man. The object of this study was the preparation of *Nosema cuniculi* parasites for use in an indirect fluorescent antibody test for the detection of circulating antibodies in infected rabbits. Carworth Farm white nice and baby rabbit kidney cell cultures were both inoculated and evaluated for this purpose.

**MATERIALS AND METHODS**

*Mouse Inoculation*

The source of inoculum used in these studies was the kidney of a clinically ill rabbit in which large numbers of *Nosema cuniculi* parasites were observed in sections by use of Goodpasture's stain. 13 CFW mice were inoculated with 2.5 mg. of cortisone acetate subcutaneously bi-weekly, one week before and over the course of the experiment. Ten of these
mice were inoculated intraperitoneally with .1 ml. of a suspension of infected rabbit kidney in phosphate buffered physiological saline, pH 7.2 (PBS), which would pass through a 20-gauge needle. Ten additional mice were inoculated intraperitoneally with the parasite alone. Five mice were used for uninoculated controls, three cortisone treated, and two untreated. Inoculated and control mice were killed at 3, 9, 15, 21, and 27 days post inoculation and their peritoneal fluids were examined for the presence of parasites by phase microscopy, giemsa, and gram stains. Tissue sections of kidneys, spleen, pancreas, intestines, heart, lungs, and brain stained with Goodpasture’s and Hemotoxylin and Eosin, were examined for the presence of parasites and lesions.

**Cell Cultures**

Primary rabbit kidney cell cultures were prepared from day-old rabbits by standard Trypsinization procedures in flasks and Leighton tubes containing cover glasses. The media used was .5% lactalbumin hydrolyste, 0.05% yeastolate and 10% fetal bovine serum in Hank's basal salt solution with penicillin potassium G. 100 units/ML; streptomycin sulfate, 100 ug/ML; polymyxin B sulfate, 25 ug/ml; amphotericin B, 50 ug/ML; and kanamycin sulfate, 8.3 ug/ml. Cultures with and without antibiotics were inoculated with the infected rabbit kidney suspension and peritoneal fluids from infected mice. The cultures were refed weekly or daily. Cultures treated for 5 to 15 minutes with EDTA solutions at 50 and 5 ug/ml concentrations and untreated control cultures were inoculated with parasites from infected cultures. Third passage rabbit kidney cells grown in rose chambers were inoculated with parasites from infected cultures.

After three passages in cell cultures, parasites were purified by differential centrifugation at 20xg for 3 minutes to remove cellular debris followed by 1,000 xg for 15 minutes to sediment the parasites. Cultures were monitored for the presence of parasites by examining supernatant fluids by phase microscopy and sediments from culture fluids by giemsa and gram stains. Cell monolayers on cover glasses were fixed for one hour with methanol and stained overnight with giemsa stain.

**Preparation of Slides**

Eight circular 6 mm. wells (4 in each of 2 rows) were drawn with a yellow ink pen (Tech-Pen, Marktex Corp., Englewood, New Jersey) on glass slides. One drop of fluid containing a concentration of parasites adjusted to give 200-300 parasites per high power microscope field (400x), was placed in each well with a tuberculin syringe. The suspension was allowed to air dry. The slides were stored at -20°C until ready for use.

**Procedure for Indirect Fluorescent Antibody Test**

Slides with frozen parasites were either thawed at room temperature for 15 minutes or in a 37°C water bath on a rack for 10 minutes. The slides were washed with distilled water and air dried. A drop of the test serum or diluted test serum was placed into each well and the slides were incubated for 30 minutes in a 37°C water bath on a rack. After 30 minutes, the slides were gently shaken to remove the excess serum. The slides were rinsed in distilled water, dipped twice in PBS (pH 7.2) and placed in
PBS for 5 minutes. The slides were placed in distilled water for 5 minutes, removed, and air dried.

When slides were dry, a drop of anti-rabbit globulin, caprine origin, fluorescein labeled (Bioquest, Cockeysville, Maryland), diluted in 0.2% Evans Blue for optimum fluorescence was placed in each well. The serum and conjugate completely covered each circle. The slides were placed in a 37°C water bath for 30 minutes. The conjugate was removed by gently shaking. The slides were rinsed in distilled water, dipped twice in PBS and placed in PBS for 5 minutes. The slides were then placed in distilled water for 5 minutes. The water was removed and the slides were air dried. One drop of buffered glycerol (pH 8) was placed on each circle and cover glasses placed on the slides which were examined with a fluorescence microscope at 250 and 400x magnifications.

**Microtitration of Sera**

Twofold dilutions of rabbit sera found to be positive at the screen dilutions (undiluted, 1:16) were made by the Microtiter technique. The sera were tested by the IFAT in increasing fourfold dilutions which was necessary to obtain sharp end points. The dilutions were made in microtiter plates using 0.025 ml. droppers and diluters. Each group of sera tested included saline controls, negative serum controls, a positive low titer control, and a positive high titer control.

Antigen from a commercial IFAT *Toxoplasma gondii* test kit was tested with a positive *Nosema cuniculi* serum and conjugate from our test system. Human serum positive for antibodies to Toxoplasma in the commercial IFAT Toxoplasma test kit was tested using the Nosema antigen.

**RESULTS**

**Mouse Inoculations**

The results of intraperitoneal inoculation of mice with the *Nosema cuniculi* parasites are in Table I. Parasites and lesions were observed in the spleen, liver, pancreas, seminal vesicles, and kidneys of inoculated mice. No lesions or parasites were found in the heart, lungs, or brain of inoculated mice. No parasites or lesions were found in the control mice. Macrophages laden with parasites were found by phase microscopy and by examination of giemsa and gram stained smears of peritoneal fluids of inoculated mice.

The spleen was generally enlarged with parasites encysted within the capsule. The liver contained focal microgranulomas infiltrated with lymphocytes and small mononuclear leukocytes. Parasites were observed individually as well as encysted within the exocrine portion of the pancreas and in the peripheral fat associated with the organ. They were also within the wall of the seminal vesicles. Many parasites were free in the lumen of the renal tubules of the kidney. The number of parasites found in the tissues and peritoneal fluids of inoculated mice was considered too low to be a practical source of antigen for use in serological procedures.
Rabbit Kidney Cell Cultures

There were no detectable differences in the morphology or growth of the parasites in inoculated cultures with or without antibiotics. No differences were observed in the number of parasites produced in untreated cultures and cultures exposed to 5 or 50 ug/ML of EDTA. Upon first passage, only a small number of cells were detected to be infected in cell monolayers on cover glasses in Leighton tubes although the parasites were detected in gram stained fluids from the cultures. After four months, the cultures in flasks were destroyed by the parasite (Table II). No differences were observed in the cultures inoculated with peritoneal fluids obtained from infected mice, from those inoculated with the rabbit kidney suspensions. Cell cultures inoculated with the antigen obtained from the first passage of the parasite in tissue cultures were destroyed by the agent 4 weeks post inoculation (Table III). Additional cultures inoculated with parasites obtained from the second passage of the parasite in cell cultures were destroyed within 7 days (Table IV). Aerobic and anaerobic sterility cultures made from the tissue culture fluids and suspensions of the parasites were all negative for bacteria.

Rose Chambers

The third passage rabbit kidney cell cultures were composed of a mixture of fibroblastic and epithelial-type cells with equal susceptibility. The first day after inoculation, parasites were observed in the fluid and embedded in the cytoplasm of some of the cells. At day 2 post inoculation, an intracytoplasmic array consisting of bead-like structures smaller than the inoculated parasites was observed on either side of the nucleus. The structures increased in size by the third day and began to resemble mature parasites. By day four, an elongated form of the parasite was aligned end to end within the cytoplasm of the tissue culture cells along-side the cyst-like structures on both sides of the nucleus. By day seven, all the cells were lysed and mature parasites had been released into the culture medium.

Evaluation of Anti-Rabbit Globulin Conjugate and Evans Blue for Nonspecific Fluorescence

The parasites observed after incubation in wells with conjugate undiluted and diluted 1:10 had a large amount of nonspecific fluorescence. In the 1:10 dilutions, less than 5% of the cells fluoresced a yellow-green and no nonspecific fluorescence was noted in the dilutions of 1:20 and above (Table V).

Titration of Conjugate

Parasites, incubated first with serum obtained from a rabbit naturally exposed to nosematosis and then with anti-rabbit globulin conjugate, fluoresced a solid, bright yellow-green. When serial dilutions of the conjugate were tested, parasites at the end point dilutions had a bright halo of yellow-green fluorescence around their periphery with a reddish-brown internal structure. In the negative dilutions of the conjugate, the
organisms were stained a reddish-brown by the Evans Blue (Table VI).
A dilution of 1:100 was the positive end point dilution and a dilution
of 1:90 was selected as the test dilution for this conjugate in the IFAT.
The same end point was established with another system using *E. coli*
and rabbit antiserum specific for that organism. Sharp endpoint titers
with serial dilutions of rabbit sera were obtained when fourfold dilutions
and positive yellow-green fluorescence of 50% of the organisms in the
field were used.

*Microtitration of Sera*

Sera from 50 rabbits were studied. The sera were obtained from five
different sources. The test results on sera collected from a litter of nat-
urally-exposed young rabbits and their dam is shown in Table VII. Nose-
matosis was diagnosed in two of the offspring from this dam's previous
litter. One of the rabbits was inoculated with antigen and had more than
a tenfold rise in titer to the agent. All the rabbit sera from this group
were positive in the IFAT.

Sera from 18 animals obtained from an investigator that had collected
paired sera on several rabbits for another project which were kept in the
same hutch as the naturally infected and hyperimmunized rabbits, were
tested by the IFAT. Ten animals had detectable titers to *N. cuniculi*
while eight animals were negative (Table VIII). Seroconversion from
negative to positive was detected in three of the rabbits. When one of the
rabbits from this group which became clinically ill was necropsied, *N.
cuniculi* parasites were found in its tissues.

Blood was collected from a litter of ten young rabbits, five of which had
a titer of greater than 1:16 while five were negative. The sera of three
rabbits from another source were negative in the IFAT.

Two additional litters of five rabbits each were negative for antibodies
by the IFAT. The rabbits were mixed, placed in separate isolation units
and five animals were inoculated intraperitoneally with antigen obtained
from rabbit kidney cell cultures. The five inoculated animals all de-
veloped antibodies detected with the IFAT and the five uninoculated ani-
mals were negative.

When antigen from a commercial IFAT Toxoplasma test kit was in-
cubated with a positive *Nosema* serum and conjugate from our test sys-
tem, a negative test was obtained. When human antiserum to Toxo-
plasma and anti-human gamma globulin conjugate from a commercial
IFAT test kit was applied to *Nosema* antigen, no yellow-green fluores-
cence was observed which indicated a negative IFAT.

**DISCUSSION**

The two primary objectives of this study were achieved: 1) an indirect
fluorescent antibody test capable of detecting antibodies to *Nosema
cuniculi* in rabbits was developed and 2) large quantities of the parasite
were produced in a standard primary rabbit kidney cell culture system.
This made possible the detection of antibodies to *Nosema cuniculi* in rab-
bbits which were actively infected or had previous contact with the agent.
Since lesions of this disease can be present without detectable parasites and parasites can be present without detectable lesions, this procedure makes possible the identification of infected rabbits by the monitoring of paired serum samples, and the location of infected colonies by routine serological testing of easily obtained blood samples. The availability of a simple cell culture system for growth of the parasite makes a careful study of its life cycle possible. A ready source of purified parasites is now available for detailed morphological studies. The results obtained in this study were similar to those obtained by Chalupsky, although the methods used were different.

REFERENCES

### TABLE I

**Experimental Nosematosis in Mice**  
**With and Without Cortisone Exposure.**

<table>
<thead>
<tr>
<th>Euthanatized (days) after exposure to antigen</th>
<th>Cortisone exposed parasite* observed</th>
<th>No cortisone parasite observed</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0/2</td>
<td>0/2</td>
<td>0/1#</td>
</tr>
<tr>
<td>9</td>
<td>1/2*</td>
<td>0/2</td>
<td>0/1</td>
</tr>
<tr>
<td>15</td>
<td>1/2*</td>
<td>2/2</td>
<td>0/1#</td>
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<tr>
<td>21</td>
<td>2/2</td>
<td>2/2</td>
<td>0/1</td>
</tr>
<tr>
<td>27</td>
<td>0/2</td>
<td>0/2</td>
<td>0/1</td>
</tr>
</tbody>
</table>

+ Number of animals positive for nosematosis / Number in group.

* One animal died in this group. The animals that died were not necropsied because of cannibalism.

# Control animals receiving cortisone.

The mice were inoculated intraperitoneally with a kidney homogenate prepared from a rabbit kidney containing visible Nosema parasites.
TABLE II

Results of the Inoculation of Primary Rabbit Kidney Cell Cultures With a Rabbit Kidney Homogenate Obtained from a Rabbit with Naturally Occurring Nosematosis.

<table>
<thead>
<tr>
<th></th>
<th>Months Post Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Cells on Cover Glasses</td>
<td></td>
</tr>
<tr>
<td>Supernatant Fluids from Leighton Tubes</td>
<td>1+*</td>
</tr>
<tr>
<td>Cells in Flask</td>
<td></td>
</tr>
<tr>
<td>Supernatant Fluids from Flask</td>
<td>1+</td>
</tr>
</tbody>
</table>

- No parasites were observed
1+ Few parasites were observed
4+ Many parasites were observed and the monolayers were destroyed

* These cultures were discarded after 3 weeks
## TABLE III

Results of the Inoculation of Primary Rabbit Kidney Cell Cultures with *Nosema* Antigen Obtained After One Passage in Rabbit Kidney Cell Cultures.

<table>
<thead>
<tr>
<th>Weeks Post Inoculation</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracytoplasmic Parasites</td>
<td>-</td>
<td>1+</td>
<td>3+</td>
<td>4+</td>
</tr>
<tr>
<td>Parasites in Supernatant</td>
<td>1+</td>
<td>1+</td>
<td>3+</td>
<td>4+</td>
</tr>
</tbody>
</table>

- No parasites were observed.

1+ Few parasites were observed.

3+ Numerous parasites were observed.

4+ Large numbers of parasites were observed and the monolayers were destroyed.

## TABLE IV

Results of the Inoculation of Primary Rabbit Kidney Cell Cultures with *Nosema* Antigen Obtained After Two Passages in Rabbit Kidney Cell Cultures.

<table>
<thead>
<tr>
<th>Days Post Inoculation</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracytoplasmic Parasites</td>
<td>-</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td>3+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>Parasites in Supernatant</td>
<td>-</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td>3+</td>
<td>4+</td>
<td>4+</td>
</tr>
</tbody>
</table>

- No parasites were observed.

Infectivity of cells or parasites observed in the supernatant fluids were recorded from 1+ to 4+, with 1+ the least number observed and 4+ the most parasites seen. On day 6, approximately 100% of the cells in the cultures were infected. The monolayers were destroyed within 1 week.
### TABLE V

**Evaluation of Nonspecific Fluorescence of the Anti-rabbit Conjugate, Parasites and Evans Blue.**

<table>
<thead>
<tr>
<th>Conjugate Dilutions</th>
<th>Undil</th>
<th>1:10</th>
<th>1:20</th>
<th>1:30</th>
<th>1:40</th>
<th>1:50</th>
<th>1:60</th>
<th>1:70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjugate</td>
<td>4+</td>
<td>1+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Conjugate and Evans Blue</td>
<td>4+</td>
<td>1+</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

- 4+ Large amounts of nonspecific fluorescence noted.
- 1+ Less than 5% of the organisms were fluorescing a yellow-green.
- - No fluorescence was observed.
- * No yellow-green nonspecific fluorescence. Parasites fluoresced reddish-brown due to Evans Blue.

### TABLE VI

**Titration of Anti-rabbit Conjugate for the Optimum Dilution.**

<table>
<thead>
<tr>
<th>Dilution of Anti-rabbit Conjugate in Evans Blue</th>
<th>1:20</th>
<th>1:30</th>
<th>1:40</th>
<th>1:50</th>
<th>1:60</th>
<th>1:70</th>
<th>1:80</th>
<th>1:90</th>
<th>1:100</th>
<th>1:110</th>
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</thead>
<tbody>
<tr>
<td>Fluorescing Parasites</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilution of Rabbit Serum*</td>
<td>1:16</td>
<td>1:16</td>
<td>1:16</td>
<td>1:16</td>
<td>1:16</td>
<td>1:16</td>
<td>1:16</td>
<td>1:16</td>
<td>1:16</td>
<td>1:16</td>
</tr>
</tbody>
</table>

- * The previous litter of the dam of this rabbit had nosematosis.
- 1+ Positive fluorescence, a bright yellow-green around the agent.
- - No bright yellow-green fluorescing organisms observed.
TABLE VII

Indirect Fluorescent Antibody Test Titers to Nosema cuniculi of Sera from a Litter of Young Rabbits and Their Dam Whose Previous Litter had Two Animals with Nosematosis.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Undil</th>
<th>1:4</th>
<th>1:16</th>
<th>1:64</th>
<th>1:256</th>
<th>1:1024</th>
<th>1:4096</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>3</td>
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</tr>
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</tr>
<tr>
<td>9*</td>
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<td>-</td>
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<td>9a*</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Bright yellow-green fluorescence.
- No yellow-green fluorescence.

* This animal was inoculated with a kidney suspension obtained from a rabbit with nosematosis. Three weeks later the titer was 1:1024 (9a).
# TABLE VIII

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>*Date Sera Collected</th>
<th>Undil</th>
<th>1:16</th>
<th>1:64</th>
<th>1:256</th>
<th>1:1024</th>
<th>1:4096</th>
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<td></td>
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</tr>
<tr>
<td></td>
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<td>-</td>
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<tr>
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<td>9-28-70</td>
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<td>+</td>
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<td></td>
</tr>
</tbody>
</table>

* Rabbits were in the same hutch as naturally infected rabbits.
- No positive fluorescence of *N. cuniculi*.
+ Positive fluorescence of *N. cuniculi*.

* This sera was obtained from another investigator, who collected paired sera from some rabbits for another project. The date is the day the rabbit was bled. Note that paired sera samples are recorded.
TABLE VIII (continued)

*Indirect Fluorescent Antibody Test Titers to *Nosema cuniculi* of Sera from Rabbits Naturally Exposed to Nosematosis.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Date Sera Collected</th>
<th>Undil</th>
<th>1:16</th>
<th>1:64</th>
<th>1:256</th>
<th>1:1024</th>
<th>1:4096</th>
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* Rabbits were in the same hutch as naturally infected rabbits.
- No positive fluorescence of *N. cuniculi*.
+ Positive fluorescence of *N. cuniculi*.

* This sera was obtained from another investigator, who collected paired sera from some rabbits for another project. The date is the day the rabbit was bled. Note that paired sera samples are recorded.
An indirect fluorescent antibody test for antibody in unknown serum was developed for swine dysentery. The test was dependent upon the immunofluorescence of spirochetes which were obtained by impression smears of the colonic mucosa of swine on the first day of diarrhea. This was the time when the spirochete population was observed to be most numerous. Also used in the test as antiporcine globulin of rabbit origin.

Most of the exposed nonmedicated swine, which developed and recovered from a diarrhea characteristic of swine dysentery, developed a serum titer which ranged from 1:2 of 1:16. Exposed medicated swine which developed a diarrhea because of the ineffectiveness of the drug, also had a serum titer; whereas, exposed swine medicated with effective drugs which prevented a diarrhea, developed little or no serum antibody. There appeared to be a relationship between the severity and duration of diarrhea and the development of a serum titer.

A direct fluorescent antibody test was developed utilizing serum from hyperimmunized swine. This test had the advantage of reducing non-specific fluorescence, but the disadvantage in that the conjugated serum had a low titer of immunofluorescence.
INTRODUCTION

The Mycoplasmas (formerly called PPLO organisms) are minute organisms that are like bacteria in containing both types of nucleic acid (RNA and DNA) and appearing seldom or never to require a live host cell in which to replicate. They are sometimes mistaken for viruses, however, because (1) their small size renders them filtrable through many bacteria retaining filters, and (2) their fastidious nature in some cases is so marked as to make them extremely difficult to propagate in cell-free media.

Within the order Mycoplasmatales there are two recognized genera among the organisms isolated from animals: (1) the genus *Mycoplasma* which includes all of the recognized animal pathogens, and (2) the genus *Acholeplasma*, for which the pathogenic status is not known. These two genera differ in one important cultural characteristic, that being requirement for sterol (usually supplied by serum) in the medium. *Mycoplasma* spp. are sterol-dependent, whereas the *Acholeplasma* spp. are not. For our purposes, the genus *Mycoplasma* conveniently may be further divided into (1) the so-called classical-type or large-colony-type Mycoplasmas, and (2) the so-called tiny-colony-type or T-mycoplasmas. The T-mycoplasmas possess a urease enzyme and apparently require the presence of urea in the medium for their replication. Most animal sera supply at least a marginal amount of urea for their growth. Depending on the makeup, the pH and the buffering capacity of the growth medium, classical-type mycoplasmas and T-mycoplasmas each may at times form colonies which appear like those usually associated with the other group.

Until recent years, the importance of mycoplasmal diseases of domestic animals was thought to be quite limited. Of the few known animal diseases of mycoplasmal causation, the two most important—contagious bovine pleuropneumonia and contagious agalactia of sheep and goats—were thought not to have occurred in the United States in this century. Prior to 1950, the two clinical endemic diseases known to be caused by mycoplasmas both affected avian species and were caused by the same organism, *Mycoplasma gallisepticum*.

As many of the relatively spectacular infectious diseases of animals were identified as to causative organisms, attention focused on the more insidious infectious diseases—those of multiple etiology, those which
are of economic importance but hard to recognize clinically, and those
due to organisms which are difficult to cultivate in vitro. Most mycoplas-
mal diseases may be included among the latter classifications. Economically
important mycoplasmal diseases of animals which have been studied
to any extent affect swine, chickens, turkeys and cattle. These have been
the subject of several recent reviews. The host species for which
mycoplasmal diseases have not been described are those with which little
study has been done. It is likely that a number of mycoplasmas and my-
coplasmal diseases will be discovered yet as we search for these organ-
isms in other host species. Several mycoplasma species or serogroups
have been isolated from dogs, cats and horses, for example, but little is
known of the pathogenic potential of mycoplasmas in those host species.
In the United States, little attention has been accorded the mycoplasmas
of sheep and goats, but recent evidence of infection with mycoplasmas
indistinguishable from the agent of caprine pleuropneumonia at widely
separated locations should serve as a stimulus to research with those
organisms.

Other, more detailed reports on procedures for the isolation and identi-
fication of mycoplasmas are or will soon be available. The purpose
of this report is to provide a simplified, more readily accessible guide to
these procedures for veterinary diagnosticians who have never worked
with mycoplasmas but would like to start an effort in that direction. It is
hoped that this will encourage further isolation and identification activity
which, in turn, will help delineate the role played by the mycoplasmas
in other diseases of domestic animals.

MEDIUM AND MEDIUM SUPPLEMENTS

Most general purpose bacteriological media (meat infusion and/or
peptone base) are suitable for propagating some animal mycoplasma
species provided they are supplemented with serum. A fresh frozen
yeast extract (available from most cell culture medium suppliers) or
suitable dried yeast product like Albimi yeast autolysate* must also be
added for good growth of some mycoplasma species. The amount
of serum required for optimum growth depends on (1) the kind of serum,
(2) the medium used, and (3) the organism to be grown. The amount
usually added is 10 to 20%, and even less will suffice for laboratory
adapted strains of many species.

Two very simply prepared media, Difco Heart Infusion Broth or Albimi
Mycoplasma Broth Base to which 15 to 20% sterile serum is added, sup-
port the growth of many animal mycoplasmas. They appear to support
good growth of more species than unmodified Difco PPLO Broth supple-
mented with a like amount of serum. The latter medium is much im-
proved if 10% fresh yeast extract is added (as in Hayflick medium).

* Pfizer Diagnostics, Flushing, New York.
All of the above basal media are autoclave-sterilized and cooled to 50°C, after which serum is added (and fresh yeast extract where applicable). These media can be recommended as possible media for conducting a minimum isolation effort. Two factors should be considered in preparing these media that would probably be different from the preparation of other bacteriological media in a diagnostic laboratory. In most cases the media should be prepared with water that is suitable for cell-culture medium, fortunately a readily available item in most diagnostic laboratories today. Secondly, solid medium should be prepared with one of the agars which experience indicates is suitable for the mycoplasmas being sought. Difco Purified Agar*, though expensive, is much more suitable for growing mycoplasmas than is Difco Noble Agar*, although the latter if often recommended. Oxoid Ionagar No. 2** is an even better choice, at least when working with Mycoplasma hyopneumoniae and certain, if not all, of the T-strains. Most mycoplasma media are adjusted to a pH of about 7.5 or above, but a pH below 7.0 supports better growth of the T-strains and even some of the larger colony mycoplasmas. In media to be used for isolation purposes, it is possible to use a compromise pH of about 7.0 in order to obtain entirely adequate growth of both T-strains and classical mycoplasmas. Ten percent HCl or NaOH solutions are commonly used to adjust medium pH.

For those interested in preparing media that will grow the more fastidious animal mycoplasmas, a review of the recent literature dealing with the mycoplasma species being sought is suggested. Mycoplasma hyopneumoniae (also called M. suipneumoniae), for example, is recognized as being one of the animal mycoplasmas which is quite difficult to cultivate in artificial media. The diagnostician wanting to prepare a medium which would enable him to isolate this organism from infected pigs should consult references that deal with the subject in depth. Several other organisms have reputations for being unusually fastidious. Mycoplasma dispar* of cattle is one of these. It was isolated a number of times from cattle in Great Britain on a medium formulated to support growth of M. hyopneumoniae. Organisms which have the unusual colony appearance typical of M. dispar have been isolated in this country, but have not been positively identified. Mycoplasma synoviae of chickens and turkeys is also credited with being hard to isolate, but this is due partly to the fact that it requires the addition of reduced nicotinamide-adenine-dinucleotide (NAD or DPN) to the medium* and that it grows very poorly or not at all on media supplemented with horse serum. As the diagnostician gains experience in working with mycoplasmas he will likely want to try several different isolation media, the number and complexity depending on the amount of time available to him for working with mycoplasmas and the fastidiousness of the strains he is attempting to isolate. Some of

* Difco Labs., Detroit, Mich.
** Distrib. by Flow Laboratories, Rockville, Maryland
the more complex media that have been used successfully for isolating animal mycoplasmas include those described in papers by Ross and Karmon, Barber and Fabricant, and Frey, et al. A medium that has been used by a number of investigators for the isolation of animal T-strain mycoplasmas was described by Taylor-Robinson, et al.

It is necessary to have bacterial inhibitors in media to be inoculated with materials containing bacterial contaminants. Thallium acetate (1:2,000 to 1:10,000) and penicillin (100 to 1000 units/ml) in combination are most commonly used. Thallium acetate is inhibitory to T-strains and certain classical mycoplasmas, however.

Liquid media should be prepared every few weeks, or stored frozen. Solid medium should also be used within a few weeks (or preferably a few days) of preparation.

SERUM SUPPLEMENTS

As mentioned earlier, serum or another source of sterol is required for in vitro growth of all Mycoplasma spp. Horse serum has been most widely used in mycoplasma media in the past. Swine or rabbit serum may be even better choices for isolation of a wide range of animal mycoplasmas. Difco Serum Fraction A is of value in media used for antigen production with adapted strains but is not a good choice of supplement for isolation media. One must keep in mind that mycoplasma infection leads to the production of growth-inhibiting antibodies, so that untested serum may actually prevent growth of mycoplasmas originating in the host species from which the serum was obtained. Serum should be used while reasonably fresh (perhaps heat-inactivated or acid-treated) or stored frozen until shortly before use.

PREPARATION AND HANDLING OF INOCULUM

A convenient protocol to use in recovering mycoplasma infected tissues is outlined in table 1. A protocol of this nature helps to circumvent problems associated with mycoplasmacidal substances in tissue suspensions and the differences in growth rate among mycoplasma species. Inoculations should always be made as soon as possible after receipt of specimen, or the swab, tissue, or tissue suspension should be frozen, preferably at -30 C or below. At that temperature, mycoplasmas survive freezing in protein-containing material for months up to years. Swabs should be frozen in growth medium or other serum-containing solutions. At the higher temperatures of household-type freezers, survival time of most strains is shortened to several days up to a few months.

CONDITIONS OF INCUBATION

Anaerobic or microaerophilic incubation for the first day increases the isolation rate of certain strains of mycoplasmas without interfering with the growth of other strains, such as the T-mycoplasmas, which sometimes appear to be inhibited by continued anaerobic incubation. Carbon dioxide (5 to 10%) appears to be necessary for the growth of some
strains, irrespective of whether incubation is under aerobic or anaerobic conditions.

Agar plates should be incubated under conditions of high humidity to prevent drying of the medium surface during prolonged incubation. If a high humidity incubator or closed jar is not used, then the plates should be put in a small container such as a plastic bag, with a pledget of wet cotton.

IDENTIFICATION PROCEDURES

At present the problems associated with identification of animal mycoplasmas are sufficiently acute to discourage diagnosticians from attempting any isolation procedures. Hopefully diagnostic reagents for animal mycoplasmas will become much more readily available over the next few years. The most urgently needed reagent for mycoplasma identification is high quality hyper-immune serum to be used in the several serological procedures available.

The simplest identification procedure is a colony inhibition technique based on the method of Clyde, in which colony formation is inhibited around a filter paper disc containing homologous antiserum. Unfortunately identification by this method is not always possible due to strain differences within certain species. A growth-inhibition procedure used with broth cultures, immunofluorescence (IF), agglutination, gel diffusion, complement fixation, and other serological procedures have been used by researchers with the mycoplasmas. None of these procedures has been widely adopted by diagnosticians partly because some of them are of unsuitable specificity or are too laborious to perform, but primarily because they, like the colony inhibition procedures, require hard-to-obtain hyperimmune sera. Most researchers who have used IF as well as other serological techniques have eventually settled on one of the IF techniques as the mainstay of their identification protocol. Some of the prominent advantages of IF procedures are that (1) cloning of strains is not necessary and mixed cultures can be identified, and (2) only a few colonies are required rather than a large amount of antigen. Several authors have described methods for in situ staining of mycoplasma colonies with FITC conjugated antiserum. The technique described by Lehmkuhl reduces background fluorescence to a minimum and is very sparing of conjugated antiserum. The colonies are grown on thin layers of agar on glass slides (incubated in Petri dishes), which are then air-dried for several hours in an incubator or other low-heat source before staining. The thin film that remains after drying is not at all absorbent, which allows specifically fluorescing colonies to be seen more readily. Staining is accomplished in small plastic cylinders, use of which allows for staining with at least 8 conjugated antisera per slide.

The use of FITC-conjugated antiserum to rabbit globulin in an indirect IF procedure would save preparing conjugates of many antisera, but indirect IF procedures with mycoplasmas do not seem to have been very successful.
The use of only non-serological tests for identifying mycoplasmas is not recommended for diagnostic laboratories. There is too great a likelihood of more than one species being present in low passage cultures to place much reliance on the biochemical-biophysical characterizations, colony morphology observations and protein separation techniques. Also there are instances when low passage strains do not exhibit the biochemical reactions characteristic of higher passage strains of the same species. Biochemical tests, growth characteristics and colony morphology are sometimes reliable enough so that an experienced observer can make a tentative identification, however.

Two simple staining techniques are of value in identifying mycoplasma isolants. A widely used stain described by Dienes is of value in differentiating mycoplasma colonies from small or aberrant bacteria colonies, and a stain described by Shepard and Howard is used to verify small colony strains as being T-mycoplasmas. The latter procedure consists of combining 1 volume of 10% urea with 1 volume of 0.8% MnCl2 and adding a drop or two to the top of the colony. After a short delay, T-strain mycoplasma colonies turn a very dark brown.

For many laboratories a shortage of antiserum and/or time limits their observations to the tentative identification, but such identification is oftentimes of invaluable aid in making a diagnosis. The ability to make such identification is dependent on long experience, however. Such experience will not be wasted if diagnostic reagents later become available, because the experienced mycoplasmologist can limit to a minimum the number of antiserum required for positive identification.

It is easy to make a start in mycoplasma isolation work. Then, by gradual progression, all aspects of isolation and identification eventually seem easy. The start is the most important step.

REFERENCES
20. Standardized Methods of Veterinary Microbiology. To be published by National Academy of Sciences. Washington, D.C.,
TABLE 1
ISOLATION FROM TISSUES

1. Prepare 1/10 tissue suspension in culture medium.
2. Make further dilutions of 1/100 and 1/000.
3. Inoculate plates with all dilutions immediately, Incubate 1/100 and 1/1000 broth dilutions; freeze 1/10 dilutions.
4. Incubate all plates overnight anaerobically (with 5 to 10% CO2), then for several additional days in a CO2 incubator or candle jar.
5. As soon as colonies are discernible, cut out a block of agar containing part of the streaked area, put it in a tube of broth and refrigerate. Repeat at later times, adding new blocks to the same tube. Freeze for storage of 1 week or more.
6. Streak new plates with broth from step 3, after 1 to 2 and 3 to 5 days' incubation.
SUMMARIZING DATA ON BOVINE ABORTIONS WITH THE AID OF A COMPUTER

William T. Hubbert, D.V.M.*; Gordon D. Booth, Ph.D.**; and Wesson D. Bolton, D.V.M.**

Careful recording of all available facts in cases of bovine abortion was pointed out earlier as a basic tool in our search for cause and effect relationships. A simple method for recording pertinent data suitable for computer tabulation was used to evaluate available information on 3812 abortions which were examined in 5 northeastern diagnostic laboratories during 1960-1970. The above study was largely "retrospective" inasmuch as the data were gleaned from existing files. We are reporting here mainly "prospective" results obtained in the Vermont Laboratory during 1971-1972 at which time an attempt was made to collect appropriate data on each of 273 cases as they were submitted.

MATERIALS AND METHODS

History data were recorded on a summary form which was modified slightly from a form described earlier (Fig. 1). The earlier form did not provide space for multiple diagnoses. The coding for breed of dam was the same as used earlier. The coding for fetal death diagnoses was modified to permit recording of multiple diagnoses (Fig. 2). Whenever a case with multiple diagnoses was recorded in the computer tabulation, it was necessary to locate the particular summary form to tabulate the individual diagnoses (hand sorting). Otherwise, the data were tabulated as described earlier.

RESULTS AND DISCUSSION

Characteristics of the cows that aborted are summarized in Table 1. Characteristics of the aborted fetuses are summarized in Table 2. The bovine fetal death diagnoses which were recorded and the corresponding crown-rump lengths are summarized in Table 3.

The characteristics of the cows that aborted (Table 1) were similar to those reported from Vermont for the past decade. For every parameter except year and month of abortion, the statistics were not reported for approximately half of the cases. Information regarding type of breeding, previous pregnancies, etc., must come from the herd records and these data should be an integral part of the material submitted to the diagnostic laboratory for examination. In addition, data on the number and

*National Animal Disease Laboratory, North Central Region, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010
**Department of Animal Pathology, University of Vermont, Burlington, Vermont 05401.
sex of the fetuses aborted should be included if there were multiple fetuses and all were not submitted. In retrospect, the 5 northeastern laboratories found that the history data recorded was often incomplete.

To improve the quality and quantity of history data submitted, those utilizing the diagnostic laboratory must be informed of the precise information desired. Distribution of sample copies of the summary form (Fig. 1) with covering instructions to all concerned should provide a convenient checklist. Once the program is initiated, positive reinforcement of those contributing the data is needed to ensure continuous flow of data. One method of reinforcement is periodic (monthly, quarterly, annual) summary reports to all contributors with tabulations of all data submitted, not just laboratory findings. With careful planning and continued emphasis on the value of complete and accurate history data, a network of cooperators in the field who will take the time to submit the needed information can be developed.

The distribution of fetuses by number (single or multiple) and sex was similar to that reported previously (Table 2A). Although these were mainly "prospective" data, the high percentage of incomplete records on fetal number and sex again emphasizes the difficulties involved in establishing new habit patterns needed to collect these data.

Crown-rump length (CRL) and weight of the aborted fetuses in relation to observed gestation length generally correspond to expected values (Table 2B). Although a reliable estimate of gestational age can be derived from the weight of a normal bovine fetus, the weight of an aborted fetus may be affected by decomposition, dehydration, edema, or emaciation. Therefore, CRL should be used in conjunction with weight when estimating gestational age of fetuses which appear normal grossly and should be given greater reliance whenever apparent discrepancies arise. Also, the time of abortion is not fixed in relation to the time of fetal death and growth of the fetus may have been retarded before death. So, CRL can be used to estimate the time of fetal death, but is a less accurate indicator of gestation length at the time of abortion. Using CRL as an indicator, there was no significant seasonal relationship between gestation length and abortion. The mean varied from 38.2 (140-170 days) for August to 57.5 (200-230 days) for June (Table 2C).

Considerable differences in mean CRL were apparent between diagnoses (Table 3). Although the potential of this simple measurement as an aid in understanding the epidemiology of fetal disease is easily recognized, greater numbers are needed before more definitive conclusions can be drawn. It has been hypothesized that mummification occurs only in fetuses before approximately 200 days gestation corresponding to the time when unkeratinized skin is present with its greater permeability to water. Additional CRL measurements on mummies would help to test this hypothesis.

The method of summarizing abortion data which we have presented has the advantages of requiring a minimum of writing (all input through numerical coding) and a maximum of machine tabulation. A facility must
be available which can prepare computer punch cards and, of course, an arrangement with a computer center to process them. There are 11 computer centers nationwide serving the Dairy Herd Improvement Program which should be able to prepare handy "print outs" of the tabulations and other related calculations. At present, the method does not permit summaries or comparisons of diagnostic techniques used, types of specimens examined, or detailed pathologic descriptions. These and other disadvantages certainly can be overcome as our studies increase in complexity.

The organ weight summary (Figure 3) is an example of a method of gathering additional facts related to the aborted bovine fetus which is amenable to computer processing. Expected values for the organ weights have been published including values for the dried eye lens which were proposed as an additional guide to estimating gestational age of the fetus. Abnormal organ weights may be detected in a variety of fetal syndromes of infectious and noninfectious origin.

SUMMARY

Data on the characteristics of 273 Vermont cows that aborted during 1971-1972, the aborted fetuses, and the laboratory diagnoses relative to these abortions are summarized with the aid of a form designed for computer tabulation. Advantages and problems associated with introducing such a system into recording diagnostic laboratory data relative to bovine abortions are discussed. A method for recording fetal organ weights is presented.

REFERENCES

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<td>(02) Guernsey</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>(03) Jersey</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>(04) Ayrshire</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>(05) Brown Swiss</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(08) Aberdeen Angus</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>(09) Hereford</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(12) Charolais</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(16) Unknown</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td></td>
<td>273</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>G. Number of pregnancy</th>
<th>First</th>
<th>Fourth</th>
<th>Second</th>
<th>Seventh</th>
<th>Third</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>2</td>
<td>11</td>
<td>1</td>
<td>7</td>
<td>244</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>273</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>H. Number of living calves</th>
<th>None</th>
<th>One</th>
<th>Two</th>
<th>Three</th>
<th>Six</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>248</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>273</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>109</td>
</tr>
</tbody>
</table>
### TABLE 2

**Characteristics of the Aborted Fetuses**

**A. Number and sex**

<table>
<thead>
<tr>
<th></th>
<th>Single</th>
<th>Triplet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>51</td>
<td>Unknown</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td>Unknown</td>
</tr>
<tr>
<td>Unknown</td>
<td>133</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twin</td>
<td>3</td>
<td>2</td>
<td>34</td>
</tr>
</tbody>
</table>

**Total = 273**

**B. CRL - gestation length - fetus weight relationships**

<table>
<thead>
<tr>
<th>CRL (cm.)</th>
<th>Range</th>
<th>Median</th>
<th>No.</th>
<th>Gestation length (days)</th>
<th>Range</th>
<th>Median</th>
<th>No.</th>
<th>Fetus weight (gm.)</th>
<th>Range</th>
<th>Median</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4-3.7</td>
<td>2.4-20</td>
<td>3.7</td>
<td>3</td>
<td>45-90</td>
<td>65</td>
<td>65</td>
<td>3</td>
<td>50-340</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10.8-20.3</td>
<td>10.8-20</td>
<td>12.9</td>
<td>7</td>
<td>90-90</td>
<td>90</td>
<td>90</td>
<td>3</td>
<td>50-340</td>
<td>150</td>
<td>150</td>
<td>7</td>
</tr>
<tr>
<td>21.5-30.5</td>
<td>21.5-30</td>
<td>26.4</td>
<td>18</td>
<td>90-180</td>
<td>120</td>
<td>120</td>
<td>10</td>
<td>50-340</td>
<td>1021</td>
<td>1021</td>
<td>18</td>
</tr>
<tr>
<td>31.7-40.6</td>
<td>31.7-40</td>
<td>38.1</td>
<td>31</td>
<td>90-210</td>
<td>150</td>
<td>150</td>
<td>18</td>
<td>150-9535</td>
<td>2837</td>
<td>2837</td>
<td>31</td>
</tr>
<tr>
<td>41.8-50.8</td>
<td>41.8-50</td>
<td>45.7</td>
<td>27</td>
<td>90-210</td>
<td>180</td>
<td>180</td>
<td>13</td>
<td>150-9535</td>
<td>4880</td>
<td>4880</td>
<td>26</td>
</tr>
<tr>
<td>53.3-59.6</td>
<td>53.3-59</td>
<td>57.0</td>
<td>13</td>
<td>150-210</td>
<td>210</td>
<td>210</td>
<td>7</td>
<td>150-9535</td>
<td>10896</td>
<td>10896</td>
<td>13</td>
</tr>
<tr>
<td>61.0-70.0</td>
<td>61.0-70</td>
<td>63.5</td>
<td>20</td>
<td>150-240</td>
<td>210</td>
<td>210</td>
<td>16</td>
<td>150-9535</td>
<td>13847</td>
<td>13847</td>
<td>20</td>
</tr>
<tr>
<td>71.1-76.2</td>
<td>71.1-76</td>
<td>72.3</td>
<td>11</td>
<td>210-255</td>
<td>225</td>
<td>225</td>
<td>7</td>
<td>210-260</td>
<td>20430</td>
<td>20430</td>
<td>11</td>
</tr>
<tr>
<td>81.3-91.5</td>
<td>81.3-91</td>
<td>86.4</td>
<td>7</td>
<td>210-260</td>
<td>240</td>
<td>240</td>
<td>5</td>
<td>210-260</td>
<td>30872</td>
<td>30872</td>
<td>7</td>
</tr>
</tbody>
</table>

|        | 137   | 82     | 133 |

**C. Fetal crown-rump length (mean ± SE cm.) in relation to month of abortion**

<table>
<thead>
<tr>
<th>Month</th>
<th>No.</th>
<th>CRL</th>
<th>Month</th>
<th>No.</th>
<th>CRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>9</td>
<td>57.0 ± 5.5</td>
<td>Jul.</td>
<td>23</td>
<td>47.2 ± 6.6</td>
</tr>
<tr>
<td>Feb.</td>
<td>4</td>
<td>42.2 ± 5.5</td>
<td>Aug.</td>
<td>10</td>
<td>38.2 ± 2.3</td>
</tr>
<tr>
<td>Mar.</td>
<td>19</td>
<td>45.5 ± 4.2</td>
<td>Sep.</td>
<td>2</td>
<td>48.3</td>
</tr>
<tr>
<td>Apr.</td>
<td>8</td>
<td>50.2 ± 7.5</td>
<td>Oct.</td>
<td>11</td>
<td>40.1 ± 4.7</td>
</tr>
<tr>
<td>May</td>
<td>17</td>
<td>48.2 ± 3.9</td>
<td>Nov.</td>
<td>23</td>
<td>46.4 ± 4.2</td>
</tr>
<tr>
<td>Jun</td>
<td>7</td>
<td>57.5 ± 11.6</td>
<td>Dec.</td>
<td>15</td>
<td>47.6 ± 6.2</td>
</tr>
</tbody>
</table>

|       | 64   | 73     |      |
WITH THE AID OF A COMPUTER

#### TABLÈ 3
Bovine Fetal Death Diagnoses Recorded and Corresponding

Crown-Rump Lengths

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>No.</th>
<th>Crown-rump length mean ± S.E. (cm.)</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial diseases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(003) Corynebacterium sp.</td>
<td>14</td>
<td>51.6 ± 6.8</td>
<td>8</td>
</tr>
<tr>
<td>(004) Escherichia coli</td>
<td>1</td>
<td>26.6</td>
<td>1</td>
</tr>
<tr>
<td>(005) Leptospirosis</td>
<td>6</td>
<td>58.1 ± 5.1</td>
<td>4</td>
</tr>
<tr>
<td>(006) Listeriosis</td>
<td>4</td>
<td>50.1 ± 12.9</td>
<td>4</td>
</tr>
<tr>
<td>(012) Pseudomonas sp.</td>
<td>1</td>
<td>25.4</td>
<td>1</td>
</tr>
<tr>
<td>(014) Staphylococcosis</td>
<td>9</td>
<td>48.9 ± 15.4</td>
<td>4</td>
</tr>
<tr>
<td>(015) Streptococcosis</td>
<td>16</td>
<td>44.5 ± 4.2</td>
<td>10</td>
</tr>
<tr>
<td>(016) Vibriosis</td>
<td>2</td>
<td>21.5</td>
<td>1</td>
</tr>
<tr>
<td>(017) Other, single species</td>
<td>4</td>
<td>36.2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Mycotic diseases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(030) Aspergillosis</td>
<td>4</td>
<td>53.3 ± 2.5</td>
<td>3</td>
</tr>
<tr>
<td>(031) Candidiasis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(032) Mucormycosis</td>
<td>2</td>
<td>66.0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Viral diseases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(062) Bovine viral diarrhea (BVD)</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(063) Infectious bovine rhinotracheitis (IBR)</td>
<td>3</td>
<td>66.1 ± 17.8</td>
<td>3</td>
</tr>
<tr>
<td><strong>Noninfectious causes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(090) Traumatic</td>
<td>1</td>
<td>48.3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Clinical manifestation, cause unknown or unspecified</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(110) Clinical manifestation unspecified</td>
<td>175</td>
<td>47.0 ± 2.1</td>
<td>88</td>
</tr>
<tr>
<td>(112) Anomaly, unspecified</td>
<td>1</td>
<td>38.1</td>
<td>1</td>
</tr>
<tr>
<td>(114) Decomposed</td>
<td>16</td>
<td>34.1 ± 1.1</td>
<td>4</td>
</tr>
<tr>
<td>(120) Mummy</td>
<td>8</td>
<td>27.3 ± 4.4</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**SUMMARIZING DATA ON BOVINE ABORTIONS**

**FIGURE 1**

**BOVINE FETAL DEATH SUMMARY**

<table>
<thead>
<tr>
<th>Station/Year/Number</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>5</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STATION ACCESSION NO.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DAM'S HISTORY**

- **Type breeding**
  - 11 (1-AI, 2-natural, 3-both, 4-unk.)

- **Gestation (days)**
  - 25 26 27

- **No. services for this conception**
  - 12 13 (3-0 pasture, 3-1-unk.)

- **Month of last service**
  - 14 15 16 17 (month/year)

- **No. of pregnancy**
  - 29 30

- **No. living calves**
  - 32 33 (if unk., put 0)

- **Date aborted**
  - 19 20 22 23 (month/year)

**FETUS IDENTIFICATION**

- 1-single, 2-twin, 3-triplet, etc., 6 NUMBER UNKNOWN

- **Sex** (1-male; 2-female; 3-unknown; 4-twins, one of each sex)

- **5-other with 3 or more fetuses**

**Individual measurements**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Crown-rump length (cm.)*</th>
<th>Weight (to nearest gm.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>44 45 46</td>
<td>48 49 50 51 52</td>
</tr>
<tr>
<td>54</td>
<td>56 57 58</td>
<td>60 61 62 63 64</td>
</tr>
<tr>
<td>66</td>
<td>68 69 70</td>
<td>72 73 74 75 76</td>
</tr>
</tbody>
</table>

**DIAGNOSIS** (Use number from list. If multiple diagnoses, insert 0, 1, 2, 3 at left and put individual diagnoses below.)

---

*Use chart to convert from inches and pounds.*
### FIGURE 2

**Bovine Fetal Death Diagnoses**

<table>
<thead>
<tr>
<th>Infectious Causes</th>
<th>Myotic Diseases</th>
<th>Parasitic Diseases</th>
<th>Viral Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial Diseases</strong></td>
<td><strong>Mycotic Diseases</strong></td>
<td><strong>Parasitic Diseases</strong></td>
<td><strong>Viral Diseases</strong></td>
</tr>
<tr>
<td>001 Brucellosis</td>
<td>030 Aspergillosis</td>
<td>045 Toxoplasmosis</td>
<td>060 Bluetongue (BT)</td>
</tr>
<tr>
<td>002 Chlamydiosis</td>
<td>031 Candidiasis</td>
<td>046 Trichomoniasis</td>
<td>061 Bovine enteric (REV)</td>
</tr>
<tr>
<td>(epizootic Bovine Abortion)</td>
<td>032 Mucormycosis</td>
<td>047 Trypanosomiasis</td>
<td>062 Bovine virus diarrhea (BVD)</td>
</tr>
<tr>
<td>003 Corynebacterium sp.</td>
<td>033 Other, single species</td>
<td>048 Other, metazoan</td>
<td></td>
</tr>
<tr>
<td>004 Escherichia coli</td>
<td>034 Other, multiple species</td>
<td>049 Other, protozoan</td>
<td>063 Infectious bovine rhinotracheitis (IBR)</td>
</tr>
<tr>
<td>005 Leptospirosis</td>
<td>035</td>
<td></td>
<td>064 Malignant catarrhal fever (MCF)</td>
</tr>
<tr>
<td>006 Listerials</td>
<td>036</td>
<td></td>
<td>065 Parainfluenza-3 (PI-3)</td>
</tr>
<tr>
<td>007 Mycoplasma sp.</td>
<td>037</td>
<td></td>
<td>066 Other</td>
</tr>
<tr>
<td>008 Mycoplasmosis</td>
<td>038</td>
<td></td>
<td></td>
</tr>
<tr>
<td>009 Nocardiosis</td>
<td>039</td>
<td></td>
<td></td>
</tr>
<tr>
<td>010 Pasteurella sp.</td>
<td>040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>011 Proteus sp.</td>
<td>041</td>
<td></td>
<td></td>
</tr>
<tr>
<td>012 Pseudomonas sp.</td>
<td>042</td>
<td></td>
<td></td>
</tr>
<tr>
<td>013 Salmonellosis</td>
<td>043</td>
<td></td>
<td></td>
</tr>
<tr>
<td>014 Staphylococcosis</td>
<td>044</td>
<td></td>
<td></td>
</tr>
<tr>
<td>015 Streptococcosis</td>
<td>045</td>
<td></td>
<td></td>
</tr>
<tr>
<td>016 Vibriosis</td>
<td>046</td>
<td></td>
<td></td>
</tr>
<tr>
<td>017 Other, single species</td>
<td>047</td>
<td></td>
<td></td>
</tr>
<tr>
<td>018 Other, multiple bacterial species</td>
<td>048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>019 Other, bacterial and mycotic species</td>
<td>049</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Noninfectious Cause Known to Be:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>080 Genetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>090 Traumatic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 Chemical (toxic)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Clinical Manifestation with Cause Unknown or Unspecified**

<table>
<thead>
<tr>
<th>Cause Unknown, Clinical Manifestations Unspecified</th>
</tr>
</thead>
<tbody>
<tr>
<td>110 Cause Unknown, Clinical Manifestations Unspecified</td>
</tr>
<tr>
<td>111 Anomaly, cardiac</td>
</tr>
<tr>
<td>112 Anomaly, unspecified</td>
</tr>
<tr>
<td>113 Anoxia</td>
</tr>
</tbody>
</table>
### FIGURE 3

**BOVINE FETAL ORGAN WEIGHTS (gms.)**

<table>
<thead>
<tr>
<th>Station/Year/Number</th>
<th>STATION ACCESSION NO.</th>
</tr>
</thead>
</table>

#### ABDOMINAL ORGANS

- Adrenals (both)  
  - 11 12 13
- Kidneys (both)  
  - 15 16 17 18
- Liver  
  - 20 21 22 23 24
- Spleen  
  - 26 27 28 29

#### CERVICAL, CRANIAL, AND THORACIC ORGANS

- Brain (total)  
  - 31 32 33 34
- Rhombencephalon*  
  - 36 37 38
- Heart  
  - 40 41 42 43
- Lungs  
  - 45 46 47 48 49
- Thymus  
  - 51 52 53 54
- Thyroid  
  - 56 57 58
- Lens, both, wet  
  - 60 61 62 63
- Lens, both, dry**  
  - 65 66 67 68

*Cerebellum, pons, and medulla oblongata.

**>48 hrs. at 80°C.
The economic impact of abortion in beef and dairy cattle cannot be overemphasized. Knowledge of the causes of abortion is a required step in establishing control measures. With these goals in mind, personnel at the South Dakota Animal Disease Research and Diagnostic Laboratory encouraged veterinarians in South Dakota and bordering states to submit specimens of bovine abortions and stillbirths for laboratory examination.

MATERIALS AND METHODS

Specimens examined, laboratory procedures applied to these specimens, and the criteria for diagnoses have been described (Kirkbride et al.).

RESULTS

Specimens of fetal tissues, placentas and entire fetuses from 3692 cases of bovine abortion and stillbirth were examined. The results for each year are given in Table 1. The relative yearly incidence of the infectious causes of abortion is shown in Figure 1.

Infectious bovine rhinotracheitis (IBR) virus infections accounted for almost 38% of all diagnoses (531/1402). In 47 cases, aborting cows had serum antibody titers of 1:1000 or greater for leptospirosis and these were arbitrarily diagnosed as leptospiral abortion. Gross and microscopic lesions consistent with epizootic bovine abortion were present in 45 cases. Miscellaneous bacteria were associated with 104 cases (Table 2). Congenital anomalies were observed in 155 fetuses (Table 3), and circumstantial evidence of dystocia was detected in 31 cases. *Trichomonas fetus* was isolated from the abomasal content of 1 fetus.

The percentage of diagnosed cases varied each period: 23.3% (1968-69), 23.5% (1969-70), 43% (1970-71), 37.5% (1971-72 and 43.8% (1972-73). The cause of abortion or stillbirth was determined in 1402 cases (37.97%) over the 5-year period.

DISCUSSION

The known or suspected causes of abortion are infection, hereditary factors, teratogenesis, malnutrition, hormonal imbalances, en-
vironmental factors and toxins. Infection was associated with nearly 87% of the cases diagnosed in this survey. The inability to demonstrate lesions (gross or microscopic) or infectious agents in 2290 cases (62% of examined cases) indicates that a high percentage of abortions may be caused by factors other than infection. However, leptospirosis is an example of an infection known to cause abortion but leave no consistent lesions, and the causative organism can seldom be demonstrated in the fetus or placenta. There may be other such infections.

IBR virus was the most commonly diagnosed cause of abortion each year of the survey, with the highest incidence during the 1970-71 period (24.4% of all cases examined). The percentage of abortions diagnosed as mycotic placentitis during the 1972-73 period was almost double that of any other year (Fig. 1). Unusually heavy rains (25.35 in. vs 18.63 in. normal rainfall, Brookings, South Dakota) during the summer and fall of 1972 may have affected the quality of hay and silage crops and increased the number of fungal spores in these feeds. This factor appears to be associated with an increase in the number of mycotic abortions. This rainy season may also have been a factor in the increase in percentage of abortions diagnosed as *Listeria monocytogenes* or leptospiral infections.

No changes were made in the procedures for diagnosing listeria abortions throughout the survey but the percentage of listeria abortions diagnosed each year varied from 0% (1969-1970, and 1970-1971) to 3.5% (40 diagnoses, 1972-1973) of all cases. The epizootiologic factors involved in the occurrence of this infection are unknown.

Since December 1972, *Leptospira hardjo* antigen along with *L. pomona*, *grippotyphosa*, *icterohemorrhagiae* and *canicola* antigens, has been used routinely in the microagglutination (MA) test at the South Dakota laboratory. Until that time, *L. pomona*, *icterohemorrhagiae* and *canicola* antigens were used routinely, and others only on specific request of the attending veterinarian. The number of bovine abortion cases in which the dam had a leptospira MA titer of 1:1000 or greater at the time of abortion is given in Table 4. In 1972-73 there were 31 such cases, 19 (61.3%) of which were *L. hardjo*.

Because testing bovine sera for *L. hardjo* antibodies had been done on only a few cattle prior to 1972-73, no comparisons can be made as to the relative incidence of leptospiral abortions from year to year. The 12 cases in which the aborting cow had a titer 1:1000 or greater to *L. pomona* represents 2.1% of the total of 584 diagnoses made in 1972-73. This compares closely to the incidence of leptospiral abortions in previous years and suggests that the apparent increase in leptospiral abortions in 1972-73 was due to the change in diagnostic procedure rather than the climatic conditions.

Blood from the aborting cow was submitted in only 244 (21.3%) of the 1148 cases in 1972-73. Of these, 31 (12.7%) were positive at a dilution of 1:1000 or greater. Interpolation of these figures reveals that if dam's blood were submitted in every abortion case the number of leptospiral abortion diagnoses could be 4 to 5 times the 47 made in 5 years.
Bovine abortion is a challenge for practicing veterinarians and livestock producers. It remains an even greater challenge for diagnosticians. Diagnostic laboratory personnel have the knowledge and techniques to identify most of the known infectious causes of abortion, if proper specimens are submitted for examination. Noninfectious causes of abortion remain a vast field to explore and conquer.

REFERENCES
TABLE 1--Results of Examination of 3692 Bovine Abortion and Stillbirth Specimens in 5 Years.*

<table>
<thead>
<tr>
<th>Diagnosis or agent</th>
<th>1968-69</th>
<th>1969-70</th>
<th>1970-71</th>
<th>1971-72</th>
<th>1972-73</th>
<th>Total</th>
<th>% of all diagnoses</th>
<th>% of all cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBR virus</td>
<td>34</td>
<td>47</td>
<td>197</td>
<td>126</td>
<td>127</td>
<td>531</td>
<td>37.88</td>
<td>14.38</td>
</tr>
<tr>
<td>Fungus</td>
<td>8</td>
<td>16</td>
<td>36</td>
<td>10</td>
<td>88</td>
<td>178</td>
<td>12.70</td>
<td>4.82</td>
</tr>
<tr>
<td>Vibriosis</td>
<td>8</td>
<td>21</td>
<td>20</td>
<td>28</td>
<td>39</td>
<td>116</td>
<td>8.27</td>
<td>3.15</td>
</tr>
<tr>
<td>C. pyogenes</td>
<td>2</td>
<td>9</td>
<td>11</td>
<td>46</td>
<td>48</td>
<td>116</td>
<td>8.27</td>
<td>3.14</td>
</tr>
<tr>
<td>Listeriosis</td>
<td>2</td>
<td>0</td>
<td>14</td>
<td>40</td>
<td>56</td>
<td>116</td>
<td>4.00</td>
<td>1.42</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>10</td>
<td>31</td>
<td>47</td>
<td>3.35</td>
<td>1.12</td>
</tr>
<tr>
<td>EBA</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>9</td>
<td>23</td>
<td>45</td>
<td>3.21</td>
<td>1.22</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>20</td>
<td>1.43</td>
<td>0.54</td>
</tr>
<tr>
<td>BVD-MD virus</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0.21</td>
<td>0.08</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>2</td>
<td>7</td>
<td>21</td>
<td>41</td>
<td>33</td>
<td>104</td>
<td>7.42</td>
<td>2.82</td>
</tr>
<tr>
<td>bacteria**</td>
<td>4</td>
<td>7</td>
<td>36</td>
<td>48</td>
<td>60</td>
<td>155</td>
<td>11.05</td>
<td>4.20</td>
</tr>
<tr>
<td>Anomalies**</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>12</td>
<td>9</td>
<td>31</td>
<td>2.11</td>
<td>0.84</td>
</tr>
<tr>
<td>Undetermined</td>
<td>211</td>
<td>357</td>
<td>460</td>
<td>617</td>
<td>645</td>
<td>2390</td>
<td>---</td>
<td>62.01</td>
</tr>
<tr>
<td>TOTAL</td>
<td>275</td>
<td>473</td>
<td>808</td>
<td>988</td>
<td>1148</td>
<td>3692</td>
<td>---</td>
<td>100.00</td>
</tr>
</tbody>
</table>

*From July 1 to June 30 of each period. **See Tables 2 and 3.

TABLE 4--The Number of Bovine Abortion Cases in which the Dam had a Microagglutination Titer 1:1000 or Greater at the Time of Abortion.

<table>
<thead>
<tr>
<th>Year</th>
<th>Pomona</th>
<th>Ictero</th>
<th>Hardjo*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>(July 1 through June 30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1968-69</td>
<td>1</td>
<td>--</td>
<td>--</td>
<td>1</td>
</tr>
<tr>
<td>1969-70</td>
<td>2</td>
<td>1</td>
<td>--</td>
<td>3</td>
</tr>
<tr>
<td>1970-71</td>
<td>2</td>
<td>--</td>
<td>--</td>
<td>2</td>
</tr>
<tr>
<td>1971-72</td>
<td>8</td>
<td>2</td>
<td>--</td>
<td>10</td>
</tr>
<tr>
<td>1972-73</td>
<td>12</td>
<td>--</td>
<td>19</td>
<td>31</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25</td>
<td>3</td>
<td>19</td>
<td>47</td>
</tr>
</tbody>
</table>

*Prior to December, 1972, L. hardjo antigen was used only on specific request of the submitting practitioner.
RESULTS OF A FIVE-YEAR SURVEY

Fig. 1  Diagnoses of causes of bovine abortion expressed as a % of the total number of cases examined over a 5 year period.

TABLE 2—Miscellaneous Bacteria Associated with Bovine Abortion (3692 cases examined during a 5-year period).

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>TOTAL</th>
<th>BACTERIA</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus sp.</td>
<td>38</td>
<td>Serratia marcescens</td>
<td>4</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>22</td>
<td>Aeromonas hydrophila</td>
<td>2</td>
</tr>
<tr>
<td>Streptococcus,</td>
<td>14</td>
<td>Nocardia asteroides</td>
<td>1</td>
</tr>
<tr>
<td>beta hemolytic</td>
<td></td>
<td>Salmonella typhimurium</td>
<td>1</td>
</tr>
<tr>
<td>Pasteurella sp.</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5</td>
<td>Staphylococcus epidermidis</td>
<td>1</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>4</td>
<td>TOTAL</td>
<td>104</td>
</tr>
</tbody>
</table>
TABLE 3—Congenital Anomalies Found in Examining 3692 Bovine Abortion and Stillbirth Specimens in 5 Years.

<table>
<thead>
<tr>
<th>ANOMALY</th>
<th>TOTAL</th>
<th>ANOMALY</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocephalus</td>
<td>80</td>
<td>Nephroblastoma</td>
<td>3</td>
</tr>
<tr>
<td>Osteopetrosis</td>
<td>15</td>
<td>Ectopia cordis</td>
<td>3</td>
</tr>
<tr>
<td>Arthrogryposis</td>
<td>14</td>
<td>Achondroplasia</td>
<td>2</td>
</tr>
<tr>
<td>Hypotrichosis</td>
<td>4</td>
<td>Anencephalia</td>
<td>2</td>
</tr>
<tr>
<td>Kyphoscoliosis</td>
<td>3</td>
<td>Other</td>
<td>26</td>
</tr>
<tr>
<td>Lymphomatosis</td>
<td>3</td>
<td>TOTAL</td>
<td>155</td>
</tr>
</tbody>
</table>
ADVANCES IN THE DIAGNOSIS OF BOVINE ABORTION

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SUMMARY

The use of serological tests on the serums of 170 aborted bovine fetuses for the detection of parainfluenza-3 (PI3) and 8 bovine enterovirus serotypes (BES) increased the possible total diagnoses due to all causes from 29 to 85% in 1972 and from 34.3 to 91% in 1973. Parainfluenza-3 antibodies occurred in 53% and 60% of 1972 and 1973 diagnoses respectively. The antibodies were detected in various combinations with infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD), bacterial infections and with each other. Often evidence was presented to verify that antibodies were of fetal origin. The significance of fetal infection with PI3 and BES as factors in abortion may be questioned in light of incomplete evaluation of all viral and toxic factors. However, the knowledge that intrauterine infection has taken place lends support to a viral diagnosis where other more pathogenic factors cannot be identified.

The diagnosis of bovine abortion has been a frustrating responsibility for the laboratory diagnosticians as well as for the practitioners in the field. Various estimates on the number of positive diagnoses arising from laboratory examinations have ranged from approximately 25 to 35% of the fetuses submitted.

Studies on the ability of the fetus to develop its own antibody in response to antigenic stimulation are based on work done by others (Osburn, 1973) and in our own laboratory. Infectious bovine rhinotracheitis (IBR) and parainfluenza-3 (PI3) can be successfully de-
detected in nasal and tracheal smears taken on the farm, fixed in acetone for 10 minutes, and examined in the laboratory using the fluorescent antibody smear technique (FAST). They can also be detected in tissue sections of lung or placenta by fluorescent antibody tissue section technique (FATST). We also test tonsillar tissue for bovine virus diarrhea (BVD) both by FATST and fluorescent antibody cell culture technique (FACCT). The paper to be presented here is based largely upon the ability of the fetus to produce antibodies to specific antigens, particularly to PI3 and to bovine enterovirus serotypes (BES) as well as to BVD and IBR.

METHODS

The work reported here includes only the advances made in the use of fetal serology in the diagnosis of bovine abortion. Time does not permit the coverage of all aspects of bovine abortion. As is customary in most laboratories, our diagnosticians record vaccinations in the herd, repeat breeding, illness in the herd, illness in the cow since it was bred, the number of abortions in the herd and whether or not it was artificial or natural breeding. They also record twinning and fetal crown rump length, weight, sex and gestational age.

A bacterial examination is made of the fetus following necropsy and the gross pathologic findings recorded. Stomach contents are submitted to darkfield examination for leptospira and vibrio and are cultured both aerobically and anaerobically. Cultures are also made for the detection of mycoplasma and for fungi when placenta is submitted. We no longer continue egg inoculations because in 3 years we detected no chlamydia or mycoplasma. It is recommended however, that where chlamydia are suspected to be a problem, egg inoculations should be done in conjunction with other examinations.

RESULTS AND DISCUSSION

We previously reported on attempts to diagnose 100 cases of bovine abortion with and without the use of fetal serology. In that report we indicated 27% positive diagnoses and 73% negative diagnoses without the use of fetal serology. In 1973 to date, we have examined 70 aborted fetuses with a total of 34.3% diagnoses without fetal serology (Table 1). In the 1972 data however, (Table 2) PI3 antibodies were found in the fetal fluids in 53 fetuses either occurring alone or with antibodies to other viruses. Antibodies to enterovirus serotypes occurred in the fluids of 41 fetuses. PI3 antibodies occurred alone in only 24 cases but were widely associated with a large variety of other agents including IBR, BVD, enteroviruses and several bacterial organisms. In 1973, PI3 occurred in the fluids of 60% of 70 fetuses and bovine enteroviruses in 60%, in varying combinations in different fetuses. Of 70 abortions, either PI3 or BES antibodies or both were detected in 57 or 81.4% of the fetuses (Table 3). In Table 4, it can be seen that BES 2, 6, and 8 are the enterovirus serotypes which occurred most frequently in the combined 1972-73 totals. But, it will also be noted that these particular serotypes were not necessarily
consistently high in occurrence in both years. Since many of the abortions which were sent to our Laboratory originated from the same herds, it is quite possible that some degree of immunity was being established against individual strains. A number of diagnoses were included in the reports for 1973 in which evidence of virus infection to different viruses was demonstrated. The diagnoses of suffocation, nutrition, myositis, and hepatomegaly were regarded generally as nonspecific diagnoses and the possible virus etiology considered a logical substitution in their place. Hydrocephalus and steroid therapy were considered significant and the diagnosis maintained. In general, the evidence of viral infection in 1972 and 1973 were quite comparable although there were a slightly higher number of fetuses with antibodies to PI3 and enteroviruses in 1973 over 1972. Utilizing the fetal serology in the diagnosis, the total numbers of diagnoses can be markedly expanded from a possible 27% in 1972 and 34.3% in 1973 to a total of 83% in 1972 and 88.2% in 1973. The grand total of possible diagnoses of abortion was 85% for 1972 and 91% for 1973.

A question invariably is raised with respect to the pathogenicity of the enteroviruses for the fetus. Experimentally, enteroviruses have caused death of chicken embryos (Kunin, 1958). Certain changes in livers of chicken embryos were readily identifiable as lesions caused by the virus (Dunne et al., 1973b). Fetal death and abortion from bovine enterovirus infection in guinea pigs was demonstrated by Moll (1964), by Van Der Maaten and Packer (1967) and confirmed in this laboratory. In studies on the fetal immune response by Schultz et al. (1973) and Dunne et al. (1973a), it was shown that PI3 antibodies and enterovirus antibodies began to develop in the fetus at about 120 days of gestation, whereas, BVD antibodies begin to be detectable between 135 to 145 days. Osburn (1973) cited the development of antibodies to Vibrio fetus, Chlamydia sp. and E. coli at 233 to 245 days of gestation. There are no antibodies recorded for fetuses which were aborted before 4 months of age, further suggesting that antibodies which occurred in fetuses later in gestation were of fetal origin rather than of maternal origin. Antibody production in the fetus was evident in certain tissues of the fetus particularly in the spleen by the appearance of antibody containing cells, morphologically identical with those cells known to have antibody producing capabilities. Also, experiments have been conducted in our laboratory which definitely indicate that dams have antibodies to many more viruses than to their aborted fetuses. This limited antibody profile suggests strongly that the fetus produces its own antibody. This is further verified by the fact that if the calf received colostrum, the antibody profile of its serum was essentially identical with that of serum of the dam. Also, if the serum of the fetus was treated with 2-mercaptopoethanol (2-ME), the greater proportion of the antibody titer disappeared indicating that the antibody present was IgM rather than IgG (Dunne et al., 1973a). The fetus is capable of producing detectable IgG antibody but only if the infection has persisted for at least 3 weeks after the capability of producing antibody was achieved. If there had been leakage of serum from the dam across the
placental barrier to the fetus, fetal serum would then have an antibody profile similar to that of the dam, and to that of the calf which had received colostrum. Other convincing evidence that the fetus actively produces antibody is the infrequent occurrence of BVD antibodies in aborted fetuses. It is well recognized that Pennsylvania herds have a relatively high incidence of BVD. Yet in the fetuses examined in our laboratory, less than 3% of the fetuses had antibodies to BVD. In a herd which is known to have the high incidence of BVD, PI3, and BES, sera were taken from 8 normal calves before they had received colostrum. Two of the 8 normal calves had antibodies to BVD whereas 4 of the dams were positive. None of the 8 fetuses had antibodies to PI3 whereas all of the dams were positive (Table 5). Seven of 8 bovine fetuses had antibodies to an average of 1.7 strains of BES whereas there were antibodies to an average of 6.1 strains in the dam. One fetus was negative for antibodies to all viruses tested.

Submission of Materials for Diagnoses.—For the most complete diagnosis, the submission of the fetus and the placenta plus a blood sample from the dam provides the greatest chance of success. Where it is not possible to submit the fetus directly to the laboratory soon after abortion, it is suggested that the fetus with the placenta be frozen and a serum sample acquired from the dam. If the serum from the dam is to be frozen, the clot should be removed prior to freezing. If it is impossible to submit the entire fetus, some information can be gathered from the fetus by submitting fetal fluids and a blood sample from the dam. It must be emphasized that freezing kills certain bacteria, particularly Vibrio and to a certain extent Leptospira. Freezing also minimizes the effectiveness of micropathology and hemolizes red blood cells and fluids to be used for serology. Fluorescent antibody tests on cryostat sections and some bacteriological studies can be made on frozen materials.

If fetal fluids are to be submitted for serology, it is suggested that they be sent only from those fetuses which are 4 months of age or older. Four fluids are useable in fetal serology. Three of these may be pooled: the abdominal, thoracic and pericardial fluids which usually contain few red blood cells. Heart blood, on the other hand, contains a lot of red blood cells and since it usually does not clot readily, the red cells can only be separated by centrifugation or static sedimentation. If this is not feasible, it is suggested that the heart blood be kept separately. Stomach fluid is desirable for the isolation of bacteria, although it is recognized that certain organisms can be swallowed by the calf during its passage through the birth canal. All of the fluids should be chilled and shipped cold either in the presence of an ice pack or frozen and shipped frozen in a well insulated container.

The tubes to be used for shipping fetal fluids should be ones which can be sealed easily and from which the fluid is not likely to leak during shipment. Sterile blood tubes are useable but the original rubber stoppers usually have a hole where the bleeding needle went in and when shipped by air the fluid can be forced out of the tubes and lost. One of the best
systems is to use disposable syringes. The needle guard can be replaced on the needle after the sample has been taken and the container, syringe and all shipped in the package. It is even better if a cork is placed on the needle rather than the guard. If there is air in the syringe, the expansion and contraction due to air shipment will have little effect and the sample invariably arrives intact. It is desirable to have about 5 ml. of fluid, but 10 is better. The greater amount enhances the chance for further work if such additional effort should be deemed necessary.

The interpretation of the results from viral tests should be weighed carefully. The FATCT for the presence of the virus in fetuses most frequently is negative in aborted fetuses except for IBR which causes abortions shortly after infection of the dam. It must be emphasized, however, that a negative test does not mean that viruses were not involved. It simply means that no virus was present. A negative FATCT may be followed by a positive serology report, because the virus usually disappears before abortion. Neutralizing antibodies which are present have reduced the infectivity of the virus but not until enough damage has been done to either the fetus or placenta to cause the abortion. Under such circumstances, the virus cannot be detected by FATCT, but antibodies to the virus can be demonstrated by serology. The question has also been raised as to what constitutes a positive and a negative titer. In our laboratory a positive hemagglutination-inhibition (HI) titer to PI3 in the fetus is 1:20. In the adult however, we consider 1:20 as suspicious. This is because there is less environmental contact and less cross-reacting factors associated with the fetus than are associated with the mature individual. Also, the fetus being at an early stage of producing antibody is very likely to have a low titer to the infecting agent. Even so, more than 65% of fetal titers for PI3 were 1:40 or higher. Serum neutralization (SN) titers for BES, BVD, and IBR are considered positive at a much lower dilution than the HI test which is quite sensitive. An SN titer of 1:6 for all viruses is considered to be positive in both the fetus and the adult.

Tests vary greatly in the time required to conduct them. Certain tests such as the FATST and HI tests can be done rather quickly. The results should be returned to the owner within the period of about 1 week or less depending upon the circumstances which tends to compound the problem but this may be shortened some by the use of microtiter system to which we are now adapting. It takes from 2 to 6 weeks to conduct serum neutralization tests.

In conclusion, the use of fetal serology offers reasonably positive information that the fetus has been infected with specific agents during its gestational period. The possibilities of maternal antibody leakage and cross reactions are recognized but appear to have minimum influence. While it is known that even normal calves may be born with antibodies to such infectious agents and that viruses other than those tested may be involved in the process of abortion, the results do strongly suggest that the agents identified may have had either a primary or secondary role in the process of abortion. The resulting information provided by these tests
does at least give the practitioner some chance to explain the cause of the abortion in a far greater number of cases than before, even though measures are not yet adequate to prevent infection.

REFERENCES

2. Dunne, H. W., Huang, C. M., Lin Whei Jun: (1973a) Bovine Enteroviruses in the Calf: An Attempt at Serologic, Biologic and Pathologic Classification. Accepted for publication. J.A.V.M.A.
## Table 1

**Diagnosis of Abortion in Cattle**

<table>
<thead>
<tr>
<th>Year</th>
<th>1972</th>
<th>1973</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Fetuses Examined</strong></td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>Infectious Bovine Rhinotracheitis (FAST and FATST)</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Bovine Virus Diarrhea (FATST and FACCT)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Bacterial Infection</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Mycotic Infection</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Noninfectious Causes</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>27 (27%)</td>
<td>24 (34.3%)</td>
</tr>
</tbody>
</table>

**FAST** - Fluorescent antibody technique  
**FATST** - Fluorescent antibody tissue section technique  
**FACCT** - Fluorescent antibody cell culture technique

## Table 2

**Evidence of Viral Infection in Aborted Bovine Fetuses Using Fluorescent Antibody Smear, Tissue Section and Cell Culture Techniques (FAST), (FATST), (FACCT)**

<table>
<thead>
<tr>
<th></th>
<th>1972 (100 Fetuses)</th>
<th>1973 (70 Fetuses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBR (8 FACCT) (2 Serology) (Alone or with others)</td>
<td>10%</td>
<td>11.4%</td>
</tr>
<tr>
<td>BVD (1 FACCT) (1 Serology) (With others)</td>
<td>2%</td>
<td>2.8%</td>
</tr>
<tr>
<td>PI3 Antibody (Alone or with others)</td>
<td>53%</td>
<td>60.0%</td>
</tr>
<tr>
<td>Enterovirus Antibody, 8 Serotypes (1 or more alone or with others)</td>
<td>41%</td>
<td>60.0%</td>
</tr>
</tbody>
</table>

**IBR** - Infectious bovine rhinotracheitis  
**BVD** - Bovine virus diarrhea  
**PI3** - Parainfluenza-3
TABLE 3
Distribution of PI3 and BES antibodies in the Body Fluids of 70 Aborted Bovine Fetuses

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3 + Bacterial Infection</td>
<td>6</td>
</tr>
<tr>
<td>+ Noninfectious Causes</td>
<td>2</td>
</tr>
<tr>
<td>PI3 Alone</td>
<td>10</td>
</tr>
<tr>
<td>+ IBR</td>
<td>2</td>
</tr>
<tr>
<td>+ IBR + BES (1 or more)</td>
<td>4</td>
</tr>
<tr>
<td>+ BVD + BES</td>
<td>1</td>
</tr>
<tr>
<td>+ BES (1 or more)</td>
<td>17</td>
</tr>
<tr>
<td>BES + IBR</td>
<td>1</td>
</tr>
<tr>
<td>BES Alone (1 or more)</td>
<td>14</td>
</tr>
<tr>
<td><strong>Total Fetuses with PI3, BES Antibodies</strong></td>
<td><strong>57 (81.4%)</strong></td>
</tr>
</tbody>
</table>

PI3 - Parainfluenza-3  
IBR - Infectious Bovine rhinotracheitis  
BVD - Bovine virus diarrhea  
BES - Bovine enterovirus serotype

TABLE 4
Parainfluenza-3 (PI3) and Bovine Enterovirus Serotype (BES) Antibodies in Aborted Fetuses with No Other Evidence of Infection

<table>
<thead>
<tr>
<th></th>
<th>1972 (15/100)</th>
<th>1973 (13/100)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3 + BES 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
## TABLE 5
Antibody Detection in the Serum of Normal Calves Taken Before Receiving Colostrum

<table>
<thead>
<tr>
<th>Viruses</th>
<th>No. of Fetuses Reacting Over Number Tested</th>
<th>Reaction in Dams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine Virus Diarrhea (BVD)</td>
<td>2/8</td>
<td>4 positive</td>
</tr>
<tr>
<td>Parainfluenza-3 (PI3)</td>
<td>0/8</td>
<td>all positive</td>
</tr>
<tr>
<td>8 Bovine Enterovirus Serotypes (BES)</td>
<td>7/8*</td>
<td>all positive**</td>
</tr>
</tbody>
</table>

* - Ave. 1.7 strains, 1 negative to all viruses tested  
** - Ave. 6.1 strains  
BVD and BES — 1:6 Positive SN titer  
PI3 — 1:20 Positive in fetus, 1:40 Positive in calves at 6 months or older and adult cattle.
BLUETONGUE VIRUS IN CATTLE: COMPLEMENT FIXING ANTIBODY RESPONSE AND VIREMIA IN EXPERIMENTALLY INFECTED ANIMALS

From Veterinary Services Diagnostic Laboratory
APHIS, USDA, P.O. Box 70, Ames, Iowa 50010

ACKNOWLEDGEMENTS
The authors thank Mr. Ralph M. Glazier and his staff for assistance in preparation of the illustrations and Mr. Charles Graham for statistical evaluation of the results.

Bluetongue is considered primarily a disease of sheep, however, cattle have been incriminated as inapparent carriers of the disease. Bekker et. al. recovered the virus from a natural infection of cattle. Du Toit repeatedly isolated bluetongue (BT) virus from 8 cattle that were naturally exposed. The isolations were all made during the summer months. The same strain of BT virus was isolated from one for 3 months. Nevill also found almost 100% bluetongue infection in cattle sampled, but only in the summer months.

Bluetongue infected cattle develop only minor clinical signs. Most animals have only a temporary loss of appetite, a slight temperature rise, and leukopenia.

The significance of cattle as a reservoir of bluetongue is dependent on length of the viremia. Bowne et. al. isolated the virus by sheep inoculation from 7 of 7 calves at PID 49. Luedke et. al. reported isolating bluetongue virus by intravenous inoculation of embryonated chicken eggs from 8 of 8 experimentally infected cattle through PID 26. Virus was reisolated from 2 of the animals tested at PID 50 by sheep inoculation with larger amounts of blood. Bowne et. al. reported that bluetongue virus isolated from the tissues of a cow that was infected and held in isolation for 105 days. Luedke cited by Bowne has reisolated BT virus from one steer up to 700 days after infection.

The correlation of viremia with complement fixing (CF) antibody titer has not been extensively investigated. Carbrey et. al. reported that 2 sheep and 3 cattle had detectable CF titers through PID 719 and 594 respectively. Virus could not be reisolated from the sheep at PID 461 and 719 or from cattle at PID 320 and 550. Luedke et. al. detected precipitating and high neutralizing antibody titers throughout the period when virus was isolated.

This experiment was performed to determine the length of the viremia as correlated with CF antibody titer of calves experimentally infected with bluetongue virus.
MATERIAL AND METHODS

Virus Strains—Three antigenically different strains of bluetongue virus were supplied* in an anticoagulant preservative solution OPG**. The strains used were 63-83B, BT-8 and 62-45S which have been described previously 19. Isolate 63-83B was obtained from a cow; BT-8 and 62-45S were isolated from sheep. The isolates have been passed in sheep 4, 11, and 9 times respectively.

Experimental Animals—Eighteen calves were used for the project; 6 purebred Jersey steers, 6 purebred Jersey heifers, 5 Holstein steers and 1 Holstein heifer. The Holsteins were from the National Animal Disease Center herd and the Jerseys were from a herd located in Iowa. The calves were 6-9 months of age at the start of the experiment and had been weaned for at least 2 months. Calves were used instead of adult cattle to facilitate handling and to better utilize the space available.

Forty-eight sheep were used for virus assay. All were mixed breed, adult sheep, 29 ewes, and 29 wethers.

All animals were from closed flocks and herds that were tested and found negative for antibody against bluetongue virus by use of complement fixation test. The animals were maintained in insect free isolation barns throughout the experiment. The sheep were held in plastic isolation cages provided with negative air pressure.

Experimental Procedure—Three groups of six calves each were used. Each group was inoculated with a different strain of virus. Each calf was inoculated with 2 ml. of OPG preserved blood, 1 ml. intramuscularly, and 1 ml. subcutaneously. Samples of blood for subinoculation into sheep were collected at approximately PID 30, 60, 90, and from 7 of the calves at about PID 120. Rectal temperatures of the sheep and cattle were determined daily. The cattle were held for 180 days postinoculation. The sheep were held for 90 days postinoculation.

Complement Fixation Test—The modified direct complement fixation test was performed as previously described by Boulanger 2 and Boulanger et al. 2.

Viral Assay—One hundred ml. of blood was collected with heparin from each calf. Bluetongue susceptible sheep were inoculated intravenously for virus assay. The blood was considered infectious if the sheep developed antibody against bluetongue virus. The blood from the 6 calves receiving the BT-8 strain was inoculated directly into the sheep. The cellular elements of the blood from the remainder of the calves were allowed to settle at 4 C., and were resuspended to the original volume in phosphate buffered saline. For sheep inoculation, serum samples were collected monthly from the sheep for the determination of bluetongue an-

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*Supplied by Dr. M. M. Jochim and Dr. H. E. Metcalf, Animal Disease Research Laboratory, ARS, Federal Center, Denver, Colorado 80225.
**OPG solution: potassium oxalate, 5 gm., phenol 5 gm., glycerin, 500 ml. and distilled water, 500 ml.
tibody titers. At least two negative subinoculations were obtained from each calf.

RESULTS

Clinical Signs—Transitory pyrexia with minimal depression was observed in the calves inoculated. A maximum temperature rise to 40.6 °C. (105°F.) was observed in 2 calves. The body temperature of the other calves did not exceed 34.9 °C. (103°F.). One calf died of ruminal tympany at PID 32.

The sheep had only a slight pyrexia. One sheep had a maximum temperature of 40.9 °C. (105.6°F.). Swelling of the muzzle of the sheep that received the BT-8 strain was observed by PID 14. Other clinical signs observed in the sheep were slight anorexia and depression at approximately PID 14.

Serology—Seventeen of the eighteen calves were positive for complement fixing antibody against bluetongue virus between PID 19 and 28. Antibody was not detected in one calf inoculated with 63-83B strain, also BT virus was not isolated from the calf (Table 1) so it was assumed that the virus failed to produce infection. Repeated subinoculations were made at PID 36 and 63 but no antibody response was obtained in the sheep. The calves reinoculated with BT-8 strain were CF positive throughout the experiment, while those given 62-45S were negative at PID 117. Similarly those inoculated with 63-83B were negative at PID 113. (Figure 1, 2, 3, and 4).

Viral Assay—Fifteen of the seventeen infected calves were viremic when the first subinoculation was made at PID 30. One calf died of ruminal tympany at PID 32 leaving 16 calves surviving at the end of the experiment. The virus was reisolated from only one calf on the second subinoculation at approximately PID 60. On the third subinoculation at PID 113 this calf was negative. (Table 1).

Statistical evaluation based on 16 animals was performed and it was determined that at least 82.9% of bluetongue infected cattle will not have a viremia that persists past 113 DPI on the basis of a 95% confidence interval.

DISCUSSION

Bluetongue virus was isolated from 16 of 17 infected animals in this experiment even though complement fixing antibody was detected at the same time. It appears that CF antibody titer persists for at least as long as the BT viremia persists. Leudke et al. II did reisolate virus at PID 2, so it is possible that cattle could have a viremia before CF antibody would be detected.

Even though calves were used in this experiment, the data should be applicable to cattle. Bluetongue virus does not appear to have a predilection for any particular age of animal. The calves should have been immunologically similar to adult animals. The experimental procedure used here cannot be compared to exposure of neonatal animals or to
EXPERIMENTALLY INFECTED ANIMALS

inoculation in utero. The long viremia reported by Bowne was produced by this type of inoculation.

The inoculum used for virus assay was purposely very large. This was to help rule out the possibility that the virus level might be fluctuating and not be detected if a small amount of blood was used. The number of insect infectious doses, if any, was not determined since the concentration of virus in the infected blood was not established. It appears that cattle act as a reservoir for bluetongue virus for only a relatively short period of time. However, as shown by Nevill, the Culicoides spp. can survive at 4°C for 53 days and this interval in addition to a 30-60 day viremia in cattle would allow overwintering of the disease throughout much of the United States. Previous work has also shown that the viremia in cattle does not usually persist after PID 50.

In this experiment CF antibody titers were detectable at least until PID 96. The complement fixation test should detect all infected animals unless they are sampled in the first 3-4 weeks after infection. Therefore the CF test may be considered a good screening test for detecting the bluetongue virus infected animals.

SUMMARY

The persistence of bluetongue virus and complement fixing antibody in calves was evaluated. Egitheen calves were inoculated with three strains of virus: 6 with the BT-8 strain, 6 with the 62-45S strain, and 6 with the 63-83B strain. One calf that was inoculated with the 63-83B strain did not develop antibody and virus was not reisolated so it was assumed this calf was not infected. Complement fixing antibody was detected in the other 17 calves between postinoculation day (PID) 19 and 28. The calves inoculated with 62-45S and 63-83B were negative for complement fixing antibody by PID 110 while the calves receiving BT-8 were still positive at PID 162 when the experiment was terminated.

Virus was isolated by sheep inoculation from 15 of the 17 calves at PID 30. One calf died of ruminal tympany at PID 32 leaving 16 calves for the remainder of the experiment. At PID 60 only one calf was positive for virus isolation and it was negative on the next isolation attempt at PID 113. Complement fixing antibody was detected in all calves at the time virus isolations were made. Statistical evaluation, based on 16 infected calves, indicated at least 82.9% of the bluetongue infected cattle will not have a viremia that persists past PID 113.

REFERENCES


<table>
<thead>
<tr>
<th>Strain of BT Virus</th>
<th>First Subinoculation</th>
<th>Second Subinoculation</th>
<th>Third Subinoculation</th>
<th>Fourth Subinoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PID</td>
<td>Infected/Total</td>
<td>PID</td>
<td>Infected/Total</td>
</tr>
<tr>
<td>BY 8</td>
<td>31</td>
<td>6/6</td>
<td>62</td>
<td>0/6</td>
</tr>
<tr>
<td>62-458</td>
<td>30</td>
<td>6/6</td>
<td>63</td>
<td>0/5*</td>
</tr>
<tr>
<td>63-838</td>
<td>36</td>
<td>3/6**</td>
<td>63</td>
<td>1/6**</td>
</tr>
</tbody>
</table>

PID - Postinoculation Day

- One calf died of rumenal tympany PID 32.
- One calf did not develop antibody and virus was not reisolated. It was assumed to have not been infected.

Table 1: Virus isolation results from bluetongue inoculated calves.
COMPLEMENT FIXING ANTIBODY TITER

Figure 2: Average and range of complement fixing antibody titers observed in serum from calves infected with Bl-8 strain.

Figure 1: Average and range of complement fixing antibody titers observed in serum from calves infected with Bl-45.5 strain.

Conjunctivitis - Bl-8 strain.
Figure 3: Average and range of complement fixing antibody titers observed in serum from calves infected with bluetongue virus - 63-83B.

Figure 4: Comparison of average complement fixing antibody titers observed in serum from calves infected with different strains of bluetongue virus.
One calf died of rumenal tympany PID 32.

One calf did not develop any antibody and virus was not reisolated. It was assumed to have not been infected.

Table 1.: Virus isolation results from bluetongue inoculated calves.
Brucella canis was first isolated and identified as a cause of abortion in the dog in 1966. A unique feature of the infection is a prolonged period of bacteremia. The etiogenic agent is mucoid in nature and this has presented problems in the development of a satisfactory antigen and test procedure for the serologic diagnosis of this disease.

This paper reports on the serologic and bacteriologic procedures developed in our laboratory for the use as aids in the diagnosis of this disease. Using these procedures, 2 naturally infected beagle colonies and 2 colonies with no history of infection were tested and hemo-cultured. In addition, 6 dogs were experimentally infected and monitored over an extended period of time.

In the experimentally infected dogs, a bacteremia was first demonstrable 8 to 23 days post challenge. A 1:50 titer was first detected 9 to 19 days post challenge. A demonstrable bacteremia persisted of 205 days to over 3 years and titers greater than 1:200 persisted for 38 to approximately 200 days after the last positive hemoculture.

In interpreting the serologic test, we consider a negative reaction at a 1:50 dilution to be indicative of no infection, a positive reaction at a 1:200 dilution as being indicative of active infection and a positive at a 1:50 or 1:100 dilution as being suspicious. Paired serums taken from 30 to 60 days apart should help clarify the status of animals classified as suspicious.
Rapid sampling by negative staining makes electron microscopy attractive for virus identification. More than 50 morphologically different types can be recognized. Direct immuno-electron microscopy allows differentiation of antigenically distinct particles and may also be used to enumerate them. This method is limited by the availability of antibodies carrying known antigenic specificity. Due to an intrinsically low contrast of single protein molecules, it is not presently practical to use the electron microscope for routinely detecting antibodies in acute or convalescent serums, particularly if the infectious agent is only weakly antigenic.
INTRODUCTION

When the microbiologist isolates a virus or a mycoplasma from an animal, he is tempted to conclude that he has adequately diagnosed the cause of illness in that animal or in that herd. When he demonstrates a significant seroconversion, he can sometimes forget that this finding may perhaps be just coincidental.

Microbial infection occurs with far greater frequency than does the disease syndrome classically attributed to that infection. For example, compare the relatively high percentage of cattle showing serological evidence of prior exposure to BVD virus with the relatively low incidence of mucosal disease. Many infected animals have escaped clinical illness.

When does infection result in disease? After exposure of the host to a prospective pathogen, a group of inter-related factors regulate the frequency of occurrence and govern the severity of any disease which may result. These factors include:

1. Hereditary predisposition to disease.
2. Environmental stress.
3. Nutritional deficiency or dietary excess.
4. Status of natural or acquired immunity.
5. Concurrent exposure to infection by more than one pathogen or opportunist.

It is exactly these complexities of animal disease processes that the veterinary diagnostician is called upon to unravel. The disease history, the clinical signs reported, the post mortem lesions observed, and these possible interrelationships must be considered before the diagnostician can assess the probable role of any microbial agent in a disease process. Only then is he able to provide the owner or the practicing veterinarian with accurate recommendations for the treatment, control, or future prevention of this disease condition.

This paper will list some well-known examples of multiple-etiology disease syndromes. Greater emphasis will be placed on some hypothet-
ical interrelationships which are encountered by the diagnostician, but not yet verified by experimental research.

**HEREDITY**

Poultry neoplastic diseases:
- Genetic resistance to Marek's disease and to diseases of the leukosis/sarcoma group have been extensively investigated.
- Genetic selection certainly plays a role in regulating resistance to these diseases although actual virus infection is not always prevented.

Adenovirus infection of foals:
- Disease problems associated with adenovirus infections may be relatively more severe in the Arabian breed.
- Actual incidence of adenovirus infection may be no higher than the average for other breeds.
- It has been suggested that the increased susceptibility of Arabian foals is associated with congenital absence of the thymus or other defect in the immune response to viral and bacterial infections.

**ENVIRONMENT**

Anthrax:
- Susceptibility to periodic flooding and alkaline pH affect the survival of *Bacillus anthracis* in soils of endemic areas.
- Men who handle contaminated wool or hides have an increased incidence of cutaneous and pulmonary anthrax infection.
- Thus the environment of the host strongly determines the possibilities of exposure to this pathogen.

Gastric ulcers of swine:
- The incidence of gastric ulcers in swine has been related to many factors including the feeding of finely ground rations, feeding of rations deficient in vitamin E, nervous temperament, and hereditary predisposition.
- However gastric ulcers are seldom observed as a significant disease problem except where swine are being fed in a close confinement facility.
- The environment therefore appears to be a most significant factor.

Tail-biting in swine:
- This behavioral characteristic is most commonly acquired by swine in close confinement with restricted access to feeders and waterers.
Suppurative polyarthritis, ascending myelitis, and miliary abscessation of the lungs by *Corynebacterium pyogenes* are common sequellae.

These conditions are seldom encountered in swine except in confinement rearing situations.

Swine dysentery:

The pathogens associated with swine dysentery survive for only limited time intervals outside of the living pig.

Swine dysentery outbreaks are more severe when close confinement fosters immediate and direct transmission from pig to pig.

Spontaneous remissions result when the cycle of reinfection is interrupted by dispersal of the host animals.

Foot rot of cattle:

True foot rot of cattle apparently requires environmental conditions conducive to mechanical injury to the interdigital area as well as soil conditions favorable for survival of *Spherophorus necrophorus* and *Bacteroides melaninogenicus*.

Suppurative polyarthritis and tendonitis mistakenly diagnosed as foot rot epidemics frequently are observed to occur about six weeks following respiratory disease episodes.

Therefore soil conditions seem unimportant as predisposing factors for the latter condition.

Of greater importance is an environment conductive to respiratory transmission of *Haemophilus somnus* or *Mycoplasma spp.* which have been associated with the polyarthritis problem.

**NUTRITION**

*Clostridium perfringens* enterotoxemia:

It is well accepted that excessive consumption of concentrate is directly associated with the occurrence of enterotoxemia in lambs and feeder cattle.

Intervals between nursing and amounts of milk consumed per feeding can influence incidence of enterotoxemia in baby pigs and calves.

Indirect effects on feed consumption of livestock can cause an "epidemic" of enterotoxemia cases following abrupt weather changes and long holiday weekends.

Edema disease and vitamin E deficiency:

Hemolytic *E. coli* enteritis and edema disease syndrome are closely associated with the post-weaning period in swine.

Lesions similar to those described for edema disease are found at necropsy of pigs on experimental vitamin E and selenium deficient diets.

Is there an accentuation of edema disease signs and lesions when there is concurrent vitamin E and selenium deficiency?

Do hepatic lesions which accompany vitamin E deficiencies possibly render the porcine liver less capable to detoxify bacterial toxins?

Is there possibly increased absorption of these toxins from the gastrointestinal tract of pigs with E deficient rations?
Histomoniasis of chickens:
Histomoniasis is commonly diagnosed in turkeys, but less frequently in chickens.
Histomoniasis has been diagnosed in our laboratory in chickens with concurrent cecal coccidiosis (*Eimeria tenella* infection) and a history of water deprivation.
Is the physiological role of the cecum in maintaining body hydration related to the appearance of cecal infection and disease of dehydrated birds?

Fat cow syndrome:
High labor costs have caused dairymen to install milking parlor facilities where it is difficult to gauge the milk production of individual cows, and also difficult to vary feeding of concentrates in relation to stage of lactation and production.
Mechanized handling of forages has made baled hay a less available item.
Results have been an excess of concentrate fed to dry cows and cows nearing the end of lactation; a deficiency in good quality roughage; and in some cases, a protein deficiency as well.
The resulting fat cow syndrome encompasses such widely divergent problems as still-born calves, post parturient metritis, non-responding ketosis, relapsing milk fevers, toxic mastitis, displaced abomasums, and sudden deaths due to perforation of abomasal ulcers.

Nutritional mismanagement results in a variety of both infectious and non-infectious disease problems that are alleviated only by correction of the basic nutritional problem.

Magnesium or calcium deficiency and *Clostridium* spp.:
It seems advisable that veterinary research be directed toward an attempt to prove or disprove the hypothesis that animals deficient in divalent cations are rendered more highly susceptible to infection and/or toxin production by members of the *Clostridium* spp.

Cows dead of grass tetany tend to undergo carcass decomposition at an accelerated rate.
Blood magnesium levels of clinically normal cows are frequently lower than those of individuals from within the same herd that exhibit clinical signs of grass tentany.
The central nervous system disorders clinically observed in hypomagnesemia do not differ greatly from those of *Clostridium* spp. toxemia.
Cattle necropsied immediately after death attributed to hypomagnesemia rather frequently have had *Clostridium perfringens* bacteremias.
High blackleg incidence may more closely coincide with grazing of rapidly growing forages than with areas of high exposure to *Clostridium chauvoei*.
Neonatal enteritis:

- Early ingestion of colostrum by the newborn calf has value over and above that normally attributed to immunoglobulin content alone.
- A supply of beneficial microorganisms competitively inhibits the establishment and growth of prospective pathogens.
- Stimulation of gastric enzyme secretion and digestive processes establishes the acid pH of the abomasum which acts as a barrier to entrance of many acid-labile pathogens into the intestinal tract.
- Overconsumption may precipitate nutritional scours, which in turn may prompt the owner to initiate unwarranted and possibly detrimental antibiotic medication.
- Hand feeding of calves at irregular intervals, feeding of too large a volume too rapidly, and contamination of nipple pails often are predisposing causes of infectious enteritis and/or enterotoxemia.
- Congregation of cows in limited areas for supplemental feeding of hay has been incriminated as one factor which enhances communicability of infectious enteritis in calves.
- Gradual introduction of milk replacer tends to reduce the severity and incidence of salmonellosis in recently-shipped dairy calves.

**IMMUNITY**

Septicemia of the newborn:

- The newborn animal which received colostrum in the first few hours of life is temporarily protected against systemic infection by pathogens against which maternal immunoglobulins are directed.
- Colostrum deprivation or colostral inadequacy is a primary factor allowing the development of septicemia, polyarthritis, polyserositis and meningitis in the newborn.
- Colostral inadequacies precipitated by movement of the pregnant female to a new environment within several weeks prior to parturition are an important factor in neonatal disease problems.

Swine erysipelas:

- Experimentation has verified the shedding of *Erysipelothrix rhusiopathiae* from infected swine, and survival of this microorganism in the soil for extended periods.
- However the soil from Iowa swine-rearing premises where there has been no recorded incidence of acute swine erysipelas for 20 years or more, and where swine populations have never been artificially immunized against erysipelas, also contains substantial populations of *Erysipelothrix rhusiopathiae*.
- Absence of acute erysipelas probably is explainable on the basis of the relative immune status of the pigs rather than the absence of exposure to virulent microorganisms.
- Therefore some factor or factors causing increased susceptibility of the host should be required to precipitate acute erysipelas outbreaks.
Listeria monocytogenes abortion of cattle:
It is suspected that cattle are orally exposed to *Listeria monocytogenes* by consumption of contaminated forages.
In two major listeriosis abortion cases confirmed at Iowa State University's Veterinary Diagnostic Laboratory, corn silage was the major feedstuff supplied to the aborting cows.
In both cases, the aborting cows were newly acquired by the owner. They had been placed on a corn silage ration as pregnant cows, and had not previously had access to this type of ration.
In one case, the aborting cows represented only a portion of the herd. The remainder, or non-aborting cows had been receiving corn silage prior to the onset of pregnancy.
It is suspected that acquired immunity of the cow can prevent fetal infection by *Listeria monocytogenes* even through non-immune cows being exposed to the same numbers of organisms frequently abort.

Greasy pig syndrome:
Chronic bacterial infections by *Staphylococcus* or *Streptococcus* spp. are associated with the severe exudative epidermitis in susceptible litters of two to three week-old pigs.
Other litters in the same farrowing house often remain unaffected.
Antibiotic therapy may produce only temporary or partial alleviation of the problem, but improved results have been obtained by use of non-specific hyperimmune swine serum.
Case histories strongly suggest that susceptible pigs may have an immune tolerance to the bacterial pathogen which they have acquired as a result of *in utero* exposure.
Or they may have a genetically determined inability to recognize the pathogen as a foreign antigen.
Onset of the problem at two to three weeks of age suggests that the pigs are temporarily protected by passively acquired maternal antibody.

BVD-mucosal disease complex:
BVD virus infects 67% or more of this nation's cattle population on the average. Most cattle recover from the virus diarrhea episode, and post-infection BVD specific antibody titers are then detectable.
Possibly two percent of BVD-infected cattle die as a result of the BVD-MD complex characterized by a depletion and necrosis of lymphoid tissues.
The BVD-MD reaction is similar in many respects to a cell-mediated delayed hypersensitivity response.
The BVD-MD syndrome has been attributed to an immune tolerance phenomenon, however not all cattle dying of the syndrome are devoid of BVD-specific humoral antibody.
Conceivably, the mucosal disease syndrome may then develop as a delayed hypersensitivity response only in those animals that are not
tolerant to BVD infection.
Failure of development of cellular mediated delayed hypersensitivity response might be the rule rather than the exception in the larger percentage of infected cattle.

**CONCURRENT INFECTIONS**

Confirmed interrelationships:

The scientific literature in veterinary medicine contains numerous reports of common disease syndromes which are the result of combined infection by more than one potential pathogen.

Some common examples that may be cited are the proven interactions of primary viral and secondary bacterial pathogens like:

1. Swine coronavirus (TGE) and *E. coli*
2. Bovine reovirus (neonatal calf scours) and *E. coli*
3. Parainfluenza 3 (shipping fever) virus and the *Pasteurella spp.*

There are the respiratory diseases of swine caused by primary *Mycoplasma hyorhinis* or *Mycoplasma hyopneumoniae* in conjunction with secondary *Haemophilus suis*, *Pasteurella*, *Bordetella* or *Streptococcus spp.*

The veterinary diagnostician must remain alert for additional syndromes which are the result of interaction by more than one infectious agent.

A valuable generalization and guideline for the diagnostician is that domestic animals frequently can tolerate a single agent infection quite easily. Mortality is ordinarily low as a result of uncomplicated viral infection. Bacterial infections can often be effectively counteracted by antibiotics in support of the animal’s natural defenses.

Disease problems characterized by high morbidity, high mortality, and a poor response to medication quite consistently prove to be multiple-etiology disease syndromes.

Some examples of commonly encountered disease syndromes which require further experimental verification by controlled research studies in order to prove their multiple etiologies can be suggested.

Swine pox and salmonellosis:

Often a change in management, an introduction of possible carriers, a change in ration, or simply movement of swine can be incriminated as predisposing causes of salmonellosis outbreaks.

In numerous cases however, these factors apparently were not involved. Instead an outbreak of swine pox has been observed to precede septicemic salmonellosis.

Observant practitioners had described this syndrome as “pox going septicemic” while the consistent presence of *Salmonella spp.* was demonstrated.

Swine pox infection apparently should be added to the list of other factors which are important triggering agents of salmonellosis.

BVD virus and *Pasteurella spp.* infections:
Published reports describe possible BVD-induced immuno-suppression which may occur in cattle during and after natural infection. Serological surveys of feedlot cattle strongly suggest that BVD susceptibility on arrival at the feedlot is a common factor consistently equated with incidence of severe illness, high treatment cost, poor profit return and higher-than-average death losses. Primary BVD infection is a very common factor associated with secondary Pasteurella hemolytica pneumonia in recently-shipped calves arriving in Iowa. It has been observed that concurrent exposure of BVD-susceptible calves to this virus and to Pasteurella hemolytica, results in a respiratory disease process that is notably non-responsive to antibiotic therapy. It is suggested that the BVD-induced immunosuppression may be responsible for the serious morbidity and mortality rates characteristic of the problem.

BVD and IBR viruses:
Many "post-vaccinal breaks" following the use of modified-live IBR vaccines in feedlot cattle can be verified as situations where IBR vaccines were used in calves naturally infected with BVD virus. Many experienced feedlot veterinarians are wary of the use of bivalent IBR-BVD vaccines in calves that they suspect are BVD-susceptible. IBR virus in exceptionally high titers (in addition to BVD virus which may be more difficult to isolate) is consistently recovered from most tissues of cattle recently vaccinated with IBR, and where dramatic gross lesions warrant a diagnosis of BVD-MD complex. It may be postulated that the BVD-infected calf responds less favorably to IBR virus exposure than does the normal BVD-immune or non-infected animal. Judicious use of modified-live viral vaccines in the future will depend on an improved awareness of the possible complications resulting from the simultaneous existence of natural viral infection.

Bovine neonatal enteritis:
Field cases have been encountered where both the bovine coronavirus and the BVD virus have been demonstrated in severe calfhood diarrhea problems. The concept of multiple-etiological disease seems especially pertinent in attempts to explain the occurrence of neonatal enteritis problems of calves.

Haemophilus somnus and Spherophorus necrophorus:
Experimental efforts to intentionally produce necrotic laryngitis by introduction of Spherophorus necrophorus into the larynx of calves have been notably unsuccessful, even when preceded by mechanical abrasion of the normal infection site. Laboratory-verified primary H. somnus infection may be an important triggering factor in necrotic laryngitis episodes.
A similar pathogenesis might possibly explain the suppurative polyarthritis and tendonitis lesion associated with so-called epidemic foot rot of feedlot cattle. Primary endothelial injury by a systemic pathogen such as *H. somnus* may well render the animals more susceptible to infection by the opportunistic *Spherophorus necrophorus* or *Corynebacterium pyogenes* pathogens which are eventually recovered from the infection site.

CONCLUSIONS

The previous topics are certainly not an all-inclusive listing of the fascinating complexities encountered in animal disease investigations. Because of the unique nature of his professional endeavor, any veterinary diagnostician could enlarge upon and probably clarify this list of possible multiple-etiological disease syndromes.

The authors are not naive enough to expect scientific agreement on the speculative proposals included in this paper. The purpose of this presentation instead has been to report laboratory observations in an attempt to encourage further speculation concerning the possible interrelationships proposed. Hopefully its presentation will stimulate as much friendly controversy among the audience as it has between the authors during its preparation.

ACKNOWLEDGMENTS

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INTRODUCTION

The digestive system of the chicken is more exposed to parasitic and infectious agents than any other system of its body. The bird eats off the floor and from potentially contaminated feed troughs. It drinks from an unsanitary water trough that may be used by hundreds of other birds. What happens to the organs of this young chicken may determine its productive efficiency for the remainder of its life.

The purpose of this paper is to present information which may help the diagnostician to differentiate diseases affecting the digestive system. A complete accurate clinical history and representative specimens of the case should be the first prerequisite.

CLASSIFICATION

The following is a partial list of diseases which affect the digestive system of the chicken, along with their preferred organ sites:

I. PARASITIC DISEASES
   A. Protozoa
      1. Coccidiosis
         a. *Eimeria necatrix*  Jejunum
         b. *Eimeria acervulina*  Duodenum
         c. *Eimeria mivati*  Small intestine, rectum, cecum
         d. *Eimeria maxima*  Jejunum
         e. *Eimeria brunetti*  Ileum, rectum
         f. *Eimeria tenella*  Cecum
      2. Histomoniasis
         a. *Histomonas meleagridis*  Cecum, liver
   B. Helminths
      1. Ascariasis
         a. *Ascaridia galli*  Intestines
      2. Capillariosis
         a. *Capillaria contorta*  Crop
         b. *Capillaria obsignata*  Duodenum
      3. *Heterakis gallinarum*  Cecum
      4. *Heterakis isolonche*  Cecum
   II. BACTERIAL DISEASES
      A. Mycobacteriosis (Tuberculosis)  Intestines, liver
      B. Colibacillosis (Hjarre's Disease)  Intestines, liver
C. Salmonellosis (Pullorum, Typhoid, Paratyphoid)  
D. Ulcerative Enteritis (Quail Disease)  
E. Streptococcosis  
F. Pasteurellosis (Fowl Cholera)  
G. Vibriosis (Vibrionic Hepatitis)  

III. MYCOTIC DISEASES  
A. Candidiasis (Candida albicans)  

IV. VIRAL DISEASES  
A. Intranuclear Inclusion Body Hepatitis  
B. Fowl Pox  

V. NUTRITIONAL DISEASES  
A. Avitaminosis A  

VI. NEOPLASTIC DISEASES  

VII. DISEASES OF UNKNOWN ETIOLOGY  
A. Avian Monocytosis  
B. Necrotic Enteritis  
C. Fatty Liver Syndrome  
D. Gizzard Erosion  

I. PARASITIC DISEASES  

A. Protozoa  
The two major protozoan-caused diseases of the chicken and turkey are coccidiosis and histomoniasis. In the chicken there are six species of *Eimeria* which can cause economic losses. The most common age incidence of coccidiosis is 3 to 6 weeks but the disease may also occur in young chickens and adult birds. Clinical signs include pale shanks and beak, ruffled feathers and loss of weight. In addition, one species, *Eimeria tenella*, will cause free-bleeding from the cecum, producing fresh blood stains in the litter. Sudden high mortality may be noted in pens where the waterers have recently flooded over. As immunity is species-specific for the particular coccidium and treatment is based on the individual species involved, a differential diagnosis of the species of coccidium is mandatory.  

1. Coccidiosis  
   a. *Eimeria necatrix*—*Eimeria necatrix* causes red and white spots on the serosa of the small intestines. In advances cases, the intestines are ballooned and contain blood-tinged exudate and cellular debris. Direct smears from the intestinal mucosa reveal schizonts in groups. Oocysts of *E. necatrix* will be found in the cecum.  

   The histopathology caused by *E. necatrix* is that of a parasitic granuloma deep in the lamina propria and usually adjacent to the muscular wall. Macrophages are seen engulfing the schizonts. Rarely do organisms appear in the epithelium, although the disease appears to begin in such cells of the deep glandular crypts. Some granulomas penetrate deep into the muscular wall and possibly predispose the birds
b. *Eimeria acervulina.* — *Eimeria acervulina* causes transverse white lesions in the intestines and are more visible from the mucosa. Direct smears of *E. acervulina* show oocysts in clusters (18.3 x 14.6 μ). The histopathology of *E. acervulina* is characterized by masses of schizonts and gametocytes on the villi tips with surprisingly little tissue reaction. Although the schizont is supranuclear in position, it is often difficult to determine because of the large numbers of the organism. This coccidium does enter deeper portions of the mucosa, but this is far less impressive than the surface colonies.

c. *Eimeria mivati.* — *Eimeria mivati,* a recently reported species of coccidia, will travel the whole length of the small and large intestines. Lesions are those of a coalescing round plaque. Direct smears of *E. mivati* reveal very small round oocysts (15.6 x 13.4 μ).

Histologically there is sloughing of the epithelium from the distal half of the intestinal villi of the small intestine. This is accompanied by edema, hyperemia, and eosinophilia.

d. *Eimeria maxima.* — *Eimeria maxima,* the largest of the coccidial oocysts (30.5 x 20.7 μ), produces a pink copious catarrhal exudate. On direct smears, the large oocysts will be found individually, not in groups.

The large size of *E. maxima* serves as an important means of diagnosis with a direct smear. However, fixation tends to shrink the organism so that size is of less value on histopathology. The lesions are confined usually to the upper half of the intestinal villi. In the epithelium, schizonts appear above the nuclei, but in far less numbers than *E. acervulina.* Many forms, usually gametocytes, appear in the lamina propria. Unlike *E. acervulina,* there is necrosis and hemorrhage. The macrogametocytes are particularly impressive—the plastid bodies stain bright red with hematoxylin and eosin (H&E) and thus are helpful in diagnosis.

e. *Eimeria brunetti.* — *Eimeria brunetti* is usually found in young adult birds and causes necrosis in the posterior large intestine. Direct smears show oocysts that are about the size (23.4 x 19.7 μ) and shape of *E. tenella.* A fibrinous enteritis with coagulation necrosis of the lower small intestine, rectum, and proximal cecum strongly suggests *E. brunetti* damage.

Histologically there is a diphtheritic membrane laden with oocysts, characterizing an acute coccidiosis. The membrane adheres tightly to the mucosa which contains scattered schizonts usually not in proportion to the number of oocysts. The mucosa is often intact.

**Differential diagnosis:** *Eimeria brunetti* infection must be differentiated from necrotic enteritis. A remarkable cystic formation and necrosis, seen in necrotic enteritis, is not so apt to occur in *E. brunetti* infection. All things considered, the differentiation is difficult as both have been considered to occur simultaneously in some instances.

f. *Eimeria tenella.* — *Eimeria tenella* causes cecal coccidiosis,
manifested by free bleeding in the cecum. Later on in the disease this may be followed by bloody cores composed of caseous exudate and dried blood. Direct smears reveal large round oocysts of *E. tenella* (22 x 19 μ).

Histologically the lesions are confined to the cecum and are essentially those of necrosis and hemorrhage. A severe reaction is caused by numerous second generation schizonts which occupy the epithelium of the deeper glands and the lamina propria. In the recovering stage, oocysts are fairly numerous and the mucosa is reduced in depth.

*Differential diagnosis*: Damage due to *E. tenella* must be differentiated from: 1) ulcerative enteritis which produces perforating ulcers of the cecum and ileum, causing focal adhesive peritonitis, and 2) histomoniasis which produces caseous cores in the cecum. However, liver lesions in ulcerative enteritis and histomoniasis will help differentiate these conditions from *E. tenella* damage. In addition, the organisms themselves are morphologically dissimilar to the extent that differentiation is simple. Any doubt can be eliminated by employing special stains as periodic acid Schiff which reveals the histomonad as PAS positive.

2. *Histomoniasis a. Histomonas meleagridis*.—Histomoniasis caused by *H. meleagridis* in the chicken tends to cause cecal lesions with far less hepatic involvement than in the turkey. In the cecum one sees a massive core of debris in the lumen; the epithelium is usually sloughed, leaving a lamina propria somewhat flattened owing to the pressure of the core.

Histologically the muscularis is markedly infiltrated by round cell types. The offending protozoan is better seen in the muscle as a pale red round body (H&E stain) in a lacuna. It is present in surprisingly large numbers. In the liver the lesion is a lymphogranuloma forming circumscribed surface erosions.

### B. Helminths

1. *Ascariasis a. Ascaridia galli*.—Ascarisasis, the most correct term for describing an increase in the number of round worms of the species *A. galli*, does not present a diagnostic problem in the chicken except when only larval forms are present.

Histologically the intestinal changes resemble those of *Capillaria obsignata* when adult ascarids are present. Mature ascarids cause lymphocytic but not much eosinophilic reaction. Larval forms are embedded deep in the mucosa. Fixation is the crux of a successful diagnosis. Tie off a section of intestine and infuse with 10% formalin until distended. Then drop this tied off piece of intestine into fixative for 24 hours.

*Differential diagnosis*: A problem is encountered in that ascarids and capillarids may exist simultaneously. Gross examination and screening techniques may be the only means of differentiation.

2. *Capillarisis a. Capillaria contorta*.—This species affects the crop chiefly of wild birds and game birds. It does resemble crop mycosis (candidiasis) in some respects and requires attention. The clinical signs
associated with crop capillariasis are primarily those of malnutrition, emaciation, and fatal anemia. There may be large masses of worms found on the surface or in the sloughing tissue. Histologically the surface of the epithelium may lift off. Under the parasite, in the dermis, are frequently aggregates of lymphocytes. The identical lesion is seen in the esophagus.

**Differential diagnosis:** Crop mycosis may be confused with crop capillaria but the parasites present easily eliminate the problem.

b. *Capillaria obsignata.*—This species of capillaria attacks chiefly young laying hens and young pullets. There is a drop in egg production for which no cause can be found, and if brown eggs, the shells may tend to pale out in color. The most effective and immediate method of making a diagnosis is to wash the mucosa under water pressure using the regular sink sprayer over a 100 mesh screen. Holding the contents in a glass plate over a light will allow a quick competent inspection of the very fine spiralling hair worms.

Histologically this parasite causes rather subtle changes not always easily associated with one's concept of parasitism. The villi become shorter and wider, while the muscle wall hypertrophies. Muscle begins to appear predominantly in the villi which is not normal. The parasite is seldom found in sections as fixation causes them to release their positions. When seen, they are deep in the lamina propria and lymphocytes are seen in great numbers. Usually the female and her eggs is a common finding.

3. *Heterakis gallinarum*—This species (the cecal worm of chickens) appears to cause no lesions of disease but on occasion *Heterakis isolonche* has been found to cause granulomas in the submucosa of the cecum and has been mistaken for *Heterakis gallinarum*.

4. *Heterakis isolonche*—This species usually occurs only in pheasants.

II. BACTERIAL DISEASES

A. *Mycobacteriosis* (Avian Tuberculosis)

Avian tuberculosis is now an uncommon disease in most poultry growing areas. At any rate, the classical granuloma of tuberculosis is seen in the fowl, usually the liver. Bacterial isolation along with acid fast stain of the microscopic tissues will confirm the diagnosis of mycobacteriosis.

B. *Colibacillosis* (Hjarre's Disease)

Colibacillosis, an acute coliform infection, manifested by an explosive "egg yolk" peritonitis, is caused by pathogenic serotypes of *Escherichia coli.* Microscopic liver lesions usually consist of large areas of confluent necrosis. The edge of such a lesion frequently contains giant cells. Birds killed early in the disease usually have a somewhat more vague but widespread lesion. Isolations of *E. coli* from bone marrow of dead birds or livers of sick birds will confirm the diagnosis.

The large mature classical granulomas in the liver and the intestine
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(referred to as Hjarre’s granuloma) is also related to the acute colibacillosis organism.

**Differential diagnosis:** Acute coliform peritonitis produces a lesion in the early phase which might be confused with so-called vibriosis. At any rate, bacterial studies separate the entity although gross pathologic examination and history are sufficient. Peritonitis is usually more severe.

Hjarre’s granuloma is identical to that of tuberculosis. Acid-fast stains will dispel any confusion.

**C. Salmonellosis (Pullorum, Typhoid, Paratyphoid)**

Salmonellosis, encompassing the diseases of pullorum, caused by *Salmonella pullorum*, fowl typhoid caused by *Salmonella gallinarum*, and paratyphoid caused by several of the salmonellae, most notably *Salmonella typhimurium*, all present diagnostic problems if bacteriology is not used. In suspicious birds, culturing of the cecal tonsils, other areas of the intestines, the gall bladder, and the ovary in brilliant green tetrathionate will usually recover a salmonella if one is present. Lesions are small and many times microscopic. There is a focal necrotic hepatitis accompanied by heterophils and lymphocytes. The cecum may or may not have cores of caseous exudate.

**Differential diagnosis:** It is not possible, without bacterial isolations to differentiate salmonellosis and pasteurellosis from each other as the lesions of the liver are similar. Colibacillosis is likewise a differential diagnosis but usually causes hepatic necrosis of a marked degree which often develops into a granuloma. Vibriosis causes similar lesions but bacteriologic studies will easily differentiate this cause. Avian monocytosis (blue comb) causes lesions identical to salmonellosis and pasteurellosis but differentiation is accomplished by the gout nephrosis of blue comb as well as the failure to isolate a bacterial agent.

**D. Ulcerative Enteritis (Quail Disease)**

Ulcerative enteritis has been described as “chicken ulcers.” Grossly, there may be focal adhesive peritonitis due to a perforation of an ulcer in the terminal ileum and cecum. The liver contains areas of hepatic necrosis without much reaction. Lesions are those of a diffuse necrosis without a definite origin.

The ulcers are sharply delineated from the adjacent mucosa which appears normal. The crater contains necrotic cells, coagulated debris, and bacterial rods usually in the deeper areas. A marked cellular reaction to the ulcer is evident and best described as serositis.

**E. Streptococcosis**

Streptococcosis, caused by *Streptococcus fecalis*, quite often will cause a bacterial endocarditis, liver infarcts, and splenic infarcts. The infarction is easily diagnosed grossly and is unremarkable on histologic examination.

**F. Pasteurellosis (Fowl Cholera)**
Pasteurellosis (fowl cholera), caused by *Pasteurella multocida*, has been mentioned previously as a differential from salmonellosis. Bacterial isolation of the bone marrow of dead birds or the liver, spleen, and other organs from recently killed birds, onto blood agar, will produce the Gram negative bipolar rod easily identified as *Pasteurella multocida*.

**G. Vibriosis (Vibrionic Hepatitis)**

Vibriosis (vibrionic hepatitis) in the acute form consists of confluent areas of necrosis of the liver. Clinical signs show a drop in egg production in the range of 25 to 35%, a loss of weight, scaly shrunken combs, and listlessness. Diarrhea in individuals or the entire flock is sometimes seen.

Gross lesions at necropsy represent inflammatory and necrotic processes. Many infected birds show no gross lesions of the liver. Yellow star-like foci are seen throughout the liver parenchyma. The heart muscle may show areas of necrosis. There may be catarrhal enteritis.

Histologically heterophils at first are the reactive cells but soon give way to greater numbers of lymphocytes. Some birds fail to recover completely and the result is a periportal infiltration of both heterophils and lymphocytes.

### III. MYCOTIC DISEASES

**A. Candidiasis**

Crop mycosis, caused by *Candida albicans*, is seen in caged birds and floor birds under poor sanitation or after antibiotic treatment. Clinical signs include a drop in egg production to 5 to 10% and a mild, white diarrhea. Brown egg shells may tint toward a whitish color. Necropsy reveals a white "turkish towel" exudate lining the crop and sometimes the first part of the duodenum. A culture of the crop lining on Sabouraud’s agar at room temperature will usually produce yeast colonies of *Candida albicans*.

Histologically the squamous epithelium seems to balloon. There is often surface debris and fairly deep erosions occur. The erosions are well demarcated and contain heterophils, lymphocytes, and unidentified debris. Cellular infiltration is present in the underlying tissue. On the surface Candida may be seen even with the H&E stain, but special stains as Gridley’s or silver methamine will bring out the yeast more clearly and make a diagnosis relatively simple.

**Differential diagnosis:** One may confuse crop mycosis with capillariasis of the crop which is a far milder condition and of course a parasite is invariably present. Avitaminosis A, now a laboratory disease, does present a non-inflammatory reaction which is just below the squamous epithelium. In this condition, keratin is common and there are no erosions. Keratin masses, so characteristic of vitamin A deficiency, are not seen in candidiasis.
Differential Diagnoses in Avian Medicine

IV. Viral Diseases

A. Intranuclear Inclusion Body Hepatitis

A recently described viral disease of the liver, manifested by intranuclear inclusion bodies, is referred to as inclusion body hepatitis or avian infectious anemia. No part of the liver is spared in this condition which consists of widespread necrosis with islets of viable hepatic cells. There is complete loss of hepatic architecture. Many cells in the necrotic areas are distended, vacuolated bodies. In others, large intranuclear inclusion bodies of the cytomegalic type are seen.

B. Fowl Pox

A virus disease of the skin, fowl pox can manifest itself in a diphtheritic form, producing plaques on the mucosa of the esophagus and the trachea. This condition is not difficult to diagnose. Virus isolation or histopathology will confirm the condition; intra-cytoplasmic inclusion bodies (Bollinger bodies) are seen on section. These are lipid positive so a so-called fat stain reveals them in a striking fashion.

V. Nutritional Diseases

A. Avitaminosis A

Vitamin A deficiency is a condition that rarely occurs spontaneously in birds on present day rations. The clinical signs of vitamin A deficiency would be extremely pale beaks and shanks, ruffled feathers, and a generally debilitated looking bird. Histologically the lesion consists of keratin sequesters, surrounded by the squamous epithelium of the esophagus. The lesion is reversible and with the immediate supply of new vitamin A the birds will recover completely.

VI. Neoplastic Diseases

Aside from the lymphomas of Marek's disease, lymphoid leukosis, erythroblastosis, and myeloblastosis, few neoplasms are seen in the avian digestive tract. Bile duct adenoma, bile duct carcinoma, and implants of the ovarian carcinoma occur in or on the liver. The implants are exceedingly common on the intestine. A point of interest—the solid neoplasms of the fowl are remarkably similar to those of mammals.

VII. Diseases of Unknown Etiology

A. Avian Monocytosis

Avian monocytosis is not primarily a disease of the digestive system, but in some birds small necrotic hepatic foci, mimicking those of salmonellosis and pasteurellosis are seen. In addition, parenchymatous degeneration of the pancreas is sometimes encountered. The most striking lesion is gout nephrosis and allows gross differentiation quite easily.

B. Necrotic Enteritis

Necrotic enteritis is an acute, catastrophic situation, generally of the...
small intestine. A sudden onset of mortality in primarily cockerels, resulting in rotting, friable intestines filled with a foul-smelling exudate is most apt to be necrotic enteritis. Birds are usually 5 to 6 weeks old and almost always on a high energy ration.

Villi tips are necrotic, the deeper glands are cystic, and a mass of fibrin and cellular debris form a pseudomembrane. A milder form, chiefly of cystic glands, is often encountered. Coccidial oocysts of *E. brunetti* are frequently associated with this condition.

**C. Fatty Liver Syndrome**

Fatty liver syndrome is manifested by subcapsular hemorrhages of the liver. The liver color is various shades of orange, it is friable, and at times greasy. Sudden mortality may arise from a flock that is otherwise normal.

In other cases, decreased egg production is the only outward sign. Gross lesions will usually make for an easy diagnosis. The hepatic cells on section are vacuolated owing to the heavy burden of fat and will confirm the diagnosis.

**D. Gizzard Erosion**

The cause of gizzard erosion is unknown and the occurrence of the disease is uncommon. It is a disease of the gizzard epithelium causing loss of this structure. The overlying keratin is accompanied by a mild reaction of heterophils and lymphocytes in the mucosa and nearby muscularis. Protozoa are sometimes seen in a fibrinoid overlay of the lesion.

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MICROTITER AND AUTOMATED SEROLOGIC TECHNIQUES FOR DIAGNOSTIC VIROLOGY

E. A. Carbrey, V.M.D., M.S. and D. R. Downing, B.S.
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An increased demand for the services of the veterinary diagnostic laboratory can only be considered a step towards better disease diagnosis. However, for the laboratory that is deluged with serum samples for a variety of confirmatory serologic tests the workload can be overwhelming because of the many tedious hand operations involved. The conversion of routine serologic tests to microtiter procedures will permit the laboratory to complete its work more rapidly and what is equally important more efficiently and economically as well.

The use of microtiter techniques has some specific advantages for the small laboratory. The plastic plate used in place of the ubiquitous culture tube is disposable so if plastic bottles are used to grow cells the total reduction in dirty glassware will permit the elimination of a dishwasher from the workforce. Since only very small volumes, 0.025 or 0.05 ml, are used in the microtiter test there is a considerable reduction in the consumption of cell cultures, antigens, and serum. The plastic plate takes much less room in the incubator so floor space may be gained through the use of smaller equipment units. The metal loops used for making microdilutions are easily cleaned by flaming while glass pipettes require careful washing and there is usually a ten percent breakage or discard loss on each pass through the washroom.

Microtiter techniques, however, have some disadvantages. There is often the problem of obtaining a useful initial dilution of the serum since the serial dilution obtained is limited to a two-fold scheme. The procedures are tedious and require careful attention due to the miniature size of the equipment used. Since the diluting process requires the rinsing of the diluting loop, the standard error is usually higher than that found with tube tests performed with careful pipetting particularly when a fresh pipette is used for each dilution. The very fact that one initially prepares all tests in duplicate is an indication that cross contamination and errors are difficult to avoid.

Microtiter techniques must be employed if diagnostic laboratories are to keep up with the demands for their service. Some of the techniques used at Veterinary Services Diagnostic Laboratory will be described here. In addition an automated procedure for the performance of viral hemagglutination-inhibition tests with a continuous flow system will be described.
Neutralization Tests

A virus neutralization test is probably the most difficult microtiter procedure to perform since it involves the production of confluent cell monolayers on the flat bottoms of the wells of the microtiter plate. If the serums to be tested are in good condition so that the cell cultures will grow in the presence of the diluted serum it is possible to dilute out the serums in the plate, add the test dose of virus, and after a suitable reaction period add the cell suspension. The plates are then placed in the incubator and a confluent cell sheet will be formed in the wells where the virus has been neutralized by the specific antibodies in the specimen serum.

An alternative procedure that must be employed where the cell cultures will not grow out in the presence of the test serums is to perform the serum dilutions and virus neutralizations in a plastic transfer plate. This plate has an identical pattern of wells to conform to those of the flat bottom plate so that the wells of the transfer plate can be inserted into them. There are tiny holes in the bottoms of each of the wells in the transfer plate so that when the two plates are together the contents of the wells in the transfer plate will mix with those of the culture plate. The holes are small enough so that leakage does not occur as long as the outside of the bottoms of the wells remain dry. Occasionally it is necessary to enlarge the hole before the fluid will be drawn by capillarity into the culture plate. This method permits the propagation of confluent cell cultures in the wells of the flat bottomed plate under ideal conditions before exposing the cells to the serum dilution-virus mixture.

The use of the transfer plate has been found necessary when testing swine serums for swine enterovirus (SE) antibodies. However, bovine serums may be tested for neutralizing antibodies against infectious bovine rhinotracheitis (IBR) and bovine virus diarrhea (BVD) by placing the cells directly in the serum dilution-virus mixture.

Equipment needed for microtiter testing may be obtained commercially. The microtiter plate is a plastic rectangle with a pattern of indented circular wells, either flat or round bottom as required, in 8 rows of 12 with a distance of 10 cm between the center of the end wells on the long side of the plate and a distance of 6.5 cm on the short side. The plates are available in either a flexible or rigid form. For cell culture the rigid plates are much easier to use. Sets of diluting loops calibrated to deliver 0.05 ml and 0.025 ml, one set for each volume delivered, are required. For dispensing diluents and antigens an automatic hand pipetting device may be used or automatic equipment capable of simultaneously loading all 96 wells may be obtained. A mechanical diluting machine is also available for the laboratory with a large number of microtiter tests to perform.

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* Cooke Engineering Co., 900 Slater Lane, Alexandria, Virginia
  • BBL, Division of Bioquest, Becton, Dickinson and Co., Cockeysville, Md.
For adequate mixing of reagents a vibrating machine is very helpful. An inverted low power microscope is essential for the examination of the plates and determining the results of the test. A carbon dioxide incubator is necessary unless special buffer systems such as Hepes buffer is used or the wells are sealed with mineral oil. Even with these aids cell growth is improved if the optimum atmosphere is provided.

The microtiter neutralization tests for IBR, BVD and SE are described below in detail. The initial procedure in all three systems is the titration of the test virus so that the correct dose of virus in 0.025 ml may be introduced into each well. Decimal dilutions of the virus suspension are prepared in culture tubes with Eagle's F-15 medium since this is impossible in the microtiter plates. Then 0.025 ml of each virus dilution is placed in wells with 0.05 ml of the cell suspension and 0.025 ml of additional diluent. The endpoint titer of the virus is determined by following the appropriate procedure described below. It is advisable to repeat the virus titration with every series of tests to insure adequate control.

Cell cultures may be propagated as previously described. The bovine turbinate cell line is used for the IBR and BVD neutralization tests and a primary embryonic swine kidney cell line for the SE test.

**Infectious Bovine Rhinotracheitis Microtiter Neutralization Test**

1. Dispense 0.25 ml of serum into 0.75 ml TRIS* buffered tryptose broth to prepare an initial dilution of 1:4 (final dilution 1-8).
2. Inactivate at 60°C for 20 minutes.
3. Place the microtiter plate on the table with short side toward the operator. Dispense 0.025 ml diluent, Eagle's F-15 with antibiotics, in vertical rows, 2 through 8 of the microtiter plate. Titrations are made across the plate in rows 2-7, leaving row 1 undiluted and row 8 for serum control (SC). The 12 horizontal rows in the plate permit duplicate test rows for 6 serums.
4. Dispense 0.025 ml inactivated serum into first two wells and the last well of each row. Perform duplicate titrations on each serum.
5. Carry out 2-fold dilutions using the diluting loops, beginning in second well. Do not touch the last well since it serves as the serum control (SC).
6. Add 0.025 ml of antigen to all wells except SC well. Virus should be titered so that between 100 and 1000 TCID50 per well are used in test. Mix well using jogger or similar agitation.
7. Allow reaction at room temperature for 1 hour.
8. During hour, use saline-trypsin-versene solution to remove cells from culture flasks. Centrifuge at 800 RPM for 8-10 minutes. Resuspend cells in Eagle's F-15 medium with 5% SPF calf serum, using the cell concentration that will give a confluent cell sheet in two

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* Syntron Division, FMC Corporation, Homer City, Penn.
* Calbiochem., P.O. Box 17, Elk Grove Village, Illinois 60007.
* Grand Island Biologics Company, Grand Island, New York
* Trizma Base No. T-1503, Sigma Chemical Co., St. Louis, Mo.
days. (Use the cell layer of one 250 ml of Falcon flask to prepare 20 ml of cell suspension.)
9. Add 0.05 ml of cells to all wells, including SC well.
10. Add 2-4 drops of mineral oil to each well.
11. Incubate at 37 C in CO2 incubator with 5% CO2.
12. Read test between 48-96 hours, usually 48-72.
13. Controls for normal and positive serums should be handled the same as field serums. Virus controls should start at the dilution used in the test, plus the next three 10-fold dilutions, using 4 wells for each dilution.

**Bovine Virus Diarrhea Microtiter Neutralization Test**
The BVD test is performed as described above except that an initial dilution of 1-8 is prepared by placing 0.15 ml of the test serum in 1.05 ml of tris buffered tryptose broth so that the final dilution in the first well will be 1-16.

**Swine Enterovirus Neutralization Test**
1. Propagate 3-5 day old flask cultures (250 ml) of a primary swine kidney cell line for seeding microtiter plates.
2. Resuspend the harvested cells from one 250 ml flask with 90 ml of Eagle’s F-15 medium supplemented with 15% fetal calf serum.
3. Inoculate each microtiter well with 0.15 ml of the cell suspension. This inoculum should produce a confluent monolayer after 2 days in a 37 C, 5% CO2 incubator with high humidity. Six microtiter plates may be seeded from each flask in this manner.
4. After microtiter cell cultures are prepared, heat inactivate test serums at 60 C for 20 minutes.
5. Place 0.5 ml of whole serum in 4.0 ml of 25% Kaolin-Earle’s medium mixture with antibiotics. This approximates an initial 1-8 dilution. The Kaolin-Earle’s medium mixture is prepared as follows:
   a. weigh 25 gms of Kaolin in a beaker and add 100 ml of phosphate buffered saline (pH 7.2). Mix thoroughly with a spatula and decant into two 50 ml centrifuge tubes. Centrifuge at 800 rpm for 10 minutes.
   b. Aspirate the cloudy PBS supernate.
   c. Resuspend the Kaolin in 100 ml of Earle’s BSS with antibiotics.
6. Allow the treated serum to adsorb for at least 20 minutes at room temperature and then centrifuge for 10 minutes at 2500 rpm.
7. Identify the microtiter transfer plates with a grease pencil indicating the virus group and serum identification.
8. Add 0.025 ml of diluent (Earle’s medium) to the first and last six rows of wells in the transfer plate (longitudinal rows = 8 wells; lateral rows = 12 wells), thus omitting the second row.
9. Add 0.025 ml of the initial dilution to the first row for the serum control, and 0.050 ml to the second row. Prepare duplicate dilutions of each specimen serum.
10. Take diluting loops calibrated at 0.025 ml and starting at the second well carry out the dilutions through the eighth well.
11. One virus-type is inoculated per plate thus allowing for six serums tested in duplicate per plate. The volume of virus inoculum used per well in 0.025 ml containing 100-500 TCID₅₀. Use 1 plate for virus and cell culture controls. The standard dilution of the virus used in the test is titrated as described previously.

12. Place the transfer plates with carriers on a Syntron vibrator for approximately 5-10 seconds using setting number 1. Allow the serum dilution-virus mixtures to neutralize one hour at room temperature.

13. Insert the transfer plate into the corresponding wells of the culture plate for inoculation. Inspect the wells of the transfer plate after withdrawal to assure complete inoculation. Add mineral oil to each well, 2 drops each from 10 ml pipette. Incubate the microtiter plates for five days before final reading.

14. Read virus controls to assure that the correct virus dose was used in the test. Observe the serum controls to detect the presence of toxicity at low serum dilutions and then read the test dilutions. The endpoint antibody titer is expressed as the highest dilution with 100% neutralization of the test dose of virus. In the event one duplicate test results in a higher antibody titer, select the low dilution value as the endpoint titer.

The heat inactivation of the test serums for the neutralization test is difficult to justify unless the advantages of destroying heat sensitive bacteria, nonspecific antibodies, or inhibitors are considered. Although an adequate CO₂ incubator makes the mineral oil unnecessary, it has been found that cross-contamination between wells is reduced when the oil is used.

The mathematical advantage of the microtiter neutralization test is best understood when it is realized that neutralization tests on 27 serums for one virus can be performed with five microtiter plates instead of six racks of cell cultures tubes containing 64 tubes each.

**Hog Cholera Fluorescent Antibody Microtiter Neutralization Test**

The hog cholera (HC) neutralization test as previously described is a tedious hand operation involving the use of at least two coverslip Leighton tube culture per serum tested at a screening dilution. The use of the eight chambered Lab-Tel™ culture slide permits the testing of four serums per slide. This minichamber culture system consists of an ordinary microscope slide with a plastic superstructure that provides eight little cell culture chambers in two rows. The cell cultures produce eight separate confluent monolayers on the surface of the slide. The serum-virus mixtures are introduced into the chambers and after overnight incubation the plastic superstructure is stripped off and the eight patches of cells are stained simultaneously with the conjugate. Reading the test is equally facilitated in that four serums can be read per slide with the test of each serum replicated.

By judicious pooling of serums when a large percentage of the tests are

* Scientific Products, 1210 Waukegan Rd., McGraw Park, Ill. 60085
expected to be negative, the output of the laboratory can be greatly increased.

**Hemagglutination-Inhibition Tests**

The hemagglutination-inhibition (HI) test, probably the most convenient tool of the diagnostic virologist, is also the easiest microtiter test to perform. The HI test may be used to detect antibody titers against several economically important animal and avian viruses: Newcastle disease, parainfluenza-3, avian influenza, and the encephalitis viruses.

When performing the parainfluenza-3 (PI-3) HI test as adapted from the macro technique a preliminary adsorption of the test serums with acid-washed Kaolin is required. This adsorption removes nonspecific inhibitors of hemagglutination that will cause erroneous indications of low antibody titers. This initial kaolin adsorption is also an essential part of the encephalitis virus (Eastern, Western, and Venezuelan) HI technique. In addition the pH must be adjusted to 9.0 and a second adsorption with gander erythrocytes must be performed. Since the specimen serums from horses infected with encephalitis may contain the virus it is essential that laboratory personnel be protected by vaccination. The microtiter test for Newcastle virus is useful for identifying the hemagglutinating virus in the allantoic fluid of inoculated eggs as well as detecting antibody titers in chicken serums.

The PI-3 HI test should be used only on paired, acute and convalescent serums since it is almost impossible to find cattle that do not carry low to medium antibody titers against PI-3 virus. The microtiter HI technique may be performed in any laboratory that can obtain fresh bovine blood.

**Parainfluenza-3 Microtiter Hemagglutination Inhibition Test**

1. Remove bovine erythrocytes from Alsever's solution and wash three times with phosphate buffered saline (PBS) pH 7.2. Dilute 1:4 by volume for brief storage at 4 C. To prepare the standard erythrocyte suspension for the test make an additional dilution of 1-60, e.g. 0.5 ml crude cell suspension in 29.5 ml (PBS).

2. Titrate the antigen by placing 0.05 ml of virus suspension in the first well and 0.05 ml of PBS in all wells down the long side of the plate. Starting with the first well carry out dilutions with the 0.05 loop (rotating the loop 180 degrees, at least 4 times). Place 0.05 ml of washed erythrocytes in each well and incubate at 4 C overnight. Prepare virus titrations in duplicate. The endpoint of the titration is the highest dilution with complete agglutination. The number of hemagglutinating units in the original virus suspension is equal to the dilution factor of the endpoint titer, e.g. if endpoint dilution is 256 then there are 256 units in 0.05 ml of antigen. The standard antigen concentration used in the test should be adjusted to contain 4-8 hemagglutinating units per 0.025 ml.

3. Prepare 1:5 dilution of test serums, 0.2 ml serum to 0.8 ml PBS.

4. Inactivate serums at 60C for 20 minutes.

5. Place approximately 0.02 grams of acid-washed Kaolin in each serum and agitate. Hold for 10 minutes at room temperature to remove nonspecific agglutinins. This amount may be measured with blunt end of
an ink pen inserted backwards in its holder.

6. Centrifuge for 15 minutes at 1500 RPM.

7. Control serums are included with each series of tests. Positive serum is used at 1:20 dilution, 0.05 serum + 0.95 PBS. Normal serum is used at a 1:5 dilution.

8. With short side of plate toward the operator, place 0.025 ml PBS in all except first vertical row of wells on microtiter plate.

9. Place 0.025 ml treated serum in first, second and last, serum control, (SC) wells of each horizontal row.

10. Carry out 2-fold dilutions beginning at second well using 0.025 ml loops. Do not touch SC well.

11. The standard antigen used in the test is titrated in 2-fold dilutions using 0.05 ml quantities. The titration is made in duplicate and is carried through four wells. The completed titration should indicate that 4 - 8 HA units of virus were used in the test.

12. Antigen, 0.025 ml, is added to all wells except serum control and the test is permitted to react for 1 hour at room temperature.

13. Standard bovine erythrocyte suspension, 0.05 ml is then added to all wells and test is placed at 4°C overnight.

14. The test is read after overnight incubation. Serum titers are expressed as the reciprocal of the highest dilution having complete inhibition of the hemagglutination of the antigen.

**Complement Fixation Tests**

The complement fixation (CF) test may be readily adapted to the microtiter technique. An excellent standard protocol is available from the National Communicable Disease Center. The technique is used for the identification of mouse brain isolates of Venezuelan, Eastern, and Western equine encephalitis viruses and for the detection of antibodies against bluetongue (BT) virus. The BT CF test is modified by the addition of calf serum to the guinea pig complement. This modified CF test was found to be more sensitive for detecting antibody against bluetongue virus in both calf and sheep serums. Antibody is detected at about three weeks after infection and persists for at least three months.

The protocol of the microtiter CF technique is too long for inclusion in this brief review. Detailed information may be obtained from the references cited.

**Automated Serologic Techniques**

The ideal system for the performance of a serologic test would involve the combination of microvolumes with a completely automated system from sampling to readout. The Technicon Auto-Analyzer* provides this type of system and it has been adapted for use with the HI tests for PI-3, Newcastle disease (ND), and Venezuelan equine encephalitis (VEE).

The AutoAnalyzer* is a train of interconnected modules (sampler, pumps, colorimeters, recorder) that automate the time consuming, step-

* Technicon Instruments Corp., Tarrytown, N. Y. 10591
by-step procedures of manual techniques. The operation of the instrument is based upon a continuous flow concept, in which reactions take place in continuously flowing, air-segmented streams.

In the automated HI system the sera are poured into small sample cups and placed on a rotating tray. As the tray rotates, a measured amount of each serum is aspirated off and pumped through a peristaltic pump into a mixing coil. A measured amount of antigen is mixed with the serum as it enters this coil; and if antibodies are present, a portion of the antigen will be inactivated corresponding to the antibody content of the serum. As the stream moves out of this coil and into the next coil a measured amount of red blood cells is added. In this coil the free antigen will agglutinate a corresponding percentage of the red blood cells.

The stream then moves through an inclined tube in which the agglutinated masses settle to the bottom of each segment. At the end of this inclined tube is a “T” connector. As the stream passes over this “T”, the agglutinated cells fall into the hole and are drawn off. The remaining stream, containing only the portion of cells that were not agglutinated, is passed through a colorimeter and the turbidity is read quantitatively. The results appear on a strip chart recorder in the form of peaks. The height of each peak represents the titer of the corresponding serum as compared to pretested standards.

The system used for VEE is a two-channel system, using one channel as a negative control, 60 - 80 serums per hour can be tested. With NDV, where a negative control is unnecessary, 120 - 160 serums per hour can be tested.

With the ND system it is approximately 15 minutes from the time the serum samples are sampled by the machine until the results can be read on the strip chart recorder and converted into actual log titers.

The results of a study comparing the automated HI technique with the microtiter technique for detecting ND antibodies are presented in Table I. The sera were collected at a local slaughter station and had HI titers ranging from less than 1-2 to 1:4096 as determined by two initial tests by the microtiter technique. The results listed represent 5 to 7 tests by each technique on 23 randomly selected serums of various titers.

The pooled sample standard deviation of the AutoAnalyzer technique was only log10 0.14 as compared with log10 0.41 for the microtiter method. This lower value for method experimental error of the AutoAnalyzer was considered evidence for a better degree of sample to sample replication of titers. The log10 0.14 corresponded to a dilution factor of 1-1.4 and the log10 0.41 to 1-2.6. The better reproducibility of the AutoAnalyzer over the microtiter method was demonstrated.

Specimen serums of high quality are required by the AutoAnalyzer if good results are to be obtained. The high cost of the equipment is another disadvantage of this system. If additional techniques such as CF and agglutination tests can be adapted for use with the AutoAnalyzer then a small diagnostic laboratory could justify the expense. Otherwise, a large
number of tests would have to be performed per day to achieve a low unit cost.

Summary
Microtiter serologic techniques provide a reasonably accurate and extremely economical means of confirming infections with animal diseases. Although precise and tedious work is involved in their application, the procedure is well accepted by technicians.

Automated procedures such as provided by the AutoAnalyzer system can eventually place low-cost serology within reach of every diagnostic laboratory.

REFERENCES


Table 1. --Comparison of Experimental Error of Microtiter and Auto-Analyzer Hemagglutination Inhibition Methods on Repeated Tests of Field Serums

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of Serums</th>
<th>Average Number of Replications</th>
<th>Pooled Sample Standard Deviation (Log10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtiter</td>
<td>23</td>
<td>7.7</td>
<td>0.41</td>
</tr>
<tr>
<td>AutoAnalyzer</td>
<td>23</td>
<td>5.2</td>
<td>0.14</td>
</tr>
</tbody>
</table>
MICROTITER SEROLOGIC TECHNIQUES
FOR DIAGNOSTIC BACTERIOLOGY
E. M. Ellis, DVM, PhD
Diagnostic Bacteriology
Veterinary Services Diagnostic Laboratory
APHIS
Ames, Iowa

The author expresses appreciation to Billie O. Blackburn, DVM, Rube Harrington, DVM and H.S. Wright, MS, for valuable assistance.

Veterinary Services Diagnostic Laboratory has the responsibility for developing or applying microbiologic techniques that are sometimes new because of an emergency disease or that are of such a complex nature that certain laboratories cannot, or do not elect to conduct them. Some of these are conducted mainly in support of control or eradication measures and are not of primary interest to diagnostic laboratories. However, as the emphasis on control programs moves toward increased state participation, it is believed that these techniques will become of importance to many laboratories.

The adaption of serologic tests to a micro technique where possible offers very obvious and distinct advantages. At a time when laboratory budgets are shrinking and needed personnel cannot be hired, savings in man hours, reagents, equipment and space are of paramount importance. The microtiter technique fulfills the above needs and although some degree of accuracy may be sacrificed, the advantages should be readily apparent.

Veterinary Services Diagnostic Laboratory (Bacteriology Section) now uses several microtiter techniques where large numbers of serums must be tested for antibody.

Microtiter Complement Fixation Test for M. Johnei Antibodies

The microtiter complement fixation test for M. Johnei antibody was adapted from work of Larsen.' The results must be interpreted with care and are most valuable when testing a large herd. Tests on serums from single animals should only be regarded as suspicious when antibody is found.

This examination is a common requirement for export cattle. If positive reactions are found and the herd is negative to the tuberculin test, titers of 1:32 are considered important. Titers of 1:16 are considered suspicious. Tuberculosis or the use of tuberculin in a herd can be the cause of confusing results when the serums are tested. Therefore, a herd history and careful clinical examination should be included to prevent errors in diagnosis. Cattle with typical Johne's disease are not difficult to
detect except for the very young in which it is believed primary infection occurs.

If a small group of cattle are examined, it is preferred that fecal cultures are collected and cultured for *M. johnei*. This examination is available in the Veterinary Services Diagnostic Laboratory and in several state diagnostic laboratories. Training is available through the area veterinarian, APHIS, and is conducted at Ames, Iowa.

Mycobactin, required as a growth substance for *M. johnei* culture, can be obtained by authorized persons from Veterinary Services, Ames, Iowa. Antigen with which to conduct the complement fixation test is available from the same source.

**Microtiter Agglutination Test for Leptospira Antibodies**

Microtiter agglutination tests for leptospiral antibodies are conducted on serums from cattle, swine, horses, sheep, goats and deer. The methodology was described by Cole *et al.* Cattle serums are examined using 12 serotypes and swine serums using 7 serotypes. The increased export of swine and cattle from the United States has created the necessity of testing an extremely large number of serums. Use of the microtiter technique has made possible the testing of these serums, with present space and personnel.

Any discussion of numbers of positive or negative serums has little meaning for two reasons: (1) There is no general agreement as to what titers represents infection and (2) serums from cattle or swine for export are screened at dilutions from 1:100 to 1:1000 depending upon the requirement of the importing country. The reasons for such variation is not apparent.

Complete instructions for conducting bacteriologic microtiter tests are included in a manual available from Diagnostic Bacteriology, Veterinary Services Diagnostic Laboratory, P.O. Box 70, Ames, Iowa 50010.

**Microtiter Complement Fixation Test for M. hyopneumoniae Antibodies**

This test, which has become important to breeders of purebred and specific-pathogen-free swine, was developed by Switzer. This technique, because of its immediate importance will be discussed in detail here.

In recent years, it has been shown that in many cases of swine pneumonia, viral etiology could not be proved, but rather, *M. hyopneumoniae* was isolated. Virus pig pneumonia (VPP) has been recognized throughout the world as one of the major diseases affecting swine. Numerous attempts to prove a viral etiology, including those of Betts, indicated that an agent measuring approximately 250 μm could be recovered from swine lungs by filtration. In an effort to clarify the status of VPP, workers have successfully recovered a small cocco-bacillary organism and proved it to be a mycoplasma. The name *M. hyopneumoniae* was proposed by Mare and Switzer.

The organism has proved to be very difficult to isolate, and produc-
tion of good antigen has been a problem.

The CF microtiter test has been applied to herds where certification regarding freedom from circulating antibody has been required. An additional problem that has arisen relates to the interpretation of the results. Their meaning has not been clear due largely to lack of confirmatory evidence of the disease. Switzer et al. have reported that titers of 1:8 or greater are generally indicative of the presence of the organism in the herd. Until further studies are completed, the test should be applied as a herd test only.

The antigen can be made available in limited amounts to laboratories requesting it through an area veterinarian, APHIS.

It is recommended that personnel receive training in the technique to ensure consistency of results.

**M. hyopneumoniae Microtiter CF Test**

**Equipment**

- Diluters, 0.025 ml
- Pipette droppers, 0.025 and 0.05 ml
- Microtitration plates
- Go-No-Go delivery testers, 0.025 ml
- Shaking apparatus
- Spectrophotometer
- Matched cuvettes
- Waterbaths, 37°C and 62.5°C (Johne’s), 37°C and 55°C (*M. hyopneumoniae*)
- Sealing tape for plates
- Tape roller
- Reading mirror
- pH meter

**Reagents**

- Hemolysin (rabbit anti-sheep serum)
- Complement
- Antigen
- Sheep blood
- Cyanmethemoglobin standard and reagent
- Pig serum, 6-8 weeks (*M. hyopneumoniae* only)

**Chemicals**

- Sodium-5,5-diethyl barbiturate
- Other common chemicals

**Preparing Stock Buffer Solution**

1. To a 2-liter volumetric flask add 1,500 ml of distilled water.
2. Add 83.0 gm of NaCl to the flask.
3. Add 10.19 gm of Na-5,5-diethyl barbiturate.
4. Mix by swirling the flask until chemicals are completely dissolved.
5. Add 34.58 ml 1 N HCl and mix by swirling.
6. Add 5.0 ml of stock magnesium calcium chloride solution. (See procedure for preparation below.)
7. Fill the flask to the 2-liter mark with distilled water and mix by inverting the flask.
8. Check the pH:
   a) Prepare a 1:5 dilution by adding 1 ml of stock buffer solution to 4 ml of distilled water.
   b) Check the pH of the 1:5 dilution.
   c) If the pH is below 7.3 or above 7.4, discard the stock buffer solution and prepare fresh.
9. Store the stock buffer solution in the refrigerator.

Preparing Stock MgCl₂ (1 molar) CaCl₂ (0.3 molar) Solution
1. Add 100 ml of distilled water to a 250-ml Erlenmeyer flask.
2. Add 20.3 gm of MgCl₂ • 6H₂O to the flask.
3. Add 4.4 gm of CaCl₂ • 2H₂O.
4. Mix by swirling.
5. Store in refrigerator.

Determining Volume of Undiluted Complement Needed
Use the formula below to determine the number of ml of undiluted C' needed to prepare a required volume of C' at a given dilution.

\[
\text{Volume (ml) of undiluted C'} = \frac{\text{Volume (ml) desired}}{\text{Reciprocal of dilution of C'} \text{ determined in C'} \text{ titration}}
\]

Example: 40 ml of 1:133 dilution of C' are needed.

\[
\text{Volume (ml) of undiluted C'} = \frac{40}{133} = 0.3 \text{ ml}
\]

Therefore, you would use 0.3 ml C' plus 39.7 ml diluent.

Preparing Hemoglobin Color Standards
1. Use a 2.0 ml pipette to add 1.0 ml of a well-mixed 2.8% RBC suspension to a test tube (15 x 125 mm).
2. Add 7.0 ml of distilled water and shake the tube until all cells are lysed.
3. Add 2.0 ml of stock buffer solution (5X) to the tube.
4. Mix the hemoglobin solution thoroughly and set it aside until it is needed.
5. Use a 2.0-ml pipette to add 1.0 ml of the 2.8% RBC suspension to a test tube (15 x 125 mm).
6. Add 9.0 ml of cold VBD to the tube with the 2.8% RBC's (0.28% RBC's).
7. Mix the 0.28% RBC suspension and set it aside.
8. Label 11 serologic tubes (13 x 100 mm or size to be used in tests) with the percentages of hemolysis shown in the table below. Label the 0% standard with the date and time of preparation.
9. Use a 2.0-ml pipette to add hemoglobin solution in the amounts shown in the table below to each tube.
10. Use a 2.0-ml pipette to add 0.28% RBC suspension in the amounts shown in the table below.
1. Mix the standards by shaking the rack.
2. Centrifuge the tubes at 600 x g for 5 minutes.
3. Remove the tubes from the centrifuge without agitation and store them in the refrigerator until they are needed.

**Preparation of Color Standards**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin solution</td>
<td>0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>0.28 percent cells</td>
<td>1.0</td>
<td>.9</td>
<td>.8</td>
<td>.7</td>
<td>.6</td>
<td>.5</td>
<td>.4</td>
<td>.3</td>
<td>.2</td>
<td>.1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Sheep Erythrocytes**

Sheep blood, drawn aseptically is preserved at 2-4°C in an equal volume of sterile Alsever's solution. Sheep blood preserved in this manner is allowed to age for 4 days prior to use.

After this stabilization period, the susceptibility of the erythrocytes to lysis by antibody and complement remains uniform for about one month, and a batch of blood may be used during this entire period, provided gross microbial contamination is avoided.

**Preparing Alsever's Solution**

1. To a 2-liter flask, add 1,200 ml distilled water.
2. Add 24.6 gm of glucose.
3. Add 9.6 gm sodium citrate (dihydrate).
4. Add 5.04 gm sodium chloride.
5. Mix by swirling the flask until chemicals are completely dissolved.
6. Adjust pH to 6.1 with citric acid.
7. Sterilize by filtration through glass filter.

**Preparation of Cyanmethemoglobin Color Standards and Calculation of Target Optical Density (Absorbance)**

1. Label 5 cuvettes for standards 80, 60, 40, 20 and 0 mg%.
2. Add to the tubes the volumes of 80-mg% standard and cyanmethemoglobin reagent shown in the following table:

<table>
<thead>
<tr>
<th>Table</th>
<th>Tube No. 1</th>
<th>Tube No. 2</th>
<th>Tube No. 3</th>
<th>Tube No. 4</th>
<th>Tube No. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cyanmethemoglobin concentration (mg%)</td>
<td>80</td>
<td>60</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Volume of 80-mg% standard (ml)</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Volume of cyanmethemoglobin reagent (ml)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
3. Wrap 5 cork stoppers in Parafilm-"M" and plug each cuvette. (Parafilm-"M" is manufactured by Marathon Products, Neenah, Wisconsin.)
4. Mix by inverting each cuvette.
5. Wipe each cuvette with a tissue to remove fingerprints; then read and record the optical density (O.D.) for each standard at 540 μ.”
6. Take the sum of the milligram percent cyanmethemoglobin concentrations of all the standards (200 mg%).
7. Take the sum of the O.D. readings of all the standards.
8. To calculate the factor, divide the sum of the concentrations by the sum of the O.D. readings.

Example:

<table>
<thead>
<tr>
<th>Concentration of standard (mg% Cmg)</th>
<th>O.D. 540 Reading of standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>80.0</td>
<td>0.460</td>
</tr>
<tr>
<td>60.0</td>
<td>0.350</td>
</tr>
<tr>
<td>40.0</td>
<td>0.240</td>
</tr>
<tr>
<td>20.0</td>
<td>0.120</td>
</tr>
<tr>
<td>0.0</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\[
\text{Factor} = \frac{200.0 \text{ mg}\% \text{ Cmg}}{1.170 \text{ (total absorbancy)}} = 170.94 \text{ mg}\% \text{ Cmg/O.D.}
\]

9. Use the factor to calculate the Target O.D. of your instrument for a 2.8% sheep cell suspension. Use the formula below:

\[
\text{Target O.D.} = \frac{35.04 \text{mg}%\text{cmg}}{\text{factor}}
\]

Example:

\[
\text{Target O.D.} = \frac{35.04}{170.94} = 0.209
\]

Preparation of Cyanmethemoglobin Reagent

1. Dilute 1 vial or tablet of reagent in twice the volume recommended on the label. This helps to overcome the "resistant cell phenomenon."

2. Store this reagent in a brown bottle (glass or polyethylene) or store in the dark at room temperature. (Do not use rubber or cork stoppers unless they are covered with parafilm, as there is a chemical reaction between the cyanide and these materials which results in contamination of the reagent.

3. Discard the reagent if it becomes cloudy or if a precipitate forms after prolonged use.

Commercial sources of certified cyanmethemoglobin standard and reagent:

- Metrix hemoglobin standard and diluent tablets from— Clinical and Diagnostics Division, Armour Pharmaceutical Co., 530 E. 31st Street, Chicago, Illinois 60616
- Hycel cyanmethemoglobin reagent and standard from— Hycel, Inc., P.O. Box 36329, Houston, Texas 77036
- GlobincaI hemoglobin standard and globin test reagent from— Charles Pfizer & Sons, New York, New York
- Hemachrome-Fe hemoglobin standard and Hemacyn diluent from— Unitek, 7901 San Fernando Road, Sun Valley, California 91350
I. Preparation of Verona1 Buffered Diluent (VBD)
   A. Add 500 ml of distilled water to a 1-liter volumetric flask.
   B. Add 10 ml of 10% gelatin (10 gm of dry gelatin dissolved by boiling in 100 ml distilled water). This will yield a final concentration of 0.1% in 1 liter. Mix thoroughly.
   C. Add 200 ml of stock buffer solution (see page 6—Preparing Stock Buffer Solution).
   D. Fill the flask to the liter mark with distilled water and mix thoroughly by inverting the flask.
   E. Check the pH of the VBD. If the pH is below 7.3 or above 7.4, discard the VBD and repeat preparation.
   F. Label the VBD, including date of preparation.
   G. Store the VBD in the refrigerator.

II. Preparation and Standardization of Sheep Erythrocyte Suspension

A. Preparation and Washing of Sheep Erythrocytes

   1. Determine the volume of packed sheep erythrocytes required for the 2.8% red blood cell (RBC) suspension.
      a. For preparation of color standards, 2.0 ml of 2.8% RBC's are needed.
      b. Determine whether a hemolysin titration is necessary. (A hemolysin titration should be performed each time a new lot of 1:100 hemolysin solution is prepared or a new lot of sheep RBC's is used.) If so, increase the volume of 2.8% RBC's needed by 8.0 ml.
      c. For the complement titration, increase the volume of 2.8% RBC's needed by 2.0 ml.
      d. If diagnostic tests are to be done, determine the volume of 2.8% RBC's required:
         1) Add 1.25 ml for each 12 serums.
         e. Find the total volume of 2.8% RBC's required by adding the volumes determined in steps a-d above.
      f. Determine the volume of packed RBC's required by setting up a proportion:
         \[
         \frac{2.8}{100} = \frac{X}{\text{number of ml of 2.8% RBC's needed}}
         \]
         \*X = number of ml of packed RBC's needed

   2. To determine the volume of preserved blood necessary to provide the required volume of packed RBC's, multiply the required volume of packed RBC's by 10 (preservative—Alsever's Solution—see appendix).

   3. Place preserved blood in round or conical bottomed 50 ml centrifuge tubes.

   4. Centrifuge the blood at 600 x g** for 5 minutes.

   5. Remove the supernatant fluid by suction.

   6. Add cold VBD to fill the tube (9 parts VBD—1 part packed RBC).

   7. Mix gently by inverting tube to resuspend the cells and centri-
fuge at 600 x g for 5 minutes.
8. Carefully remove the supernatant fluid and white blood cell layer by suction.
9. Add cold VBD to fill the tube.
10. Mix gently by inverting tube to resuspend the cells the centrifuge at 600 x g for 5 minutes.
11. Look at the supernatant fluid to see whether it is colorless. If not, the cells are too fragile and should be discarded. Obtain new preserved blood and return to step 2.
12. Remove the supernatant fluid by suction.
13. Add cold VBD to fill the tube.
14. Mix gently by inverting tube.
15. Filter the blood through 2 layers of sterile gauze into a 50-ml centrifuge tube.
16. Centrifuge at 600 x g for 5 minutes.
17. Carefully remove as much supernatant fluid as possible without disturbing the cells.
18. For each 33 ml of cell suspension needed, suspend 1 ml of packed cells in 32 ml of VBD. Use an appropriate sized Erlenmeyer flask.

B. Standardization of 2.8% RBC's—Spectrophotometric Method
1. Turn on the spectrophotometer to allow for adequate warm up time.
2. Swirl the flask containing RBC's gently to secure an even suspension.
3. Carefully pipette 1.0 ml of the 2.8% suspension into a 25-ml volumetric flask.
4. Fill the flask to the 25-ml mark with cyanmethemoglobin reagent (see appendix for cyanmethemoglobin preparation).
5. Mix well by inverting the flask at least 10 times.
6. Allow the suspension to stand at least 20 minutes at room temperature. While the suspension is standing, you should check the 40% cyanmethemoglobin standard to determine whether the optical density reading is withing + 3% of the original reading. If not, prepare new standards and calculate the Target O.D. (If cyanmethemoglobin standards have not been prepared previously, factor and target O.D. must be determined.)
7. When at least 20 minutes are up, mix the suspension again by inverting the flask.
8. Select a clean, calibrated cuvette and fill it with the sample; wipe the cuvette with a paper tissue to remove fingerprints.
9. Read the optical density of the sample against the reagent blank (0 mg%) at 540 ma, being careful to read the O.D. (logarithmic) scale. This is the Test O.D.
10. Calculate the final volume of the desired 2.8% suspension using the formula:
(O.D. of test suspension) X (original volume of test suspension-1.0 ml)

Target O.D. for a 2.8% suspension

11. Dilute the suspension with cold VBD to the desired final volume.

III. Hemolysin Titration*

A. Preparation of 1:100 Hemolysin Dilution
   1. Add 4.0 ml of a 5% phenol solution to a 125-ml Erlenmeyer flask.
   2. Add 94.0 ml of cold VBD to the flask and mix by swirling.
   3. Add 2.0 ml of glycerinized hemolysin and mix by swirling.
   4. Store the 1:100 hemolysin dilution in a refrigerator.

B. Preparing 1:1000 Hemolysin Dilution
   1. Label a 15 x 125 mm tube for the 1:1000 hemolysin dilution.
   2. Add 9 ml of cold VBD to the tube.
   3. Add 1 ml of 1:100 hemolysin dilution and mix.

C. Preparing Further Hemolysin Dilutions
   1. Label six 15 x 125 mm tubes with the final hemolysin dilutions shown in the first column of Table 1.
   2. Use a 5.0-ml pipette to add the volumes of VBD shown in Table 1 to the tubes.

Table 1. Preparation of Further Hemolysin Dilutions

<table>
<thead>
<tr>
<th>Final Hemolysin Dilution</th>
<th>1:1000 Hemolysin Dilution, ml</th>
<th>VBD, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1500</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>1:2000</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1:2500</td>
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<td>3.0</td>
</tr>
<tr>
<td>1:8000</td>
<td>1.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

D. Preparing 1:500* Dilution of Complement (C')
   1. Measure out 99.80 ml of cold VBD and add it to a 125-ml flask.
   2. Obtain lyophilized C' and reconstitute with blood serum obtained from a 6-8 week old pig. Place reconstituted C' in ice bath. (For the M. hyopneumoniae CF test, this is considered undilute C'.)
   3. Draw up undiluted C' in a 1.0 ml pipette beyond the 0.6 ml mark.
   4. Wipe the tip of the pipette and return the excess C' above the 0.6-ml mark to the stock container.
   5. Deliver 0.25 ml of C' dropwise into the VBD in the flask.
   6. Mix by swirling the flask gently.
   7. Place the diluted C' in the refrigerator for at least 20 min-
utes. (Diluted C' should be used within 2 hours.) During this 20-minute waiting period, continue with E1.

E. Preparing Sensitized RBC's for Hemolysin Titration
1. Place seven 13 x 100 mm tubes in the rack. Label the first tube 1:1000, label the remaining six tubes with the final hemolysin dilutions shown in Table 1.
2. Add 1.0 ml of the standardized 2.8% sheep RBC suspension to each of the 7 tubes (prepared IIB11).
3. Add slowly, with constant swirling, 1.0 ml of the 1:1000 hemolysin dilution to the sheep RBC's in the 1:1000 tube.
4. Mix the 1:1500 hemolysin dilution with a pipette and add, with constant swirling, 1.0 ml of the dilution to the sheep RBC's in the 1:1500 tube.
5. For each of the five remaining tubes, mix the corresponding hemolysin dilution with a pipette and add 1.0 ml to the sheep RBC's.
6. Shake the rack for mixing and incubate the 7 tubes in a 37°C water bath for 20 minutes to sensitize the cells. While the tubes are incubating, continue with step F1.

F. Setting up Hemolysin Titration
1. Label 7 serologic tubes (13 x 00 mm) with the following hemolysin dilutions: 1:1000, 1:1500, 1:2000, 1:2500, 1:3000, 1:4000, and 1:8000.
2. Add 0.4 ml of cold VBD to each tube.
3. Add 0.4 ml of the dilute complement to each of the 7 tubes (prepared in steps D1-D7).
4. Shake the rack for mixing.
5. Add 0.2 ml of RBC's sensitized with the same hemolysin dilution as the label on the tube to each tube (prepared step El-E6).
6. Mix each tube by shaking.
7. Incubate the tubes in a 37°C water bath for 1 hour, shaking once after the first 30 minutes of incubation.
8. While the tubes are incubating, prepare hemoglobin color standards. (See appendix for the procedure.)

G. Determining Hemolysin Dilution Needed for sensitization of 2.8% RBC's
1. Centrifuge the tubes at 600 x g for 5 minutes.
2. Compare each tube with the hemoglobin color standards (prepared in step F8).
   a. If the tube matches a standard, read and record the percent hemolysis.
   b. If the tube does not match a standard, interpolate to the nearest 5% and record the reading.
3. Plot on ordinary arithmetic (linear) graph paper the amount of hemolysis obtained with each dilution of hemolysin. (See Figure 1.)
4. Draw a line through the points plotted.

FIGURE 1. HEMOLYSIN TITRATION

*To prepare the hemolysin dilution scale: let the left end of the scale be 0, and lay off a suitable length for the 1:1000 dilution. Other dilutions are represented as fractions of this length. Thus 1:1500 = \( \frac{3}{5} \) of 1:1000, 1:2000 = \( \frac{2}{5} \) of 1:1000, 1:2500 = \( \frac{2}{5} \) of 1:1000, 1:3000 = \( \frac{3}{5} \) of 1:1000, 1:4000 = \( \frac{4}{5} \) of 1:1000, and 1:8000 = \( \frac{8}{5} \) of 1:1000. (Arithmetic graph paper, 20 x 20 to the inch is most suitable, using a 6-inch horizontal distance for the 1:1000 dilution.)

5. Examine the graph for a “plateau,” that is, the level at which increasing the amount of hemolysin produces no marked increase in percent hemolysis. (See Figure 1)

6. Read the second dilution on the plateau as the hemolysin dilution to be used for subsequent RBC sensitization (optimal hemolysin dilution).

IV. Complement Titration*

A. Preparing Complement

1. Measure out 99.80 ml of cold VBD and add it to a 125-ml flask.
2. Obtain undiluted C' and place it in an ice bath.
3. Draw up undiluted C' in a 1.0 ml pipette beyond the 0.6 ml mark.
4. Wipe the tip of the pipette and return the excess C' above the 0.6-ml mark to the stock container.
5. Deliver 0.20 ml of C' dropwise into the VBD in the flask.
6. Mix by swirling the flask gently.
7. Place the diluted C' in the refrigerator for at least 20 minutes. (Diluted C' should be used within 2 hours.) During this 20-minute waiting period, continue with B1.)

B. Preparing Sensitized RBC's
1. Add 1 ml of standardized 2.8% RBC suspension to a 13 x 100 mm tube (prepared II B 11).
2. Prepare optimal hemolysin dilution (III G 6) from the 1:100 stock hemolysin solution.
3. Add 1 ml of optimal hemolysin dilution to the RBC's with rapid swirling.
4. Incubate for 20 minutes in a 37°C water bath. While the RBC's are incubating, continue with step C1 below.

C. Setting Up Complement Titration
1. Label 2 sets of serologic tubes (13 x 100 mm) 1-4.
2. Add VBD in the amounts shown in Table 2 to both sets of tubes.
3. Add 1:500 dilution of C' in amounts shown in Table 2.

<table>
<thead>
<tr>
<th>Table 2. Complement Titration</th>
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<tr>
<td>Reagent</td>
</tr>
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</tr>
<tr>
<td>30.35</td>
</tr>
<tr>
<td>1:500 dilution of C'</td>
</tr>
<tr>
<td>Sensitized RBC's</td>
</tr>
</tbody>
</table>

4. Add 0.2 ml of sensitized RBC's to each tube (prepared step B4).
5. Shake the rack for mixing and place it in a 37°C water bath for 15 minutes.
6. When 15 minutes are up, remove the rack and shake to resuspend unlysed RBC's.
7. Return the rack to the water bath for an additional 15 minutes to give a total of 30 minutes of incubation.

D. Reading Percent Hemolysis
1. Remove the rack from the water bath and centrifuge the tubes at 600 x g for 5 minutes*.
2. Compare each tube in the one set with the hemoglobin color standards. If the tube matches a standard, read and record the percent hemolysis. If the tube does not match a standard, interpolate to the nearest 5% and record the reading (comparisons may be made with a spectrophotometer at a wavelength of 540 mu).
3. Read and record the percent hemolysis in the duplicate set.
4. Determine the average percent hemolysis for each pair of tubes containing the same volume of C'.
5. For each of the four pairs of tubes (containing C'), plot on probability by logarithmic 2 cycle log graph paper the volume of the 1:500 dilution of C' in ml against the corresponding percent of hemolysis. (See Figure 2.)
6. Examine the graph to see whether two of the points fall on the left side of the vertical “50” line and two on the right. If so, continue with step 7. If more than two points fall on the left side of the vertical “50” line, repeat the C' titration using a dilution of C' lower than 1:500 (1:400). If more than two points fall on the right side, repeat the C' titration using a dilution of C' higher than 1:500 (1:600).
7. Join the two points plotted for tubes 1 and 2 and mark the midpoint of the line joining them.
8. Join the two points plotted for tubes 3 and 4 and mark the midpoint of the line joining them.
9. Draw a line between the two midpoints. (See Figure 2.)
10. Determine the slope of the line which joins the two midpoints:
    a. From any point near the left end of the line joining the two midpoints, measure horizontally to a point 100 mm to the right.
    b. Measure the vertical distance in millimeters from that point upward to the line joining the two midpoints.
    c. Divide the vertical distance by 100 mm to obtain the slope. If the slope is 0.44 ± 10%, continue with step E1 below. If the slope is not within ±10%, repeat the C' titration. (Reproducible results are obtained only when the slope of this line falls within ±10% of 0.44.)
E. Determining the Dilution of C' Needed for Diagnostic Test
1. From the intersection of the vertical “50%” line with the line joining the two midpoints, draw a dotted horizontal line to the vertical axis on the left. (See Figure 2.)
2. Read the volume in ml of the 1:500 dilution of C'. This volume contains one 50% hemolytic unit of C' (C'H50).
3. Determine the volume containing five C'H50, by multiplying the volume containing one C'H50 by five. (Five C'H50 in 0.4 ml is the quantity required for diagnostic test.)
4. Calculate the dilution of C' necessary to obtain five C'H50 in 0.4 ml using the following equation:

\[
\text{dilution of C' used in titration} \times \frac{\text{volume containing 5 C'H50}}{0.4} = \text{x} \times \text{dilution of C' needed for 5 C'H}
\]

Example: The volume containing 5 C'H50 at 1:500 dilution is
1.3 ml (5 x 0.26 ml).
The dilution of C' necessary to obtain 5 C'H50 in 0.4 ml is calculated as follows:

\[
\begin{align*}
a. \quad & \frac{500}{1.3} = \frac{X}{0.4} \\
\text{b.} \quad & 1.3X = 500 \cdot 0.4 = 200 \\
\text{c.} \quad & X = \frac{200}{1.3} = 154
\end{align*}
\]

d. The dilution of C' needed is 1:154.

V. Diagnostic Test: Micro Method

1/6 Serum Inactivation and Plate Labeling

1. Inactivate undiluted unknown serum, a known positive serum and a known negative serum for 30 minutes in a 62.5°C water bath. During this incubation period, continue with steps A2-3 and B1-3.

2. Label the rows of wells on plates for diagnostic test according to Table 3 (each square represents a well).

<table>
<thead>
<tr>
<th>Serum Dilutions</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<th>12</th>
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<tbody>
<tr>
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<td></td>
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<tr>
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<tr>
<td>1:4</td>
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<tr>
<td>1:8</td>
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<td></td>
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<tr>
<td>1:256</td>
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</tr>
</tbody>
</table>

3. Number 9 wells on an additional plate for reagent controls according to Table 4.

B. Antigen Preparation

1. Determine the volume of test antigen required by multiplying the number of wells receiving test antigen by 0.025 ml.
Allow excess for pipetting (5 ml).

2. Dilute test antigen to dilution stated on the label, using cold VBD and mix thoroughly.

3. Store the antigen at 0°C until needed.

C. Serum Dilution

1. When the serum has been in the water bath for 30 minutes (step A1), remove and allow to cool to room temperature.

2. Use a 0.025 ml dropper pipette to add 0.025 ml of cold VBD to 1 row of 8 wells for each serum being tested (see Table 3).

3. Add amounts of VBD specified in Table 4 to the reagent control wells.

4. Using 0.025 ml loop (microdiluter), pick up 0.025 ml of each undiluted serum and transfer to the well labeled 1:2; twirl the loop rapidly for 4 seconds to mix. Continue the dilution process in each successive well through 1:256. Repeat for each serum.

D. Diluted Complement Preparation

1. Determine the volume of diluted C' required for the test by multiplying the number of wells in the test by 0.05 ml. Allow some excess for pipetting (5 ml).

2. Calculate the volumes of VBD and C' needed to prepare the required volume of dilute C' containing 5 C'H50 as determined in the C' titration in tubes. (See the appendix for the formula).

3. Add the calculated volume of VBD to a small flask.

4. Add the calculated volume of undilute C' dropwise to the VBD.

5. Mix gently to avoid foaming.

6. Allow the dilute C' to stand at 0°C for at least 20 minutes. Continue with step E1.

E. Reagent Addition and Incubation of Diagnostic Samples

1. Use a 0.025 ml dropper pipette to add 0.025 ml VBD to each 1:4 well. This is the serum anti-complementary (AC) well (Table 3).

2. Use a 0.025-ml dropper pipette to add 0.025 ml of the diluted test antigen (step B3) to wells labeled 1:8 through 1:256 and to the reagent control wells designated in Table 4.

3. Use a 0.05 ml dropper pipette to add 0.05 ml of diluted C' containing 5 C'H50 to wells 1:4 through 1:256 and to the reagent control wells specified in Table 4.

4. Use a 0.025 ml dropper pipette to add 0.025 ml of diluted C' to the reagent control wells specified in Table 4.

5. Turn on mechanical vibrator before placing plates on the vibrator to mix. After mixing 1 minute, remove plates before turning off vibrator. This procedure should be followed each time plates are shaken.

6. Cover the plates with clear acetate sheets to minimize eva-
poration and incubate overnight (16 to 18 hours) at 4°C. Do not stack plates.
7. The following morning remove the plates from the cold and leave at room temperature while completing steps E8 to E13.
8. Prepare sensitized cells. Determine the volume of sensitized cells needed for the test by multiplying the total number of wells in the test by 0.025 ml and adding excess for pipetting (5 ml).
9. Remove the 2.8% standardized RBC suspension from the refrigerator and shake it gently to secure an even suspension (step II B 11).
10. To a flask, add a volume of standardized cells equal to half the volume of sensitized cells needed.
11. Add an equal volume of optimal hemolysin dilution (step III G 6) to the standardized cells with rapid swirling.
12. Incubate the cells in a 37°C water bath for 20 minutes.
13. Remove the sensitized cells from the water bath.
14. Use a 0.025 ml dropper pipette to add 0.025 ml of the sensitized cells to each test well and to the reagent control wells designated in Table 4.
15. Add 0.025 ml of 1.4% unsensitized RBC's (equal quantities of 2.8% RBC's and VBD) to the reagent control wells designated in Table 4.
16. Use a 0.025 ml dropper pipette to add 5 drops of the 30% hemoglobin color standard to 1 well and 5 drops 90% hemoglobin color standards to a second well on the plate containing the reagent controls.
17. Cover each plate with 3-inch transparent plastic or cellophane tape.
18. Shake the plates until the cells are suspended (approximately 1 minute).
19. Place the plates in 1 37°C incubator for 15 minutes. Do not stack the plates.
20. Remove plates and shake on shaker to resuspend unlysed cells. Return to incubator for an additional 15 minutes to give a total of 30 minutes incubation.

F. Reading the Recording Test Results
1. Centrifuge the plates for 5 minutes at 300 x g. If centrifuge carriers are not available, let plates stand in the refrigerator for 2 to 3 hours until the cells settle.
2. Read and record the results of reagent controls by comparing the percent hemolysis with the color standards. Interpret results based on Table 5.
3. Compare the reagent control readings with those in Table 4 to see whether they are acceptable. If the controls are not acceptable, disregard the test results and repeat the test.
4. If the reagent control readings are acceptable, read and re-
cord the percent hemolysis in each well in the test. Use the signs shown in Table 5 for recording the readings.
5. The RBC's should be completely hemolyzed in the AC control well (1:4 dilution). If not, this serum is AC and the test results are unreliable. Record this serum as being AC.
6. Serum titer is the reciprocal of the highest dilution in which less than 30% of the RBC's are hemolyzed. Thirty percent or more hemolysis is interpreted as negative.

### Table 4

<table>
<thead>
<tr>
<th>Well No.</th>
<th>Purpose of Control</th>
<th>VBD</th>
<th>Ag</th>
<th>C'</th>
<th>SRBC'S</th>
<th>Normal RBC'S</th>
<th>Acceptable limit of hemolysis on controls</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Ag anticomplementary</td>
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<td>.025</td>
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<td>Ag-hemolysin-hemolytic</td>
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<td>.05</td>
<td>.05</td>
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Number 9 wells on one plate. These are the reagent controls for the test.

### Table 5

<table>
<thead>
<tr>
<th>Percent Hemolysis</th>
<th>Interpretation</th>
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</thead>
<tbody>
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<td>0% - 29%</td>
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</tr>
<tr>
<td>30% - 90%</td>
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</tr>
<tr>
<td>91% - 100%</td>
<td>-</td>
</tr>
</tbody>
</table>
Summary

Microtiter serologic techniques have proved to be a saving in time and materials. Their introduction into the diagnostic laboratory has made possible the testing of considerably more specimens than would be possible using previous test tube techniques.

References

**Obtained by centrifugation at 2,000 rpm in an SB 1 International Centrifuge with a head having a radius of 13 cm. or at 1,700 rpm in an SB 2 International Centrifuge with a head having a radius of 19 cm. The general case formula for obtaining the number times gravity is $1118 \times R \times (rmp)^2 \times 10^{-9} (R \text{ radius of centrifuge head in cm.}).$

*A hemolysin titration should be performed each time a new lot of sheep RBC's or hemolysin is used.

*Lyophilized complement rehydrated in swine serum diluted 1:500 generally will yield 30% to 80% hemolysis for optimally sensitized cells. To obtain the correct percent hemolysis with a less active complement, it may be necessary to use a 1:400 dilution for the titrations. With a very potent complement, it may be necessary to use a 1:600 dilution.

*Complement must be titrated each time a diagnostic test is performed.

*See footnote on page 8 for the general case formula for obtaining the number times gravity.
A MICROTITER TECHNIQUE FOR THE COMPLEMENT FIXATION TEST FOR ANAPLASMOSIS

Martin, W. H. and Ritchie, W. H.

I. Introduction

The complement fixation technique for anaplasmosis that has been used until this year was adapted as a standard procedure in 1958.1

Developments in antigen preparation and purification since that time have provided the laboratory with a diagnostic product enabling us to develop more precise methods of test. Antigens used in the old tube method contained hemoglobin, cell protein, and particulate material and a relatively small percent of antigen. This antigen, when used in standard microtiter equipment, quickly clogged the micropipettes and microdiluters. Ameralt and Roby2 first described a method by which anaplasma marginale could be released from an infected erythrocyte using the French Pressure Cell (FPC) in a highly purified form. Stained sides of the released organism show only dark staining bodies characteristic of the intracellular A. marginale organism. The released organism (antigen) has been shown to fix complement when highly diluted.

In developing a technique for testing for anaplasmosis, the general method of standardizing reagents described in "Standard Diagnostic Complement-Fixation Method and Adaptation to Micro Tests" was followed. Slight changes were made in cell concentration, diluents and methods of incubation, however, methods of reagent standardization were approximately the same.

The microtiter technique has been employed at Beltsville since April 1973. Prior to this time, an evaluation was made on its accuracy and efficacy using the tube test as a standard. Routine diagnostic samples as well as numerous samples collected from animals experimentally inoculated with A. marginale over the past 5 years were used for this study. Selected herds also were tested having high, medium and low incidence of the disease as shown by the standard test.

Agreement between the two techniques on all samples tested was 96 percent. Of the 7,120 samples tested, the results were identical on all positive (4+) samples. A few samples showing suspicious (1+, 2+ or 3+) reactions on the tube test were negative on the microtiter test. Those samples showing negative reactions on the tube tests were also negative on the microtiter system.

When positive (4+) samples are titrated, the microtiter technique is usually in agreement with the tube, however, where discrepancies occur the microtiter results are usually one dilution higher. This is probably due to the use of antigen freed of extraneous matter.

Neither the standard tube nor the microtiter test can differentiate
an infected animal from a vaccinated animal. Vaccinated animals often show a persistent CF titer for as long as 23 weeks. In the case of treated animals the CF reaction may persist for as long as 9 months.

II. The Standardized Microtiter CF Test For Anaplasmosis

A. General Information

1. Antigen. The antigen for the microtiter test has been prepared from acute experimental cases of bovine anaplasmosis which has been lysed by the French Pressure cell method and washed free of all hemoglobin and cellular debris. Stained preparations of this antigen reveal only the dark staining bodies associated with clinical anaplasmosis.

The antigen is diluted in veronal buffer containing calcium and magnesium and employed in the test at its optimum dilution based on standard checkerboard or box titrations using positive serums.

2. Serum. Clear phenolized serum at a final concentration of 0.25 percent and free of hemolysis is tested for the presence of the specific *Anaplasma marginale* antibody. Inactivated serum (58°C for 35 minutes) is diluted in veronal buffer solution (1-5) for the test.

3. Complement. Normal guinea pig serum is used as complement. A pool of complement for laboratory use can be produced in the following manner: A minimum of 15 healthy, mature guinea pigs are used which have been fasted 18 hours prior to bleeding. The animals are exsanguinated by severing the neck vessels and the blood collected in centrifuge tubes and allowed to clot. The clot is loosened from the inner surface of the tube by means of a rigid wire and then centrifuged. The clear, hemolysis free serum (complement) is poured off, sealed in airtight ampules, and stored at -60°C or lower.

4. The Hemolytic System.

a. The Hemolytic System consists of complement as described which consists of equal parts of spectrophotometrically standardized washed sheep red blood cells and an optimum dilution of hemolysin. Hemolysin and sheep cells are mixed in equal parts and are placed in the 37°C water bath *ten minutes before using*.

5. The total volume of reagents in this test is 0.125 ml. Standard microtiter "U" plates, preferably those having a curved lip surface is used in this procedure.

B. Reagents Used in the Complement-Fixation Test.

1. Veronal buffer stock solution Preparation.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (C.P.)</td>
<td>85.0 g</td>
</tr>
<tr>
<td>5,5 diethylbarbituric acid</td>
<td>5.75 g</td>
</tr>
<tr>
<td>Sodium 5,5 diethylbarbiturate</td>
<td>3.75 g</td>
</tr>
<tr>
<td>Magnesium Chloride (MgCl₂ • 6H₂O)</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Calcium Chloride (CaCl₂ • 2H₂O)</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Distilled Water—q.s.</td>
<td>2000 ml</td>
</tr>
</tbody>
</table>

5,5 diethylbarbituric acid is insoluble in cold water and only slightly
soluble in boiling water. It is therefore important that the buffer solution be made in the following manner:

Measure approximately 2,000 ml of distilled water into a graduate. Pour about 500 ml of this volume into a 1,000 ml beaker and put on a hot plate. To this add the 5,5 diethylbarbituric acid and bring to a boil and continue boiling until all the crystals are dissolved. To the remaining 1,500 ml of distilled water add the Sodium Chloride, the Sodium 5,5 diethylbarbiturate, the Magnesium Chloride and the Calcium Chloride. It may be necessary to warm the solution to dissolve all the constituents. Mix the two solutions thoroughly by pouring from container to container. Then bring the volume of the liquid to exactly 2,000 ml by the addition of distilled water. Place in Erlenmeyer flasks and sterilize at 15 pounds pressure for 20 minutes. The pH of the diluted buffer solution should be 7.3-7.4.

Used as follows:
Serves as a diluent (1 + 4 dilution) throughout the test for serum, antigen, complement, hemolysin and sheep cells.

3. Alsever's Solution.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium citrate</td>
<td>12.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4.2 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20.5 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

(a) The sodium citrate and sodium chloride are dissolved in 800 ml distilled water and sterilized at 15 pounds pressure for 15 minutes. Dissolve the dextrose in 200 ml distilled water and sterilize by Seitz filtration. Add aseptically to the sterile saline-citrate solution.
(b) Dispense aseptically 150 ml of the sterile solution into 500 ml Erlenmeyer flasks which are marked at the 300 ml level.


(a) A normal adult sheep is bled from the jugular vein into a 500 ml flask containing 150 ml of Alsever's fluid. (Blood collected using aseptic precautions.)
(b) When sufficient blood is collected to raise the level in the flask to the 300 ml mark, the needle is then withdrawn from the vein, and the site is swabbed with 70 percent alcohol.
(c) The blood and preserving fluid are mixed thoroughly and stored at 4°C in the refrigerator. This is the stock supply of sheep red blood cells, and it keeps satisfactorily for a period of 3 weeks.

III. Spectrophotometric Standardization of Reagents for the Complement-Fixation Test

A. Spectrophotometric Standardization of Sheep Erythrocytes

1. The sheep erythrocytes in Alsever's solution is thoroughly mixed by gentle rotation each day that the cells are to be used.
2. Sufficient blood to meet that days needs is aseptically removed by means of a sterile pipette and placed in a sterile conical centrifuge
tube.

3. Centrifuge for 10 minutes at 1000 g. and remove the supernatant by suction and wash cells 3 times with veronal buffer.

4. Record the volume of packed cells after the last wash and prepare a suspension of cells exceeding the usual “2 percent” by resuspending the cells in veronal buffer to make 40 volumes of the suspension. For example, if the packed cell volume is 2.4 ml the total volume of the suspension should be $2.4 \times 40 = 96$ ml.

Standardize the suspension spectrophotometrically at 540 millimicrons using the Coleman Jr. Model 6/20 with a 12 mm adapter and calibrated 12 X 75 cuvettes. Using a 1.0 ml pipette, transfer exactly 0.3 ml of the cell suspension to each of 2 tubes and lyse the erythrocytes by adding 1.7 ml distilled water. When lysis is complete read the optical density of the lysate against a cuvette of water as a blank. Mean readings of 0.600 + 0.005 are acceptable. Correct to this optical density by adding (or removing) veronal buffer, the required final volume of the suspension being determined according to the relation $V_2 = V_1 \times \text{OD}_1$ where $V_1$ is the total volume of the suspension yielding optical density $\text{OD}_1$ and $V_2$ is the final volume required to yield the desired optical density of 0.600. Should $V_2$ prove less than $V_1$, centrifuge a volume of the cell suspension, remove the required amount of veronal buffer and resuspend the remaining cells and veronal buffer in the original suspension.

If another instrument is used, a RBC concentration of approximately $5 \times 10^8$ cells per ml must first be prepared. Using .3 ml of this suspension, lyse with 1.7 ml distilled water. The OD of this solution is read against a suitable blank at the proper wavelength for hemoglobin of that instrument. This is the target OD for the cell concentration to be used in this test.

**B. Hemolysin Titration.**

1. Commercially available anti-sheep hemolysin may be used for these tests.

   (a) Prepare a 1:100 dilution of hemolysin solution by adding 0.1 ml hemolysin to 9.9 ml veronal buffer.

   Higher dilutions of 1:00 or greater are prepared by making further dilutions of 1:100 as shown in the following table.

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>1 ml of Hemolysin dilution</th>
<th>Diluent ml</th>
<th>Final dilution of hemolysin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:100</td>
<td>plus</td>
<td>9.0 gives</td>
</tr>
<tr>
<td>2</td>
<td>1:1000</td>
<td>&quot;</td>
<td>1.0 &quot;</td>
</tr>
<tr>
<td>3</td>
<td>1:1000</td>
<td>&quot;</td>
<td>1.5 &quot;</td>
</tr>
<tr>
<td>4</td>
<td>1:1000</td>
<td>&quot;</td>
<td>2.0 &quot;</td>
</tr>
<tr>
<td>5</td>
<td>1:1000</td>
<td>&quot;</td>
<td>3.0 &quot;</td>
</tr>
<tr>
<td>6</td>
<td>1:1000</td>
<td>&quot;</td>
<td>7.0 &quot;</td>
</tr>
</tbody>
</table>
Lysed standardized cells (0.3 ml plus 1.7 ml distilled water) for hemolysis control. 0.600 + .005 OD.

Figure 1

<table>
<thead>
<tr>
<th>Dilution of Hemolysin</th>
<th>Ave. Optical Density</th>
<th>Percent Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1000</td>
<td>.420</td>
<td>70</td>
</tr>
<tr>
<td>1:2000</td>
<td>.370</td>
<td>62</td>
</tr>
<tr>
<td>1:2500</td>
<td>.360</td>
<td>60</td>
</tr>
<tr>
<td>1:3000</td>
<td>.345</td>
<td>58</td>
</tr>
<tr>
<td>1:4000</td>
<td>.330</td>
<td>55</td>
</tr>
<tr>
<td>1:8000</td>
<td>.240</td>
<td>41</td>
</tr>
<tr>
<td>1:12000</td>
<td>.220</td>
<td>36</td>
</tr>
<tr>
<td>1:16000</td>
<td>.190</td>
<td>32</td>
</tr>
</tbody>
</table>

(optimal hemolysin dilution 1:2500)

Place 16 12 x 75 cuvettes in a rack (each dilution to be set up in duplicate) and add .03 ml of each of the hemolysin dilutions starting with
1:1000 to each 2 tubes. Add 0.3 ml of the spectrophotometrically standardized sheep cells to each tube. Incubate (sensitize) this cell-hemolysin mixture for 10 minutes at 37°C. To each of the tubes, add 0.3 ml of 1:250 complement C' (which is approximately 1C'H$^{50}$) diluted in veronal buffer and 0.6 ml of veronal buffer.

Incubate for 30 minutes. At the end of 30 minutes, add 0.5 ml cold veronal buffer, centrifuge at 1000 g, and read spectrophotometrically using veronal buffer as a blank. Calculate the percent hemolysis by dividing the OD of each hemolysin dilution by the hemolytic control OD.

The amount of hemolysis obtained with each dilution of hemolysin is plotted on ordinary graph paper as shown in figure 1. The "optimal dilution" of hemolysin is determined from the graph by inspection.

The "optimal dilution" is that point where further increases in the concentration of hemolysin does not appreciably change the percent lysis. Read the second dilution on the plateau, i.e. the level at which increasing the amount of hemolysin produces no marked increase in percent hemolysis. Proceeding from left to right the optimal dilution is 1:2500.

**C. Complement Standardization.**

A stock dilution of normal guinea pig serum (complement) is prepared in veronal buffer at 1:30 dilution. The titrated dilution (D$_2$) of complement is ten times that of stock dilution (D$_1$).

The protocol shown in Table 1 is followed to determine hemolytic activity of the complement. The titration is conducted in 12 X 75 mm cuvettes and the results read on the Coleman Jr. Spectrophotometer 6/20. Tubes 1-6 are prepared in duplicates. The fifty percent hemolytic unit (C'H$^{50}$) is determined by plotting on log-probit coordinates, the employed volumes of D$_2$C' vs the average of the corresponding percentages of hemolysis and fitting a straight line to the experimental points as illustrated in Figure 2. A solution of C' containing 5 fifty percent hemolysis units per 0.3 ml (5.C'H$^{50}$/0.3 ml) of use in diagnostic C-F test then may be prepared by further dilution of the stock solution (D$_1$) using the factor determined as follows:

\[
\text{Dilution factor} = \frac{0.3D_2}{5V D_1}
\]

where $D_1$ = the dilution of stock C'

\[D_2 = \text{the dilution of C' used in the titration}\]

\[V = \text{the volume of D$_2$ giving 50 percent hemolysis}\]

Dilution Factor is the reciprocal of the dilution of stock C' (D$_1$) that will yield a solution containing 5 C'H$^{50}$/0.3 ml.

Under the condition of the titration, $D_2 = 10D_1$. Thus, the relation can be simplified to:

\[
\text{Dilution factor} = \frac{3}{5V} = \frac{0.6}{V}
\]

In the example given in Table 1, $V = 0.38$ ml. (See figure 2). Thus, the
Dilution Factor = 0.6/0.38 = 1.6. The solution containing 5 C'H60/0.3 ml for use in the C-F tests, therefore, would be prepared by making a 1:1.6 dilution of the stock (D1) C'. For Example, 20.0 ml of stock C' diluted with 12.0 ml of VB would prepare 32.0 ml of five-unit C'.

After the five-unit C' has been allowed to equilibrate in the refrigerator for at least 30 minutes, the accuracy of the titration is checked by carefully pipetting 0.06 ml volumes of the five-unit C' (use a 0.1 ml pipette) into three 12 X 15 mm cuvettes and adding 0.84 ml of cold VB and 0.6 ml of sensitized cells of each. The contents of the tubes are mixed and incubated in the water-bath for 30 minutes at 37°C as for the C' titration. Following incubation, 0.5 ml of cold VB is added, the contents mixed, and the tubes centrifuged to sediment the unlysed cells. Using the controls (tubes 7-9 having 100 percent hemolysis and 10) of the initial C' titration, the optical densities of the supernates are determined spectrophotometrically and the percentage of hemolysis calculated for each. The five-unit C' is deemed satisfactory for use in C-F tests if the checks yield 50-55 percent hemolysis.

D. Color Standards for Estimating Percent Hemolysis

As an alternate method for determining the percent hemolysis in place of the spectrophotometer a set of color standards may be used. Those standards should be prepared on the day the tests are to be read.

I. REAGENTS:

A. Hemoglobin solution: lyse exactly 3.0 ml of the spectrophotometrically-standardized erythrocyte suspension with exactly 9.6 ml
of distilled water. When lysis is complete, add exactly 2.4 ml of stock VB (conc. 5X), and mix well.

B. Erythrocyte suspension: dilute exactly 3.0 ml of the spectrophotometrically-standardized suspension with exactly 12.0 ml of VB (conc. 1X), and mix well.

II. PROCEDURE:

Combine the reagents as follows to simulate the given percentage of hemolysis:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Percent Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0 10 20 30 40 50 60 70 80 90 100</td>
</tr>
<tr>
<td>B</td>
<td>1.5 1.35 1.20 1.05 0.90 0.75 0.60 0.45 0.30 0.15 0</td>
</tr>
</tbody>
</table>

Standards representing degrees of hemolysis less than 70 percent are spun for 10 minutes at 1000 G; cells of the remaining standards are maintained in suspension while in use.

In reading the results of tests, tubes in which 70 percent or more of the cells are lysed are read with cells in suspension by comparison with the corresponding standards. Tubes showing less than 70 percent are spun for 3 minutes at 1000 G, and read by comparing their
supernates with those of the centrifuged standards. The reactions are recorded, interpolating to the closest 5 percent hemolysis.

E. Antigen Standardization

The antigen which is a suspension of \textit{Anaplasma marginale} in a buffer solution is put up in 2.0 and 10.0 ml ampules with the lot number and production date stamped on the vial. Because a relatively large quantity of the antigen is used in standardization using the checkerboard or block titration with serial dilutions of specific high titered and other reference antiserums the antigenic unit or dilution of the antigen will be specified with each lot of antigen.

For example the optimal antigen dilution for the present lot of antigen ABA-1 is 1:40 in veronal buffer. 0.025 ml of this dilution of antigen is used in the routine test.

IV. The Standardized Tests of Anaplasmosis

To perform the test proper, standardization of all reagents must be accomplished prior to use.

A. Antigen diluted in veronal buffer according to the optimal dilution stated with that lot.
B. Complement (C') diluted according to the results of the complement titration such that 5C'H₉₀ units are obtained.
C. Hemolysin diluted to its optimal dilution according to the titration.
D. Sheep cells standardized to OD 0.600 ± 0.005. (Equal volumes of diluted hemolysin and standardized sheep cells are mixed together and sensitized for 10 minutes at 37°C prior to use.)
E. Standard positive and negative control serums.

V. Procedure

For each serum being tested a 1-5 dilution is made in 12 X 15 tubes usually by adding 0.1 ml of the serum to 0.4 ml veronal buffer. The tubes are then placed in a water bath at 58°C for 35 minutes to inactivate the serums. Remove the rack from the water bath and allow to come to room temperature.

To each of two wells in the microtiter plate add 0.025 ml of the inactivated serum. The protocol for the test is shown in Table 2.

\begin{table}[h]
\centering
\begin{tabular}{lccc}
\hline
Reagent & Well 1 & Well 2* & Incubation** \\
\hline
Test serum & 0.025 & 0.025 & Incubate in water bath \\
diluted antigen & 0.025 & 0.025 & 1 hr. at 37°C \\
complement & 0.025 & 0.025 & \\
Veronal buffer & & 0.025 & \\
Hemolysin and erythrocyte suspension mixture & 0.05 & 0.05 & Incubate 45 minutes at 37°C \\
\hline
\end{tabular}
\end{table}
**serum control**

**Incubate sealed perforated plates by floating on surface of water bath**

The plates are removed from the water bath after the second incubation and either centrifuged or placed in the refrigerator overnight. The test is read by means of a microtiter reader using an overhead light. A positive and negative control should be included with each test series.

**VI. Interpretation**

All serum controls, those wells which did not receive antigen should be completely hemolyzed. If any serum control tube contains unhemolyzed cells it is reported anticomplementary.

Those wells which contains the test samples plus antigen may show reactions from complete hemolysis (negative reaction) to complete fixation (positive reaction).

The interpretation of those reactions falling between these two points is as follows:

- 4 plus—no hemolysis—positive
- 3 plus—25% hemolysis—suspicious
- 2 plus—50% hemolysis—suspicious
- 1 plus—75% hemolysis—suspicious
- Trace—few cells remaining—negative
- Complete hemolysis—negative

**VII. Serum Titration**

The titer of a positive serum is indicative of the stage of the disease whether it is from an acute or carrier animal. This is accomplished by serially diluting the inactivated 1-5 serum in veronal buffer in a microtiter plate.

Using a microtiter plate, pipette 0.025 ml of veronal buffer into wells 2 through 11. Into wells 1, 2 and 12 add 0.025 ml of the inactivated diluted serum. Using a 0.025 ml microtiter diluter, starting with well 2 serially dilute the serum through well 11. Well 12 is the serum control. A positive control serum should be included each time a group of serums are titrated.

The antigen and complement are added to the wells as in the test proper. Well number 12 receives only complement and hemolytic system. The titer of that serum is the highest dilution of the serum showing complete fixation (4+).

**VIII. Equipment Required**

1. Microtiter “U” plates.
2. 0.025 and 0.05 ml micropipettes.
3. 0.025 microdiluters.
4. Tape sealers.
5. Tape perforator.
6. Centrifuge carriers for plates.
7. Test reading mirror.
8. 0.025 ml loop delivery testers.
9. Coleman Junior Spectrophotometer*.
REFERENCES


* If this particular instrument is not available, the standardization for other spectrophotometers can be found on p. 19 of Public Health Monograph No. 74. Standardized Diagnostic Complement Fixation Method and Adaptation to Micro Test. Use $5 \times 10^8$ cells per ml instead of the numbers listed.
THE USE OF MICROTITER HEMAGGLUTINATION-INHIBITION IN MYCOPLASMA GALLISEPTICUM TESTING PROGRAM

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Raleigh, N.C. 27605

In 1966 Mycoplasma gallisepticum (S-6) testing was started in North Carolina. The number of tests done by our laboratory increased from 70,220 in 1965 to 286,261 in 1966. All samples were tested by the serum plate method. Positive flocks were confirmed by the Hemagglutination-inhibition test. This was performed in 12x75 mm tubes as outlined in the USDA, ARS Protocol, Standard Methods for Testing Avian Sera for the Presence of Mycoplasma gallisepticum—Antibodies (March 1966). The number of HI tests that could be done was limited. In 1966, only 786 were done. In 1967 when we tested 462,827 samples by the serum plate method, only 550 were tested by HI. There was clearly a need for a faster method.

In late 1966 we began working with microtiter by setting samples up in duplicate (tube and microtiter). The microtiter technique is taken directly from the USDA protocol, using the same proportions of serum, antigen, diluent and red cells. The principle difference is in titrating the antigen. In microtiter the dilutions are set up in duplicate rather than the method outlined by USDA. A back titration or antigen control is always set up with the HI test to insure a proper antigen dilution.

Also, the red cell suspension is increased to 0.5%. Homologous cells are used from birds negative to the serum plate test.

After we had demonstrated to our own satisfaction that the microtiter was equivalent to the tube method, we started using it exclusively.

In 1972 we tested 524,845 samples by serum plate and approximately 1,500 by microtiter HI.

MYCOPLASMA GALLISEPTICUM
Hemagglutination-Inhibition Test
Microtiter Technique

HEMAGGLUTINATION
1. Mark off two rows of 10 wells ea. for antigen titer (H.A. is done in duplicate).
2. Mark off last well in each row for cell controls.
3. Prepare in small test tube (12 x 75 mm) a starting dilution of antigen as follows:
   0.1 ml antigen plus 0.9 ml Phosphate Buffer Solution (PBS)—
   This is a 1:10 dilution.

598
4. Add 0.05 ml PBS to all wells including cell controls.
5. Add 0.05 ml antigen (1:10 dilution) with diluters to the first well in both rows, mix well, transfer diluter to second well of each row and mix, continue through the 10th well of each row. With mixture in diluter from last well, check diluter on go-no-go card, then place diluter in distilled water. If diluter checks out, antigen dilution will be 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, 1:5120.

6. Add 0.05 ml of 0.5% erythrocyte suspension to all wells using a 0.05 dropper.

7. Seal plate, shake and allow to stand at room temperature until cells in cell control gather in compact button. The titer is the highest dilution in which agglutination is complete. This dilution contains 1 H.A. unit in 0.05 ml.

8. Prepare a dilution of antigen which contains 8 H.A. units in 0.05 ml.

   Example: If the antigen titer is 1:640 then that dilution contains 1 H.A. unit per 0.05 ml. Then 640 × 8 = 80, a dilution of 1:80 contains 8 H.A. unit.

   640 ÷ 4 = 160, a dilution of 1:160 contains 4 H.A. units per 0.05 ml.

HEMAGGLUTINATION-INHIBITION

1. Prepare two dilutions of antigen, one containing 8 H.A. units per 0.05 ml and one containing 4 H.A. units per 0.05 ml. The 4 unit antigen can be prepared from the 8 unit antigen by mixing with equal parts of PBS.

2. Mark off one row of 8 wells for each test.

3. Prepare a 1:5 dilution of each sera to be tested in small test tube (12x75 mm): 0.1 ml serum plus 0.4 ml PBS or 0.05 ml serum plus 0.20 ml PBS.

4. Add 0.05 ml PBS with the 0.05 ml dropper to the first wall in each row.

5. Add 0.05 ml of 8 unit antigen to well Number 2 in each row.

6. Add 0.05 ml of 4 unit antigen to wells Numbers 3 through 8 for each row.

7. For each serum to be tested, load 0.05 ml diluter with 1:5 dilution as prepared in No. 3 and place in first well of row.

8. Mix well and transfer loaded diluter to Number 2 well, etc., through well No. 8.

9. Well No. 1 (serum dilution of 1:10) is serum control.

Well No. 2 = 1:20
Well No. 3 = 1:40
Well No. 4 = 1:80
Well No. 5 = 1:160
Well No. 6 = 1:320
Well No. 7 = 1:640
Well No. 8 = 1:1280
Well No. 9 = 1:2560
Well No. 10 = 1:5120
10. Antigen Control
   a. Mark off 6 wells for antigen controls.
   b. Add 0.05 ml PBS to Wells 2, 3, 4, 5, and 6
   c. Add 0.05 ml 8 unit antigen to Wells 1 and 2
   d. With empty diluter, mix contents of Well No. 2 and transfer to Well No. 3 etc. through Well No. 6.
   e. Well No. 1 contains 8 units
      Well No. 2 contains 4 units
      Well No. 3 contains 2 units
      Well No. 4 contains 1 unit
      Well No. 5 contains ½ unit
      Well No. 6 contains ¼ unit
   f. Mark off two wells for cell controls and add 0.05 ml PBS to each.
   g. Add 0.05 ml of a 0.5% suspension of red blood cells (chicken cells for chicken serum—turkey for turkey serum) to all wells.
   h. Seal all wells and shake thoroughly
   i. Incubate at room temperature for 30-45 minutes.

The H.I. titer is the highest serum dilution exhibiting complete inhibition of hemagglutination as indicated by flowing of cells.

Interpretation: Using a 4 unit antigen, serum having a titer of 1:80 or greater is considered positive. A titer of 1:40 is suspicious. Serum with a titer of 1:20 or less is negative.
A SIMPLE AND RAPID MICROTEST PROCEDURE FOR DETERMINING NEWCASTLE HEMAGGLUTINATION-INHIBITION (HI) ANTIBODY TITERS

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SUMMARY
A simple manual microtest procedure is described for Newcastle hemagglutination-inhibition (HI) tests. The method has features that enable diagnostic laboratories with limited resources and personnel to perform a large number of tests in a short period of time at a moderate cost. Adoption of this method of HI determination could prove most beneficial in vaccine evaluation if virulent Newcastle disease became widespread beyond the point of feasibility for total eradication.

INTRODUCTION
Since 1970, virulent strains of viscerotropic Newcastle disease virus have produced severe worldwide losses in poultry. Attempts to reduce these losses in endemic areas have usually included vaccination with a variety of Newcastle vaccines. Results of vaccination efforts have been variable; some report excellent protection against the lethal effects of the disease, and others report little or no acquired protection. These differences are doubtless due to a multitude of factors that include vaccine strain used, route of administration of the vaccine, and infectivity titer of the vaccine.

Because of the variability of results achieved by vaccination, a determination of serologic response must be made after vaccination. Negative or inadequate serologic findings could allow time for additional vaccinations with improved or different procedures before a flock at risk is exposed to a virulent virus.

The hemagglutination-inhibition (HI) test is generally considered to be a reliable, economical, rapid means of measuring the response of poultry to Newcastle vaccination. Diagnostic laboratories use a variety of HI methodology for this purpose, with little standardization of methods or reagents. The hemagglutination (HA) antigens used in diagnostic laboratories are sometimes viable velogenic strains in egg fluids. These highly virulent strains not only are hazardous from the standpoint of possible dissemination from the laboratory, but also can serve as a frequent source of contamination in virus isolation attempts from tissues submitted to the laboratory.

Mention of product used in this report does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply approved to the exclusion of other products that may also be suitable.
This report describes the use of commercially available manual microtest equipment for determining Newcastle HI titers without excessive financial outlay or complexity. When used with an inactivated HA antigen, it enables even small laboratories to perform a large number of tests.

MATERIALS AND METHODS

REAGENTS

Saline. Phosphate (.01M) buffered saline (PBS) with a pH of 7.2 was prepared as follows:

\[
\begin{align*}
\text{Na H}_2\text{PO}_4 \cdot \text{H}_2\text{O} & \quad 7.5 \text{ gm.} \\
\text{Na}_2\text{HPO}_4 & \quad 20.5 \text{ gm.} \\
\text{Na Cl} & \quad 160.0 \text{ gm.} \\
\text{H}_2\text{O} & \text{ (distilled) to make 20 liters.}
\end{align*}
\]

Red blood cells (RBC). Blood was obtained either from the wing vein or by cardiac puncture from white rock chickens known to be free of antibodies against Newcastle disease. The blood was drawn into a syringe that contained Alsever’s solution in a volume to comprise one-third of the total after the blood was collected. The syringe was then gently rocked to mix the solution and the blood to avoid the formation of clots. The Alsever’s solution was prepared as follows:

\[
\begin{align*}
\text{Dextrose} & \quad 20.5 \text{ gm.} \\
\text{Na Cl} & \quad 4.2 \text{ gm.} \\
\text{Sodium Citrate} & \quad 8.0 \text{ gm.} \\
\text{Citric Acid} & \quad 0.55 \text{ gm.} \\
\text{H}_2\text{O} & \text{ (distilled) to make 1 liter.}
\end{align*}
\]

Sterilize by filtration.

The RBC’s were sedimented by centrifugation at a RCF of 500 for 5 minutes. The supernate was then poured off. The cells were gently re-suspended in a quantity of PBS slightly in excess of the original blood-Alsever’s mixture. The cells were again sedimented and re-suspended three times. After the last centrifugation at a RCF of 500, the supernate was aspirated but the sedimented RBC’s were not disturbed. A quantity of packed cells was removed with a pipette and added to PBS at the rate of 0.5 ml of packed cells per 100 ml of PBS (0.5% RBC). This cell suspension was used both for HA determination and in the HI tests.

HA Antigen. The formalin-inactivated antigen prepared from virus-laden egg fluids and concentrated with the aid of polyethylene glycol is described elsewhere. It can be prepared by most laboratories from the LaSota strain of Newcastle virus purchased as vaccine. The antigen offers the advantages of being stable and non-infectious without the disadvantage of rapid elution from the RBC’s.

HA Procedure. The proper dilution of the antigen to be used in preparing the antigen-saline mixture was determined as follows:

1. Prepare 1:10 and 1:15 dilutions of antigen in PBS.
2. Fill a microtest plate (clear, round bottom) with 50 ul (.05 ml) of
PBS in each well.

3. Add 50 ul of the 1:10 dilution to the first well in a row with a 50 ul microdiluter.

4. Add 50 ul of the 1:15 dilution to the first well in a second row.

5. Pass 50 ul quantities down the rows to achieve 2-fold dilutions (1:20, 1:40, etc., and 1:30, 1:60, etc.) through 1:20480 and 1:15360.

6. Add 50-ul of a 0.5% RBC suspension to each well with a modified Coombs rinser (twelve-channel dispenser).

7. Observe the plate after a control well that contains only 5 ul of PBS and 50 ul of RBC suspension exhibits a distinct “button” of RBC’s in the bottom.

8. Record the last dilution in each row where there is complete hemagglutination and no button formation.

9. Obtain an average of the reciprocals of the highest agglutinated dilution in the row that began with the 1:10 dilution with the reciprocal of the next highest dilution that did not agglutinate in the row with the 1:15 dilution. For example, in the row of dilutions that began with 1:10, the last well to hemagglutinate was 1:1280. In the row of dilutions that began with 1:15, the last well to hemagglutinate was 1:960. The positive reciprocal (1280) is averaged with the next negative reciprocal (1920) to result in an average HA titer of 1:1600. Thus a 1:160 dilution of the antigen in PBS resulted in an antigen-saline mixture that contained 10 HA units. This mixture was used for making the serum dilutions as the virus-saline was used in other similar methods.²

**HI Procedure**

Blood samples were usually obtained with syringe and needle either from the wing vein or by cardiac puncture and placed in tubes that had been treated with a silicone solution to aid in clot release.³ The blood samples were held either at room temperature or at 37° C until clotting was adequate or free serum was observed. After chilling at 4° C (sometimes overnight), the serum was harvested with disposable glass Pasteur pipettes and placed in microtest plates with a 0.4-ml well volume. A mimeographed pattern similar to the layout of the wells in the plate was used to identify a particular serum in the serum storage plate. Each plate has the capacity for as many as 96 sera for immediate use or for storage at below-freezing temperatures. The plate was covered with a plastic sealing sheet if it was stored.

The purpose of the serum-storage plate was not only to occupy minimum freezer space, but also to allow the simultaneous pickup and dilution of 12 sera. The pickup and diluting was accomplished with commercially available microdiluter handles that held 12 microdiluters (Figure 1).

The plates were prepared for serum dilution by adding 50 ul of antigen-saline mixture to each well. An additional 50 ul were added to each well in the first row into which the serum was later introduced for the initial 1:10 dilution. Plates were filled with a modified Coombs rinser (also
known as a 12-channel dispenser) connected to a 10-ml automatic syringe and modified by the use of a calibrated manifold known to deliver 50-μl quantities (Figure 2). Metal manifolds are now commercially available for use on the rinser. The procedure is as follows:

1. Ten-μl microdiluters mounted in a multi-microdiluter handle were wet and blotted in the usual manner. The 10 μl microdiluters were cut at a length adequate for picking up individual sera from the tubes or vials if it was needed.

2. They were then used to remove 10 μl of the antigen-saline mixture from the first row, leaving 90 μl in each well of that row.

3. After these microdiluters were rinsed and blotted, they were used to transfer 12 sera from the serum-storage plate to the first row in the HI test plate (containing 90 μl of antigen-saline).

4. The first row contained 90 μl of a 1:10 dilution of the sera after the microdiluters were rotated and then withdrawn.

5. A handle fitted with 50-μl microdiluters was then used to pass the diluted sera from the first row to the other rows (Figure 3). This resulted in 2-fold dilutions (1:10, 1:20, 1:40, etc.). There were only 40 μl of the 1:10 dilution left in the first row after this process, resulting in a volume error in only that dilution. All of the diluters rotate as the knob is turned.

6. After the sera were diluted in that manner, the 12-channel dispenser was used to add 50 μl of a 0.5% suspension of chicken RBC's to each well in the plate.

7. The RBC sedimentation pattern was observed with a mirror stand after approximately 30 to 40 minutes at room temperature or when a positive serum control row inhibited hemagglutination and exhibited buttons (Figure 4). Adequate mixing of the RBC's with the diluted serum resulted from their addition without additional agitation.

8. The dilution of serum where there was inhibition of agglutination (button formation) was considered to be the end point. The reciprocal of this dilution (i.e., 320 for a 1:320 dilution) was reported as the titer without the multiplication of this value by the number of HA units used in the test. The demarcation between wells with inhibition and those with agglutination was clear and required no intermediate reading values. A known positive and negative serum were included in each serum-storage plate or in each day's testing to assure that the reagents and procedure were performing satisfactorily.

RESULTS AND DISCUSSION

The titers obtained with the microtest procedure as described in this report were usually identical or within one dilution of those that were obtained with the conventional macrotest methods. The macrotest method was similar to the microtest method except that macrotest volumes were 10 times as great. The macrotest dilutions were made with glass pipettes instead of microdiluters in disposable plastic plates (Linbro 96 U-WS).

Up to 700 sera that had been previously transferred to the storage
plates were tested in approximately 7 hours by one experienced technician when stock antigen and washed RBC's were prepared in advance.

This manual method of Newcastle HI testing has proved to be a very satisfactory compromise between the old tube method and the highly automated microtest method. With the latter method, equipment costs are 10 times as great as with this manual method, a cost that can be prohibitive for many laboratories.

It has been our experience that chicken sera obtained from either experimental or field flocks require no pretreatment to remove non-specific inhibitors. Perhaps the use of 10 HA units of antigen serves to overwhelm any low level of inhibitors that may be present. Turkey sera present no unique problems and can usually be tested by the same procedure and with the same reagents as those used to test chicken sera. When chicken RBC's are used, occasionally a turkey serum will produce non-specific hemagglutination at the low dilutions. The cell clumping has a different appearance than the hemagglutination due to antigen. This non-specific hemagglutination can usually be avoided, if the HI titers necessitate it, by using turkey instead of chicken RBC's.

One large diagnostic facility (Georgia Poultry Laboratory) has been using this microtest procedure for approximately one year with favorable results. The savings in time, space, and reagents coupled with the lack of subjectivity in reading the reaction make it ideal for most poultry diagnostic and serology laboratories.

REFERENCES


Figure 1. A microdiluter handle equipped with 10 ul microdiluters is used to transfer 12 sera from the serum storage plate to the test plate.
Figure 2. A Coombs rinser modified with a calibrated manifold is used to fill the test plates with antigen-saline and to add the suspension of red blood cells.
Figure 3. Two microdiluter handles, one equipped with 50-$\mu$l diluters (front) and one with 10-$\mu$l diluters (back).
Figure 4. An actual test plate as viewed on the mirror stand. Dilutions were made from the top to the bottom of the plate.
USE OF MICROTITER AND AGAR-GEL DIFFUSION TECHNIQUES IN SCREENING AVIAN SERUMS FOR INFLUENZA A AND MYXOVIRUS YUCAIPA ANTIBODIES

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**Dept. of Veterinary Microbiology, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77840

This report is concerned with the results and the comparison of techniques used in screening avian serums obtained for two different surveys. In one, the microtiter hemagglutination-inhibition (HI) test was used and the other the agar-gel diffusion technique was used.

Antigens used in the surveys were made from three avian influenza virus strains—1) A/Turkey/Calif/meleagrium/64 which is antigenically similar to A/Turkey/Canada/1/63 (Wilmont Strain), the A/Turkey/Mass/3740/65 strain, A/Turkey/Engl/1/66, and the A/Turkey/Wisconsin/1/66 strain; 2) A/Turkey/Calif/AC-3/65 and 3) A/Turkey/Calif/5142/64 (2,4,5,6). The influenza A virus strains were obtained from Dr. R. A. Bankowski, University of California at Davis. Also Myxovirus yucaipa, a paramyxovirus, was obtained from Dr. Bankowski. This is a virus that was isolated from chickens in California that had a mixed infection with Infectious Larygotracheitis (1). One isolation of this virus from turkeys has been reported in Canada.

Of the two surveys, one was conducted in Texas from September 1, 1967 to September 1, 1969. Serums were obtained from blood samples of turkey breeding flocks submitted to the Pullorum/typhoid testing laboratory at Texas A&M University. In the two year period over ½ million samples were submitted to the laboratory from 290 breeder flocks. The large number of blood samples presented a problem in that an attempt was made to collect serums from 10% of the blood samples submitted. Storage space was soon lacking and to attempt to do that many HI tests would have been very time consuming. A compromise was made and 20 individual serum samples, plus 2 composite samples, of 5 serums each were collected from each flock. A total of 5,800 individual serum samples, plus 2 composite samples, of 5 serums each were col-

A From the Animal Disease Diagnostic Laboratory and the Department of Microbiology, Pathology and Public Health, School of Veterinary Medicine. Submitted as paper No. 5506 journal series, Purdue University, Agricultural Experiment Station, West Lafayette, Indiana.
lected from each flock. A total of 5,800 individual and 580 composite serum samples were collected.

It was not possible to run the HI tests as the serums were being collected, so the twenty vials of serums and 2 composite samples from each flock were placed in plastic bags containing a paper that gave the flock identification. The outside of the plastic bags were marked numerically and they were placed in a freezer. As time would allow, sets of serums were randomly removed and tested. By removing serums randomly from the freezer, some serums were not tested for more than a year after being collected. Microtiter HI tests were used in this survey.

The second survey was conducted in Indiana by obtaining serums from turkeys, submitted for disease diagnostic work, at the Southern Indiana Animal Disease Diagnostic Laboratory at Dubois. There was only one turkey breeder flock in southern Indiana so serums collected were from birds usually younger than 24 weeks, and most often, obtained from birds between 10 and 24 weeks of age.

One thousand, three hundred thirty-nine serum samples were collected from September 1, 1969 to September 1, 1973. The serums were from 420 flocks representing 2,873,877 birds. Statistically the number of serum samples only represents approximately .05% of the total number of birds.

The HI microtechnique described by Sever (9) was used with a constant-virus decreasing-serum method. The virus was titrated in 0.05 ml quantities of 0.85% NaCl to determine the dilution containing 8 hemagglutination (HA) units in 0.05 ml. The serums tested were screened by using 2-fold dilutions of 1:2 and 1:4, made in 0.025 ml volumes of pH 7.2 phosphate buffered saline (PBS). An equal amount of virus (4 HA units) was added to each serum dilution. To this was added 0.05 ml of a 0.5% suspension of washed turkey red blood cells (RBC).

There were five different technicians, a graduate student, an undergraduate student, as well as myself that did the microtiter HI tests over a period of 3 to 6 years. Each individual's technique had to be evaluated. It was difficult for two of the individuals to consistently get valid results, so most of their tests had to be rerun.

Of the serums collected from the 290 turkey breeder flocks, all serums, single and composite samples, from each of the 205 flocks were tested at 1:2 and 1:4 dilutions against an influenza A antigen, A/Turkey/Calif/meleagrium/64 also tested against the two other influenza A strains, A/Turkey/Calif/AC-3/65 and A/Turkey/Calif/5142/66. Any serums from any flock in which any titers were found—such as a partial reaction in the 1:2 dilutions, then all serums from that flock were then tested against all four antigens plus Newcastle disease antigen and carried through dilutions to 1:128.

After completing approximately 12,600 HI tests it was decided to do HI tests on only the two composite serums from the remaining flocks. So only the two composite serums of the remaining 85 flocks that had
not been tested against the A/Turkey/Calif/meleagrium/64 and Myxovirus yucaipa viruses were done unless some HI activity was found. Also, only the composite serum samples from 203 remaining flocks that had not been tested against A/Turkey/Calif/AC-3/65 and A/Turkey/Calif/5142/66 were completed. In all, there were 13,800 microtiter HI tests recorded.

If the HI tests had been attempted to have been done by the tube test, it would have been very impractical because of the time required; and impossible, because quantities of serums would not have been sufficient to run the tests. Now the HI microtiter technique appears to be obsolete in screening serums against influenza A viruses, when compared to the agar-gel diffusion techniques.

RESULTS OF FIRST SURVEY
Serums from all birds were negative when tested against the three influenza A antigens. However, serums of 12 flocks from 3 different ranches did have HI activity against Myxovirus yucaipa.

Table No. 1 shows that the first serum samples were obtained from Ranch No. 1 on November 29, 1967 and the last samples on July 11, 1969. Serums were tested approximately 3 months, and up to 14 months, after the samples were obtained.

HI titers were generally very low, ranging from a partial reaction a 1:2 dilution. Partial reactions in the 1:2 dilutions seemed significant.

The composite samples had significant titers in 4 flocks, however the one flock in which the composite titers were <1:2, only 3 of 20 individual samples had significant titers.

Table No. 2 shows results of tests of serums from Ranch No. 2. Serums from flocks in both years of testing, as on Ranch No. 1, had HI antibodies to Myxovirus yucaipa.

Also, all of the composite serums had significant HI titers that could have identified a reactor flock.

Table No. 3 shows results of tests of serums from Ranch No. 3. Serums of birds had significant titers in both years of testing—even though serums were obtained in the same calendar year. The first on May 13, 1968 and the last on November 20, 1968. Again HI titers were low, and again all of the composite serums had significant titers.

Twelve flocks of turkeys from 3 widely separated ranches had HI antibodies to Myxovirus yucaipa. To date, this virus has not been reported from turkeys in the United States and apparently, infections in turkeys are very mild (8).

Results are significant in not finding any HI antibodies against the Influenza A strains, especially in view of the flock problems that have been found in California, Canada and Minnesota with the avian influenza A viruses (2,4,5).

USE OF AGAR-GEL DIFFUSION TECHNIQUES
The agar-gel diffusion technique as described by Dr. Charles W. Beard
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(3), with some slight modifications, was used to test serums.

The antigen was made by inoculating 9-day-old chick embryos with A/Turkey/Calif/AC-3/65 and in 24 hours chorioallantoic membranes were harvested. Membranes were washed in PBS, placed in a blender, ground, and placed through 2 freeze-thaw cycles, formalized, and 1 ml aliquots were put in small screw cap vials.

The diffusion medium was 0.6% Ionagar No. 2 (Colabs) containing 1:10,000 thymol, with 8.0% NaCl buffered to pH 7.2. Sodium Azide was also, occasionally used as a preservative, since at that point and time thimerosal was not available. Agar was put in plastic petri dishes and wells were cut with a home-made well cutter.

The cutter was made by gluing expended 22 caliber long rifle cartridges to a flat metal surface (7). Wells were arranged so that six wells for serums were placed around a center well containing the antigen. Forty unknown serums could be tested on each plate along with a known positive and a negative control serum. Serums were placed in wells by using a capillary tube, fitted with a small rubber bulb, for each serum.

Serums from two birds, of the 1,339 serums tested from one flock, reacted to produce precipitin lines. The two serums were tested by the microtiter HI tests to attempt further identification. Apparently antibodies would not cause any inhibition when tested against each of the 3 avian influenza strains previously described. One of the positive serums was completely used and very little of the other remains, so it may never be known what virus strain caused the antibody production.

Screening the serums by use of the macrotiter (tube) tests would have been impossible because of the small amounts of serums that were available.

Again, it also appears that the HI macro and microtiter tests are obsolete for use in screening avian serums to determine influenza A activity since the agar-gel technique has been developed. It is much easier to use the agar-gel technique since once the antigen is made, it is much more stable than antigens used in the HI tests. Also, the agar-gel test will determine the presence or absence of antibodies in serums of apparently all known influenza A virus strains.

However, the HI tests are needed to help identify the specific influenza A virus strains, as well as to screen and identify antibodies of other myxoviruses.

It appears that avian influenza has not been a problem in Texas and is not a current problem in Indiana.

REFERENCES


Table No. 1

Results of HI Tests of Turkey Serums from Ranch 1 with Antibodies to Paramyxovirus Yucaipa

<table>
<thead>
<tr>
<th>Ranch-</th>
<th>No. of Blood Samples Submitted</th>
<th>Date Serum Submitted To Lab</th>
<th>Date Serum Tested</th>
<th>HI TITERS</th>
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A Partial reaction in 1:2 dilution
B Insufficient serum
Table No. 2

Results of HI Tests of Turkey Serums from Ranch 2 with Antibodies to Paramyxovirus Yucaipa

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<th>Ranch-Flock</th>
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*Partial reaction in 1:2 dilution

Table No. 3

Results of HI Tests of Turkey Serums from Ranch 3 with Antibodies to Paramyxovirus Yucaipa

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*Partial reaction in 1:2 dilution
MICROTESTING FOR AVIAN SALMONELLOSIS

J. E. Williams and A. D. Whittemore

Southeast Poultry Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, 934 College Station Road, Athens, Georgia 30601

SUMMARY

Conventional macrotest serological procedures for avian salmonellosis, other than for pullorum disease and fowl typhoid, have not been adequately sensitive. The sensitivity of serological procedures for the detection of salmonella infections in chickens and turkeys has been enhanced by microagglutination (MA) and microantiglobulin (MAG) procedures.

Microtest methodology and equipment was adapted for routine use in the laboratory for both the MA and MAG procedures. The tests were carefully evaluated with experimental and field sera. Recommended procedures for the application of the MA and MAG test methods for the detection of salmonella infections in both chickens and turkeys are described in this report.

Information regarding research developments leading to the application of microtest methods for the serological detection of salmonella infections in avian species and additional information on specific equipment mentioned here can be found in earlier publications. This report will be devoted entirely to recommended procedures for laboratory applications of the microagglutination (MA) and microantiglobulin (MAG) tests for the detection of flocks that are or have been infected with salmonella organisms under experimental or field conditions. Attention is presently being directed to the detection of salmonella infections belonging to serological groups B, C, and D. These infections represent a major proportion of the salmonellae naturally infecting avian species in the United States. It will be advantageous in the future if we can direct the organization of our thinking about salmonella infections in poultry more and more away from the pullorum-typhoid and typhimurium concept and consider salmonellosis of poultry in terms of specific sero-group infections.

MA test procedures for pullorum-typhoid and for typhimurium reveal very close agreement with conventional macrotests for these infections.

Mention of product used in this report does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply approval to the exclusion of other products that may also be suitable.
The microtest procedures have the great advantage of affording savings in time, cost, and space. Microtest antigens and test procedures have also been developed for field applications of both the MA and MAG tests as a means of accrediting flocks of chickens and turkeys as free of exposure to motile salmonella sero-group B, C, and D infections. The MA and MAG tests for the motile salmonellae are being studied as a flock sampling test rather than a bird-to-bird test although applications for the latter purpose may be called for under certain circumstances. From 5-10% of the birds in flocks under study are usually tested.

Routinely, the MA test is applied as a single-dilution test; either typhimurium (group B), group C, or pullorum-typhoid (group D) antigen suspension is used to test each serum sample. The MAG test is applied as a four-dilution test; the B, C, and D antigen suspensions are used. For the MA test, only a single 18-24 hour reading is made. For the MAG test, readings at 18-24 and 42-48 hours must be made. If positive reactors are encountered at a dilution of 1:20 or above when initial readings are made at 18-24 hours, the flocks should be deemed serologically positive, and the decision to proceed with the MAG test will be based upon the interest that may exist for more detailed information on the number of birds that have been or are presently infected. When compared with MA test results, MAG results will show considerable enhancement of titers.

The procedures to be described are based on the application of microtest methods with manually operated equipment. Equipment used in our laboratory for microtesting for avian salmonellosis and sources of supply are listed in Table 1. Automated equipment is also commercially available to carry out most of the procedures described with a minimum of effort after sera have been added to test microplates. Manufacturers will be glad to work with laboratories having this equipment in setting up protocols for specific microtest requirements.

**Microagglutination (MA) Test for Pullorum-Typhoid, Typhimurium, and Group C**

(a) The procedure for the collection and delivery of blood samples in the MA test is the same as that described in 447.1 (a) of the National Poultry Improvement Plan. A method that has proved advantageous is to transfer the serum samples from the blood clot to a microplate as recently described. Blood samples can also be collected in silicone-treated microtest plates for multiple-sample transfers.

(b) Stained MA test antigens for pullorum-typhoid, typhimurium, and group C are supplied as concentrated-stock suspensions and must be approved by USDA. These antigens are prepared on solid media and stained with tetrazolium dye as recently described. Directions for dilution are provided with the antigens. The stock as well as the diluted antigen prepared each day should be kept sealed in the dark at 5º to 10ºC when not in use.

(c) The maximum serum-dilution used for the pullorum-typhoid test must not exceed 1:50 for chickens nor 1:25 for turkeys. The maximum serum dilution for the typhimurium test must not exceed 1:25 for both
chickens and turkeys. Available data indicate that a 1:20 dilution is the most efficient for the pullorum-typhoid, typhimurium, and group C test. In all official reports on the blood test, the serum dilutions will be indicated.

(d) The recommended procedure for the 1:20 dilution is as follows:

1. Add 100 microliters (0.10 ml.) of normal saline to each well of the microplate.

2. With a 10-microliter (0.01 ml.) microdiluter or a multi-microdiluter handle equipped with up to 12 single 10-microliter microdiluters, transfer 10 microliters (0.01 ml.) of the serum sample from the collected specimen to the corresponding well of the microplate by touching the surface of the serum sample with the microdiluter and then transferring and mixing the serum with the diluent in the microplate well. The microdiluter is removed, blotted, touched to the surface of the distilled water rinse, and blotted again. Other acceptable methods of serum delivery have been described.

3. Dilute the MA test antigen with 0.5 percent phenolized saline in accordance with directions and add 100 microliters (0.1 ml.) to each microplate well with a 12-channel dispenser (Coombs rinser).

4. Seal each microplate with a plastic sealer or place unsealed in a tight incubation box as has been described. Incubate at 37°C for 18-24 hours.

(e) The recommended procedure for the 1:25 dilution is as follows:

1. Add 50 microliters (0.05 ml.) of 0.25 percent phenolized saline to each well of the microplate.

2. With a 10-microliter (0.01 ml.) microdiluter or a multimicrodiluter handle equipped with up to 12 single 10-microliter microdiluters, transfer 10 microliters (0.01 ml.) of the serum sample from the collected specimen to the corresponding well of the microplate by touching the surface of the serum sample with the microdiluter and then transferring and mixing the serum with the diluent in the microplate well. The microdiluter is removed, blotted, touched to the surface of the distilled water rinse, and again blotted. Other acceptable methods of serum delivery have been described.

3. Dilute the MA test antigen with 0.25 percent phenolized saline in accordance with directions and add 200 microliters (0.2 ml.) to each microplate well with a 12-channel dispenser (Coombs rinser).

4. Seal or place unsealed microplates in a tight incubation box as described in (d) (4) above. Incubate at 37°C for 18-24 hours.

(f) The recommended procedure for a MA test titration is as follows:

1. Add 50 microliters (0.05 ml.) of normal saline to each well of the microplate.

2. To the wells representative of the lowest dilution in the titration, add an additional 50 microliters (0.05 ml.) of normal saline, making a total of 100 microliters (0.10 ml.) in these wells.

3. With a 10-microliter (0.01 ml.) microdiluter or a multi-microdi-
luter handle equipped with up to 12 single 10-microliter microdiluters, transfer 10 microliters (0.01 ml.) of the serum sample from the collected specimen to the first well, which contains 100 microliters, and represents the lowest dilution (i.e., 1:20), by touching the surface of the serum sample with the microdiluter and then transferring and mixing the serum with the diluent in the microplate well. The microdiluter is removed, blotted, touched to the surface of the distilled water rise, and again blotted. Other acceptable methods of serum delivery have been described.

(4) Make twofold serial dilutions of each serum to any desired end titer by transferring 50 microliters (0.05 ml.) of diluted serum from one well to the next using 50-microliter microdiluters. After transfers have been made to all the wells of the series, the 50 microliters (0.05 ml.) remaining in the microdiluters is removed by blotting, touching the microdiluters to the surface of the distilled water rinse, and blotting again. A handle that will hold up to 12 microdiluters is useful in making these serial dilutions.

(5) Dilute the MA test antigen with 0.5 percent phenolized saline in accordance with directions and add 50 microliters (0.05 ml.) to each microplate well with a 12-channel dispenser (Coombs rinser).

(6) Seal or place the unsealed microplate in a tight incubation box and incubate as described in (d), (4) above. Dilutions provided by this technique start at 1:20 and increase in a twofold fashion.

(g) Read the test results with the aid of a reading mirror. Reactions are interpreted as follows:

(1) N or—(negative) when the microplate well has a large, distinct button of stained cells.

(2) P or + (positive) when the microplate well reveals no antigen button.

(3) S or ? (suspicious) when the microplate well has a small button. Suspicious reactions may tend to be more positive than negative (+) or vice versa (±) and can be so noted if desired.

Microantiglobulin (MAG) Test for Salmonella Group B, C, and D Infections

(a) See (a) in MA section above for methods of serum collection and delivery.

(b) See (b) in MA section above for information on test antigens.

(c) Four twofold serum dilutions ranging from 1:20 through 1:160 are used. The MAG test requires up to 48 hours for completion. Available data indicate that a complete reaction at the 1:40 dilution or above should be interpreted as positive; however, a positive reaction at a 1:20 dilution cannot be interpreted as negative. The final diagnostic titer to be recommended for routine use is still under investigation.

(d) The recommended procedure for the MAG test is as follows:

(1) Make four twofold dilutions (1:20-1:160) of sera as recommended for the MA test titration under item (f) in the MA section above.

(2) Dilute the MA test antigen with 0.5 percent phenolized saline
in accordance with directions and add 50 microliters (0.05 ml.) to each microplate well with a 12-channel dispenser (Coombs rinser).

(3) Place unsealed plates in a tight incubation box as has been described. Incubate at 37°C for 18-24 hours.

(4) Read the test results with the aid of a reading mirror. Reactions are interpreted as described under item (g) in the MA section above. If no positive MA reactors are observed at the time MA readings are made, proceed with steps 5-13 that follow.

(5) Balance plates and load into centrifuge carriers. Centrifuge plates for 10 minutes at 900-1500 r.p.m. The speed will vary somewhat, depending upon the carrier load. Select the speed at which there is minimal cracking of the microplates. Allow centrifuge to stop without braking so that the settled cells will not be disturbed.

(6) Decant the supernatant fluid from the wells by quickly inverting the plates individually over a sink or pan.

(7) Resuspend the packed cells by forcefully adding 100 microliters (0.10 ml.) of normal saline to each well of the microplate with a 96-channel dispenser. To do this requires that 2 equal volume deliveries be made (4.8 ml. each) with considerable force.

(8) Repeat steps 5, 6, and 7 two more times.

(9) After the second repeat of 5, 6, and 7, repeat steps 5 and 6 for a third time.

(10) Resuspend the packed cells in 50 microliters (0.05 ml.) of normal saline.

(11) With a 12-channel dispenser (Coombs rinser) add to each well 50 microliters (0.05 ml.) of the optimal saline dilution of rabbit anti-chicken globulin serum (Sylvana, Millburn, New Jersey). The optimal dilution for each lot of rabbit anti-chicken globulin serum is determined by the procedure described by Coombs and Stoker. 

(12) Place plates unsealed in a tight incubation box. Incubate at 37°C for a second 18-24 hours.

(13) Read the MAG test results in the same manner as described under item (g) in the MA section above.

REFERENCES

### Table 1.
Equipment List for Salmonella Microagglutination and Microantiglobulin Tests
(November, 1973)

<table>
<thead>
<tr>
<th>Source</th>
<th>Equipment</th>
<th>Scientific Apparatus &amp; Supply Co. Catalog</th>
<th>Cooke Laboratory Products</th>
<th>Linbro Chemical Co., Inc</th>
<th>Hughes Machine Company</th>
<th>Microbiological Associates</th>
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* This and subsequent numbers enclosed in parentheses indicate the source in the list that follows.
** Catalogue number.
*** Addresses of these companies will be found in the source list that follows.
Equipment Source List (Refer to Table 1)
This list is in alphabetical order, and is not to be interpreted to indicate preference. Latest available catalog numbers have been used. If numbers of items vary, it will be due to the use of a different catalogue edition.

(1) Croan Engineering Company
5582 McFadden Avenue
Huntington Beach, California 92649

(2) Curtin Matheson Scientific
P.O. Box 1456
Houston, Texas 77001

(3) Fisher Scientific Company
711 Forbes Avenue
Pittsburgh, Pa. 15219

(4) International Equipment Company
300 Second Avenue
Needham Heights, Massachusetts 02194

(5) Scientific Products
1430 Waukegan Road
McGaw Park, Illinois 60085

(6) Arthur H. Thomas Company
P.O. Box 779
Vine Street at Third
Philadelphia, Pa. 19105

Addresses of Sources Listed at the top of Table 1
Cooke Laboratory Products
Division of Dynatech Laboratories, Inc.
900 Slaters Lane
Alexandria, Va. 22314

Hughes Machine Company
Rogers Mill Road
Route 2
Danielsville, Georgia 30633

Linbro Chemical Company, Inc.
681 Dixwell Avenue
New Haven, Connecticut 06511

Microbiological Associates, Inc.
4733 Bethesda Avenue
Bethesda, Maryland 20014

The Sylvana Company
22 East Willow Street
Millburn, New Jersey 07041
### Table 1.
Equipment List for Salmonella Microagglutination and Microantiglobulin Tests
(November, 1973)

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* This and subsequent numbers enclosed in parentheses indicate the source in the list that follows.
** Catalogue number.
*** Addresses of these companies will be found in the source list that follows.
THE USE OF THE MICRO-MODIFIED DIRECT-CF-TEST IN THE DETECTION OF IBR AND BVD ANTIBODIES

A. K. Eugster and A. Angulo*

The serological procedure was most commonly used for the detection of antibodies to the IBR and BVD virus is the serum neutralization test (SN). The passive hemagglutination (HA) test has also been applied for the detection of antibodies to IBR virus. In addition, a Modified Direct CF-Test (MDCF) for BVD was developed by Ruckerbauer, et al. All of these tests give available information on the humoral immune status of an animal to these two viruses.

In serological diagnoses of infection a negative to positive conversion or at least a fourfold rise in antibody titer between a serum sample taken during the acute state of the infection and a second sample taken during convalescence must be demonstrated. Unfortunately these "paired" serum samples are difficult to secure from the field. Furthermore, in cases of viral abortions, an acute and convalescent serum can generally not be obtained because the initial infection may be asymptomatic and may occur a long time before abortion takes place.

Since the MDCF Test was shown to give a good indication of the stage of the infection, i.e., the CF-antibodies to BVD persisted for a much shorter time (30 weeks) than SN-antibodies (years), this test was adopted at the Texas Veterinary Medical Diagnostic Laboratory (TVMDL).

MATERIALS AND METHODS

Sera: Specimens consisting of clotted blood or serum with varying degrees of hemolysis were submitted by practicing veterinarians. Sera showing extensive hemolysis or contamination were not tested since the frequency of anti-complementary activity of sera in "poor" conditions was high. The sera were heat inactivated at 56°C for thirty minutes.

Cell Culture: Secondary and tertiary fetal bovine kidney cells and fetal bovine spleen cells were grown on Hanks Lactalburmin Hydrolysate (HLH) medium with 10% calf serum. The cells were maintained on HLH medium containing 2% heat inactivated fetal calf serum. The growth and maintenance medium contained 100 IU Penicillin G and 100 μG Streptomycin.

Antigen-Production: Sixteen oz. bottles containing a full monolayer of fetal bovine kidney or spleen cells were inoculated with IBR (Colorado) and BVD (NADL) viruses, respectively. The viruses were allowed to absorb to the cells for one hour before being overlaid with maintenance medium. The bottles were frozen at -70°C as soon as the cell monolayers

* From the Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas 77840.

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showed a complete cytopathic effect (4 + CPE). The bottles were frozen and thawed three times and then subjected to two low speed centrifugations (480 and 3000 x g) in a refrigerated centrifuge. The virus particles were concentrated by filtering the supernatant through a dehydrated gel coated membrane filter* which is impermeable to IBR and BVD viruses. We tested the filtrate and found it to be free of viruses. The filter containing the absorbed virus particles was dissolved in 2-3 ml of 3% sodium citrate solution and veronal buffer was added to give a final concentration of about 1/3 of the original volume. Lately we have used uncentrated virus-containing tissue culture fluid instead of veronal buffer (Fig. 1).

**MDCF-Test Proper:** The procedure was described by Ruckerbauer, et al. was followed (Fig. 1). Complement was supplemented with 5% normal unheated calf serum. This was obtained from a healthy five month-old calf found to be free of antibodies to IBR and BVD by the SN-Test. The serum was separated from the clot within one hour after bleeding, distributed into 1 ml aliquots and frozen at -70° C. at once. In our experience this serum could not be thawed and refrozen more than once without losing its complement supplementing activity. A positive control serum of a known titer was always included.

**RESULTS**

During 1971, 924 and 633 sera were tested by the SN-Test for IBR and BVD antibodies, respectively. Thirty-six percent of these sera were positive for IBR and 22% for BVD. Positive in this case means a serum titer greater than 1:8 using 100 TCID50 of either IBR or BVD virus. During part of 1972 we used the passive hemagglutination (HA) test. The percentage of positive sera remained about the same. The passive HA is a very rapid test but has variables which need to be carefully controlled for an accurate test.

In 1973 we tested 3,318 sera for IBR and 1,184 for BVD antibodies using the MDCF-Test and found 15% and 11%, respectively, positive (Table 1). In our laboratory we considered a serum MDCF-titer of 1:4 or less as negative, from 1:8-1:32 as suspicious, and 1:32 or higher as positive.

Serum samples from healthy animals with a request for evaluating the immune status of animals after a vaccination were tested by the SN-Test rather than the MDCF-Test. The MDCF-titers in these cases were either low or negative while the SN-Test on the same sera did show antibody levels to be present. For this reason we always meet requests for a particular vaccine evaluation with the SN-Test. A sample taken at the time of abortion due to IBR may be negative or have only a low titer. About ten days post partum a significant MDCF-titer (1:32 or higher) will be present in the serum if the abortion was due to IBR.

**DISCUSSION**

Our objective is to use a test that would give an indication of the stage of the infection to IBR and BVD viruses. This is ideally achieved by test-
ing paired samples. We were unable to obtain paired samples in most instances. In most cases practicing veterinarians could not obtain the "acute" sample, and in abortion cases the initial infection is mostly asymptomatic and may occur a long time before abortion takes place. The use of the SN-Test in testing "unpaired" samples has the disadvantage from the diagnostic standpoint that it detects long lasting antibodies (up to five years). Therefore, the results of this test on a single serum sample are of little help in diagnosing the disease. The antibody titer may give an indication of a recent infection, but no definite conclusions can be drawn. In our experience, the passive HA-Test behaved similarly to the SN-Test in this regard.

The diagnosis of recent infections by detecting the primary immunologic response which is mainly that of the IgM class of immunoglobulin has been reported in a number of viral infections. Madalengartia reported that the antibodies produced against Venezuelan Encephalitis virus early in the infection were sensitive to the reducing action of 2-Mercaptoethanol (2-ME). However, five to six weeks later the antibodies were resistant to 2-ME treatment. Similarly, Angulo reported that the primary immunologic response against PI3 virus was sensitive to the action of 2-ME. The treatment of serum with 2-ME for differentiating the IgM from the IgG class of immunoglobulins has been useful in detecting recent rubella infection.

An indication of the stage of infection can also be obtained by using the MDCF-Test. The IgM class of antibody response predominate early in the infection, however, they do not persist for more than a few weeks. This class of immunoglobulin is readily detected in the MDCF-Test because it is more efficient in fixing the complement than the long standing or persistent IgG class of antibodies. This characteristic could also probably explain the failure of the MDCF-Test to detect significant antibody titers (higher than 1:8) in animals that had been vaccinated with IBR or BVD vaccine six weeks or longer before testing. The use of 2-ME treated serum for the MDCF-Test is currently being investigated to further support the validity of these observations.

The nature and mechanism of action of the factor in the unheated normal calf serum which is necessary for complement fixation in heated cattle serum is not known. Some experimental evidence, however, suggests that this heat labile factor is closely related to the first component of complement. Boulanger and Bannister suggested that the labile factor contributes to the formation of globulin aggregates which might form the nucleus necessary for the interaction of antigen-antibody complex and complement.

We encountered the following difficulties and/or problems using the MDCF-Tests:

1. A low percentage of sera show anticomplementary activity (AC).
2. Occasionally an antigen batch is AC. We find it unrewarding to try to correct this; rather we discard the particular batch and produce new
by inoculating a different stock and a different lot of serum in the
growth and maintenance medium.

3. It is difficult to concentrate the virus-laden fluids extensively with-
out losing activity, and therefore one may not operate on the optimal
peak of CF-activity of the virus.

4. The MDCF-Test may be negative at the time of abortion. A second
sample about 10 days post partum should therefore be requested if
the sample at the time of abortion is either negative or shows only a
low titer (1:16 or below).

The requirements of a good serological test for diagnostic purposes
are:
1. The tests must be sensitive and specific.
2. Results of the tests should be quickly obtained and easily inter-
   preted.
3. The test should be convenient to perform for large numbers of speci-
   mens.
4. The test should conserve time, labor and reagents as much as possi-
   ble.

All of these requirements are met by the MDCF-Test. In addition, the
MDCF-Test gives an indication of the stage of infection, thereby testing
of a single or "unpaired" serum sample can become meaningful.

SUMMARY
The Modified Direct CF-Test (MDCF) was used on 4,502 sera for deter-
mining antibody levels to IBR and BVD viruses. The main advantage of
using this test over the serum-neutralization test, or passive hemagglu-
tination tests is that it gives a better indication of the stage of the infec-
tion in an animal.

REFERENCES
1. Bindrich, H., "Untersuchungen ueber die Virusarten der Rhino-
   tracheitis und des Ex. coitale ves. bov. mittels der Fluoreszenmi-
2. Vengris, V. and Mare, C.J., "A Micro-passive Hemagglutination
   Test for the Rapid Detection of Antibodies to IBR Virus," Can. J.
3. Ruckerbauer, G., Girard, A., Bannister, G. and Boulanger, P., "Stud-
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   1971.
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5. Madalengartia, J., "Hemagglutination—Inhibiting Antibody Sensi-
   tive to 2-Mercaptoethanol as a Means of Detecting Recent Vene-
TABLE I

TEST RESULTS FOR IBR AND BVD ANTIBODIES IN SERA
SUBMITTED TO TVMDL (1971-73)

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<tr>
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<td>36</td>
<td>2300</td>
<td>34</td>
<td>3318</td>
<td>15</td>
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<td>BVD</td>
<td>633</td>
<td>22</td>
<td>1301</td>
<td>18</td>
<td>1184</td>
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* Sept. 1 - Aug. 31
SUMMARY

Forty-five dairy herds were selected statistically at random from Calumet County, Wisconsin, representing five percent of approximately 900 dairy herds in the county. Blood samples were drawn from all adult animals and tested against antigens of six serotypes of leptospiroa known to be indigenous to Wisconsin, they being *L. pomona*, *L. icterohemorrhagiae*, *L. hardjo*, *L. autumnalis*, *L. grippotyphosa*, and *L. canicola*. The 45 herds contained a total of 1,593 head or an average of 35.4 animals per herd. The statewide average is 29.7 adult animals per herd. One sample was broken enroute to the laboratory resulting in 1,592 samples tested.

Results are summarized as follows:
1. 25 herds or 55.5941% of the herds were negative for all six serotypes. These herds contained 821 head or 51.7% of the total animals.
2. 20 herds or 44.5% of the herds had one or more positive animals. These herds contained 771 head or 48.3% of the total animals.
3. A total of 148 animals or 8.7% of all cattle tested were positive for one or more serotypes.

*Reactions by Serotypes (1,592 samples)*

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<tr>
<th>Serotype</th>
<th>No. Positive</th>
<th>No. Negative</th>
<th>% Positive</th>
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<td><em>L. pomona</em></td>
<td>81</td>
<td>1,511</td>
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<tr>
<td><em>L. icterohemorrhagiae</em></td>
<td>1</td>
<td>1,591</td>
<td>0.06</td>
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<tr>
<td><em>L. hardjo</em></td>
<td>0</td>
<td>1,592</td>
<td>0.0</td>
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<tr>
<td><em>L. autumnalis</em></td>
<td>46</td>
<td>1,546</td>
<td>3.0</td>
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<td><em>L. grippotyphosa</em></td>
<td>73</td>
<td>1,519</td>
<td>4.6</td>
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<tr>
<td><em>L. canicola</em></td>
<td>21</td>
<td>1,571</td>
<td>1.3</td>
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</table>

For the purposes of this summary, any reaction at the 1:100 dilution or greater was considered positive.

TEST PROCEDURE

The microscopic agglutination microtiter test (MAMT) was employed utilizing live antigens grown on Difco's Bacto-Leptospira Medium Base EMJH. Materials and reagents necessary are as follows:
1. Disposable plastic plates measuring 3.25 x 5.0 inches containing 96 round bottom wells.
2. A microdiluter calibrated to deliver 0.025 ml.
3. Plastic pipette droppers calibrated to deliver 0.025 ml.
4. One ml. pipettes and 6 x 50 mm. test tubes.
5. Live antigen of each serotype to be tested.
7. Dark field microscope with 4X objectives.

The test procedure is:
1. 2.45 ml. of buffer is measured into a test tube.
2. 0.05 ml. of test serum is added making a 1:50 dilution.
3. Add 0.025 ml. of buffered solution to each well.
4. Insert microdiluter into test tube containing the 1:50 dilution, rotate thumb wheel several times to mix and then transfer to tray wells. Mix contents in well by again rotating the thumb wheel 5 or 6 times and going to next dilution.
5. Blot microdiluter dry after each set of samples.
6. When all serums are diluted, add 0.025 ml. of antigen. Tap each tray sharply to mix antigen and serum.
7. Trays are stacked in groups for incubation for two hours at room temperature. Use a cover on the top tray to prevent evaporation.
8. Examine by dark field microscopy and interpret results.
9. All test serums are tested at two-fold dilutions commencing at the 1:100 dilution until an end titer is obtained.
10. Known positive and negative control serums are run with each batch of test serums.

DISCUSSION

A limited epidemiological study was made of the 45 herds with more attention given to the 20 herds with one or more animals showing titers. Pertinent information gathered was:
A. Six of the 20 infected herds had vaccinated with L. pomona bacterin within the past two years because of clinical outbreaks of the disease.
B. The majority of the titers were in animals from two to four years old and in over 90% they were animals that were born and raised on the farm.
C. Fifteen of the twenty farms had woodland pastures that were in most instances used for very young stock and dry cows.
D. Fourteen of the twenty farms had swampy areas with stagnant surface water in pasture areas.
E. All twenty of the farms indicated the presence of considerable numbers of species of wildlife and rodents.
F. Fourteen of the twenty herds had purchased one or more head of cattle within the past three years.
G. Hemoglobinuria was the single most mentioned symptom that could be associated with leptospirosis followed by abortion and retained placenta. Thirteen of the twenty herds had experienced some symptoms of leptospirosis in the past two years.
H. There were three herds with a high incidence of L. grippotyphosa titers—one with 100%. All were essentially closed herds and in none of the three was there evidence of clinical manifestations of leptospirosis.

This is the first such statistical survey conducted on a given cattle population in Wisconsin. The incidence of infection was not significantly different than are the percent of positives from those samples routinely submitted to the Wisconsin Animal Health Laboratories for diagnostic testing when considering the disease in toto. On an individual serotype
evaluation, the number reacting to *L. hardjo* was significantly less than is observed on a statewide basis and the reactors to *L. grippotyphosa* was considerably greater.
APPLICATION OF MICROTITER TECHNIQUES TO DETERMINE THE IMMUNO-COMPETENCE OF RAINBOW TROUT BEFORE TRANSFER TO REDMOUTH-DISEASE ENZOOTIC AREAS

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Western Fish Disease Laboratory
Bldg. 204 Sand Point NSA
Seattle, Washington 98115

INTRODUCTION

Microtiter techniques for the detection of humoral antibody in rainbow trout are finding application in experimental and practical disease diagnosis and prevention. The following experiment with immunization of rainbow trout (*Salmo gairdneri*) against Hagerman Redmouth Disease (HRM) is an example.

HRM of salmonids is a serious, often enzootic, disease in certain commercial and governmental fish hatcheries in the Western United States (McDaniel, 1971). The etiologic agent is a gram-negative bacterium which has not been categorically placed in taxonomic keys (Ross *et al.*, 1966). Although serious outbreaks in hatcheries may indicate careless managerial procedures, sometimes even under the most desirable environmental conditions for trout culture, HRM is suspected of existing as a sub-clinical infection in the population. Some hatcheries use antibiotics or drugs, particularly Sulfamerazine, to suppress these infections. However, use of this treatment prevents the marketing of the fish until six months after the administration of the drug. Other antibiotics and drugs, while effective in controlling disease outbreaks, have not been approved by the U.S. Food and Drug Administration.

Immunization of rainbow trout against HRM is one of the effective methods of prevention (Ross and Klontz, 1965, Anderson and Ross, 1972). Although still on an experimental basis, feeding or injection of a phenol-killed or chloroform-killed bacterial suspension is known to be effective in inducing significant levels of protection in fish.

Fish losses arise particularly when fish are transferred from a hatchery that is free of HRM into hatcheries or environments where the HRM is enzootic. In the following experiments, fish immunized in HRM-free areas are found to possess antibody titers detected by the microtiter technique and to have concurrent protection as demonstrated by LD-50's.

EXPERIMENTAL DESIGN

Fifteen hundred rainbow trout weighing approximately five grams apiece were kept at a HRM-free hatchery. These fish were divided into three groups and either given one subcutaneous inoculation of 1 mg per fish of bacterin, fed 1 mg per fish of bacterin in the diet for a period of two weeks, or held as unimmunized controls. The method of bacterin
preparation and feeding has been previously shown to be effective in the laboratory (Anderson and Ross, 1972). Five weeks after the single injection or three weeks after the last bacterin feeding, ten fish were sampled from each group for humoral antibody microtiter tests. Similar samples were taken at various intervals thereafter for 13 weeks. To obtain the serums, caudal peduncles were severed, and approximately two capillary tubes of blood were taken from each fish. The blood was allowed to clot, then the tubes were spun in a hematocrit centrifuge. The tubes were filed and broken, and the serum loaded on 0.05 ml microtiter looper (Cooke Engineering, Alexandria, Virginia). The washed bacterin was the antigen indicator.

Protection test were demonstrated with these groups of fish at time intervals shown in figure 2. Since the pathogen could not be introduced into the HRM-free hatchery, the fish were transported by tank truck to the Western Fish Disease Laboratory, Seattle, Washington. Here the effluent is treated with chlorine to prevent the escape of virulent pathogens into the environment. The degree of protection was established with each of these fish groups by the inoculation of known, graded doses of Hagerman Redmouth bacteria into separately held lots of fish. Probit analysis was used to calculate the LD-50's (Goldstein, 1956).

For a natural challenge, sample groups of the immunized and control fish were transported into an area where the HRM is known to be endemic. The fish were held in live boxes in the effluent below ponds which held adult fish showing typical HRM signs, i.e. darkening, lethargy, hemorrhagic lesions, etc.

RESULTS AND DISCUSSION

The advantages of using the microtiter agglutinin method for the detection of humoral antibody were two-fold for these particular tests. First, these trout were so small that a little over 0.1 ml of serum was the maximum amount obtained from severing the caudal peduncle from an individual fish. Since the microtiter loopers held 0.05 ml of serum, the sample was usually adequate for at least two microtiter tests. This helped confirm the accuracy and validity of the antibody titer tests. Secondly, the microtiter method is well-known for its ease and efficiency. Combined with the used of sampling by capillary tubes, these methods circumvent holding the blood in open test tubes and performing laborious titrations.

Trout injected with the bacterin demonstrated humoral antibody by the microtiter tests three weeks after the single injection (Fig. 1). At this time this injected group also showed a high degree of protection (Fig. 2). Both the orally-immunized fish and the unimmunized controls, however, did not demonstrate any bacterial agglutinins by the microtiter tests. The orally-immunized fish were protected, but not to the degree of the fish which had been immunized by injection.

In the natural environment challenges, no mortalities were observed in either the immunized or control groups of fish that could be directly attributed to the homologous pathogen. Further tests will be conducted to
learn more about the natural outbreaks of this disease in the environment and its effect on immunized and control fish.

BIBLIOGRAPHY

Anderson AAVLD St. Louis October 15-16, 1973

Figure 1. The mean agglutinating antibody titers for the trout inoculated with the HRM vaccine. The humoral antibody was measured by the microtiter method using the chloroform-killed bacterial cells as the antigen. No specific antibody was found in the fish fed the vaccine or in the unimmunized controls.
Figure 2. Following immunization, a rise in protection (LD-50's) was shown in the groups of fish fed or inoculated with the vaccine. The unimmunized fish consistently had LD-50's of approximately $4 \times 10^5$ bacteria per 0.1 ml on the scale shown here.
A COMPARISON OF CLINICAL MANIFESTATIONS AND PATHOLOGY OF THE EQUINE ENCEPHALIDITES: VEE, WEE, EEE

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The authors thank Mrs. Marilyn Carr and Mr. John Kirk for technical assistance.

SUMMARY
The clinical signs and lesions seen in ponies inoculated with Venezuelan equine encephalomyelitis (VEE) virus were compared to those caused by Eastern (EEE) and Western (WEE) infection. The clinical signs were similar in all three diseases. The Eastern strain of virus produced much more extensive and severe lesions than did Western. The lesions of VEE were intermediate in both extent and severity.

INTRODUCTION
The outbreak of VEE in Texas in 1971 was not unexpected. The northward movement of VEE through Central America and Mexico had been carefully followed. Plans had been formulated for implementing the necessary control measures in the event that VEE did appear in the United States. The principal control measures of vaccination and mosquito abatement were effective in controlling the epizootic and emphasis rapidly shifted to surveillance activities.

One of the primary objectives of the surveillance program was investigation of all possible equine illness cases showing signs of a central nervous system disturbance. From such cases, a variety of specimens obtained at various stages of illness was anticipated.

Observations of horses infected with VEE virus were needed to supply baseline information for evaluation of field specimens and for comparison to EEE and WEE.

MATERIALS AND METHODS
Seventeen ponies ranging from 2 to 8 years of age were inoculated with VEE virus.

The ponies were observed daily for clinical signs of illness and their temperatures were recorded.

All ponies were bled prior to inoculation and daily thereafter. Hematologic studies included total leukocyte count, differential count and packed cell volume.

Portions of the brain and spinal cord were collected at necropsy for
histopathologic examination. Sections were embedded in paraffin, cut at six microns and stained with hematoxylin and eosin.

For comparison of VEE, EEE and WEE, tissues were examined from animals that died or were euthanitized in extremis.

Field specimens submitted to our laboratory provided information on EEE and WEE.

RESULTS

Clinical signs noted in the VEE inoculates on the first and second days post inoculation (DPI) were vague and included decreased activity and slight depression. Subsequently, inappetence ranging to complete anorexia, lowering of the head, inattention to movements of the observer, decreased response to restraint or venipuncture, and somnolence were noted. Some ponies exhibiting somnolence were startled by noises of low or moderate intensity. Chewing movements, salivation and partial mastication of food were observed. Serous to purulent ocular and nasal discharges occurred in several ponies. The more dramatic signs of falling when attempting to get up, paddling, head pressing and convulsive seizures were seen only in ponies surviving 6 or more days.

All ponies inoculated with VEE virus had elevated temperatures 2 DPI. The temperature increase ranged from 1.5 to 5° F above preinoculation levels. By 5 DPI the temperature of three ponies had returned to preinoculation levels. Twelve ponies had remained febrile until necropsy 3 to 8 DPI. The other two ponies had normal temperatures on the seventh DPI. Clinical reports on field cases indicated temperatures of 103 to 105°F were common.

A decrease in circulating leukocytes was observed 3 DPI in all VEE inoculates. Leukocyte counts as low as 3000 per cu mm of blood were seen. A gradual return toward normal occurred in ponies surviving more than one week. Differential leukocyte counts revealed that the leukopenia resulted from a decrease in both neutrophils and lymphocytes. A decline of 3 to 12% the volume of packed red cells were consistent during 2 to 4 DPI. After that the results were less uniform, reflecting the clinical status of the individual pony. Similar to the leukocyte response, the packed cell volumes returned toward preinoculation values in those ponies surviving more than one week.

Gross lesions in most cases were limited to congestion of the brain and meninges. Ecchymotic hemorrhages of traumatic origin were seen in two cases with terminal convulsions.

Microscopic lesions were found throughout the central nervous system with all three types of equine encephalomyelitis virus infections. The inflammatory response involved the gray matter primarily. Neurons in various stages of degeneration and chromatolysis were common. Many were necrotic and fragmented. The severity of the accompanying inflammatory response was noticeably different with each strain of virus.

Western infections were marked by lymphocytic infiltrations and the lesions were focal in nature. Cases of EEE, on the other hand, were characterized by the presence of large numbers of neutrophils among the
inflammatory cells and lesions occurred throughout the gray matter. The lesions in acute cases of VEE were intermediate in both severity and extent of tissue injury. The cellular accumulations were composed of lymphocytes and glial cells as well as substantial numbers of neutrophils.

Other concomitant changes were also progressively more severe in WEE, VEE, and EEE respectively. These included petechial and ring hemorrhages, perivascular cuffing with leukocytes, diffuse and focal gliosis, neuronophagia and liquefaction of the neuropil.

DISCUSSION

The clinical signs observed in the VEE inoculates were not unlike those recorded for field cases of WEE and EEE. Circling has been reported as a frequent sign and was observed among the field cases.1,2 The absence of circling in the VEE ponies was attributed to their confinement in small isolation rooms.

The appearance of elevated temperatures and leukopenia during the early stages of VEE infections were consistent. These determinations could be made by diagnosticians in the field as they have been in the State-Federal Hog Cholera Eradication Program. Such information would be helpful in making a tentative diagnosis and in interpretation of other laboratory results.

Lesions produced by each of the three virus strains were similar to those previously described 3-7. The differences noted in the character of the central nervous system lesions makes histological examination of brain tissue a valuable laboratory aid for diagnosis of equine encephalomyelitis.

REFERENCES

SEROLOGIC INCIDENCE OF LEPTOSPIROSIS IN GEORGIA HORSES

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College of Veterinary Medicine, University of Georgia
Tifton, Georgia 31794

Equine leptospirosis is not considered a disease of major economic importance in the United States, however, clinical disease and high serologic reactor rates in the absence of clinical signs have been reported. The serotype commonly associated with infections in horses is pomona. Serotypes canicola, grippotyphosa, and icterohemorrhagiae have been reported less frequently. The serologic incidence of leptospirosis in 1606 Georgia horses is reported.

MATERIALS AND METHODS

Serum. The samples used were submitted to the laboratory for equine infectious anemia (EIA) testing. The sera were collected by veterinary practitioners located throughout Georgia from November, 1972 through August, 1973.

Antigen. Five-to 7-day-old cultures of pomona, hardjo, grippotyphosa, icterohemorrhagiae, canicola, tarassovi, alexi, and autumnalis in Stuart's medium were utilized as previously described.

Test procedure. Serum samples were screened at a dilution of 1:50 by the microscopic agglutination (MA) test. Agglutination titers of positive samples were determined using the MA test previously described.

RESULTS

Positive leptospiral reactions, at the screening dilution of 1:50, were found in 730 (45.5%) samples (Table 1). The predominant serotypes were autumnalis (62.7%), icterohemorrhagiae (36.9%), and pomona (30.7%). When single reactions only were considered, the incidence of autumnalis (22.1%) and icterohemorrhagiae (10.2%) remained relatively high, and pomona (4.3%) dropped significantly. At a dilution of 1:100, which is considered a minimum significant dilution, positive reactions were found in 411 (25.6%) samples. Autumnalis (43.8%), icterohemorrhagiae (30.0%), and pomona (37.2%) were still the predominant serotypes detected. These three serotypes also were predominant when only single reactions were considered.

The occurrence of multiple serotypes in horses is shown in Table 2. Of the 730 animals positive at the 1:50 dilution, 362 (49.6%) reacted to a single serotype, 188 (25.7%) to two serotypes, and 180 (24.7%) to three or more serotypes. With dilutions of 1:100 or greater, 264 (64.3%) react-
ed to a single serotype, 100 (24.3%) to two serotypes, and 47 (11.4%) to three or more serotypes.

The distribution of reactors according to serotype and titer is shown in Table 3. The incidence of *grippotyphosa* and *canicola* was high at the 1:50 dilution, but declined at the 1:100 dilution.

To determine whether leptospiral reactors were associated with clinical disease or age (Table 4), the horses were divided into three groups: 1) EIA positive, 2) EIA negative with a history of clinical illness such as fever, depression and, in some cases, death, and 3) EIA negative with no clinical history available. Leptospiral antibodies were found in 38 of 71 (53.5%) horses in group 1, 44 of 99 (44.4%) in group 2, and 648 of 1436 (45.1%) in group 3. When ages of the animals were known, no specific age group appeared to have a greater reactor rate except the 5- to 7-year-old animals in group 3.

To demonstrate whether leptospiral reactions were confined to certain areas of Georgia, the state was divided into six sections based on Georgia Cooperative Extension Service Districts. The greatest number of samples with the lowest incidence (17%) of reactors were received from the south central section (Fig.1). The percentage of reactors from other sections of the state ranged from 22 to 32%.

**DISCUSSION**

The incidence of leptospiral antibodies in Georgia horses has been reported by Shotts *et al.*, who found antibodies in 83 of 110 (74%) clinically normal horses. The predominant serotypes detected were *autumnalis*, *grippotyphosa*, *icterohemorrhagiae*, and *pomona*. Hanson *et al.* found a serologic reactor rate of 28% in Illinois horses suspected of having leptospirosis with *pomona* being the predominant serotype. In two other groups of horses, one composed of animals admitted to the veterinary clinic and the other containing animals from a race track, reactor rates were 11.7% and 6.9%, respectively.

The present study indicates that the serologic incidence is lower than that previously reported by Shotts, but is essentially the same as that reported by Hanson for horses suspected of having leptospirosis. The high serologic incidence of *autumnalis* is interesting, since a literature survey by Hanson did not indicate this serotype to be of importance.

The MA test can be a valuable technique for the diagnosis of leptospirosis in horses, however, the relationship between the results of the MA test and clinical signs in the animals needs further evaluation. Future studies should include detailed clinical histories, a second serum sample from reactors, and attempts to isolate the organism. No relationship between serologic reactions and clinical signs could be determined in this study because of inadequate clinical histories. Of the information received from the practitioners, there was no report of abortion or periodic ophthalmia.
SUMMARY
Serum samples from 1606 horses were tested for antibodies against leptospiral serotypes *pomona*, *hardjo*, *grippotyphosa*, *icterohemorrhagiae*, *canicola*, *tarassovi*, *alexi*, and *autumnalis*. Leptospiral antibody titers of 1:50 or greater were detected in 730 (45.5%) samples. The predominant serotypes were *autumnalis* (62.7%), *icterohemorrhagiae* (36.9%), and *pomona* (30.7%).

<table>
<thead>
<tr>
<th>Table 1. Serologic Distribution of Leptospiral Serotypes in Horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Ani-</td>
</tr>
<tr>
<td>Serum Dilu-</td>
</tr>
<tr>
<td>mals</td>
</tr>
<tr>
<td>1606 ≥ 1:50</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1606 ≥ 1:100</td>
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<td></td>
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</table>

Number animals (percent of positive animals)
* - Single reactions only
** - Multiple reactions only
† - Total positive reactions

<table>
<thead>
<tr>
<th>Table 2. Distribution of Test-Positive Animals According to the Number of Leptospiral Serotypes</th>
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<tbody>
<tr>
<td>Serum Dilution</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>≥ 1:50</td>
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<tr>
<td></td>
</tr>
<tr>
<td>3 or more</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>≥ 1:100</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3 or more</td>
</tr>
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</table>
**Table 3.** Leptospiral Serotypes and Titers in Positive Equine Sera

<table>
<thead>
<tr>
<th>Serotype</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
<th>1600</th>
<th>3200</th>
<th>6400</th>
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</thead>
<tbody>
<tr>
<td><em>pomona</em></td>
<td>71*</td>
<td>86</td>
<td>28</td>
<td>27</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><em>hardjo</em></td>
<td>46</td>
<td>37</td>
<td>16</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>grippotyphosa</em></td>
<td>72</td>
<td>34</td>
<td>11</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>icterohaemorrhagiae</em></td>
<td>151</td>
<td>74</td>
<td>30</td>
<td>10</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>canicola</em></td>
<td>73</td>
<td>14</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>tarassovi</em></td>
<td>47</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>alexi</em></td>
<td>25</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>autumnalis</em></td>
<td>278</td>
<td>115</td>
<td>43</td>
<td>15</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

**Total**: 763 374 138 70 19 12 7 1

* No. positive reactions

**Table 4.** Serologic Incidence of Leptospiral Serotypes According to Age and Clinical Disease in Horses

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yrs.)</th>
<th>No. Animals</th>
<th>No. Positive Animals</th>
<th><em>pomona</em></th>
<th><em>hardjo</em></th>
<th><em>grippotyphosa</em></th>
<th><em>icterohaemorrhagiae</em></th>
<th><em>canicola</em></th>
<th><em>tarassovi</em></th>
<th><em>alexi</em></th>
<th><em>autumnalis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative - EIA Positive</td>
<td>0-1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2-4</td>
<td>21</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>5-7</td>
<td>26</td>
<td>8</td>
<td>3</td>
<td>2</td>
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<td>3</td>
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<td>8-10</td>
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<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>unknown</td>
<td>41</td>
<td>24</td>
<td>12</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>38</td>
<td>19</td>
<td>8</td>
<td>9</td>
<td>12</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

| Negative - EIA Negative - Clinical Illness | 0-1        | 2             | 0                   | 0        | 0        | 0                | 0                      | 0          | 0          | 0       | 0           |
|                                             | 2-4        | 21            | 6                   | 2        | 0        | 0                | 0                      | 0          | 0          | 0       | 4           |
|                                             | 5-7        | 26            | 8                   | 3        | 2        | 2                | 2                      | 0          | 0          | 0       | 3           |
|                                             | 8-10       | 10            | 5                   | 1        | 1        | 0                | 0                      | 0          | 1          | 0       | 2           |
|                                             | unknown    | 31            | 19                  | 4        | 3        | 4                | 0                      | 1          | 0          | 11      |
| Total       | 99         | 44           | 12                  | 6        | 6        | 9                | 3                      | 2          | 0          | 23      |

| Negative - EIA Negative - Clinical History | 0-1        | 38            | 3                   | 2        | 0        | 0                | 2                      | 0          | 0          | 0       | 0           |
|                                             | 2-4        | 220           | 53                  | 10       | 7        | 8                | 17                     | 6          | 5          | 1       | 28          |
|                                             | 5-7        | 166           | 84                  | 38       | 16       | 19               | 38                     | 10         | 10         | 3       | 53          |
|                                             | 8-10       | 71            | 46                  | 13       | 9        | 9                | 21                     | 7          | 6          | 3       | 35          |
|                                             | unknown    | 55            | 40                  | 12       | 11       | 3                | 14                     | 6          | 1          | 0       | 23          |
| Total       | 1,436      | 648           | 193                 | 98       | 116      | 248              | 90                     | 50         | 34         | 405     |

Data expressed as number of reactions at dilutions of 1:50 or greater.
FIG. 1. GEOGRAPHIC DISTRIBUTION OF LEPTOSPIRAL REACTORS IN GEORGIA HORSES

REFERENCES


INTRODUCTION
A relatively high incidence of stomatitis is observed in cattle in the late fall and early winter months in Tennessee. The morbidity and mortality is quite variable. Evidence is offered that this condition is in part due to a pox virus infection with intercurrent agents affecting the severity of the disease noted.

Clinical stomatitis was observed in a group of 13 yearling steers that became ill over a period of 3 weeks. Other signs and symptoms reported were a loss of appetite, nasal and ocular discharge and weight loss. There was no evidence of diarrhea or respiratory distress. A total of 7 animals died during the period of illness.

MATERIALS AND METHODS
Tissues were fixed in 10% buffered formalin embedded in paraffin, sectioned at 6 microns and stained with hematoxylin and eosin. Histological sections of paraffin-embedded formalin fixed post mortem specimens were prepared for electron microscopic study by a modified method of "open-face" embedding as described by Rossi (1). The lesions were located and photographed by light microscopy. Their locations were then marked coverglasses removed, section rehydrated, post fixed in OsO4, dehydrated and the study foci embedded in Araldite 502 (2). Plastic sections, 2 micron thick, were examined by phase contrast microscopy. Thin sections cut on an LKB ultrotome ultramicrotome with a diamond knife were mounted on Formvar carbon-coated grids, stained with uranyl acetate, and lead citrate (3) and examined in a Phillips EM 300 electron microscope.

GROSS LIGHT, AND ELECTRON MICROSCOPIC FINDINGS
A severely emaciated animal was submitted to the Diagnostic Laboratory. Lesions noted at necropsy were confined to the oral cavity, esophagus, and abomasum. Numerous focal ulcerations of the soft palate and tongue were evident. Longitudinal ulcerations 2-3 cm in length were present in the esophagus (Fig. 1). The abomasal folds were thickened, necrotic, and undergoing ulceration (Fig. 2) Microscopically there was ulceration of the epithelium with hydropic degeneration at the margins of the ulcers (Fig. 3). Numerous intracytoplasmic inclusions were present in the areas of hydropic degeneration (Fig 4). The ultrastructure of the intracytoplasmic inclusions consisted of electron dense viruses that varied from round forms measuring approximately 165 nm in diameter to...
ovoid forms measuring 106 nm in diameter and 233 nm in length (figs. 5 and 7). These measurements are consistent with previously reported data for Bovine Papular Stomatitis virus (12). The nuclei of these cells contained numerous tubular structures which were 80 nm in diameter (Figs. 5 and 6).

DISCUSSION

Bovine Papular Stomatitis (BPS) was first reported in the United States in 1960 (4). It was described as a relatively mild disease in calves characterized by proliferative lesions in the mouth which resembled papules. Since that time there have been case reports of more overt illness (5 and 6). BPS experimentally induced produced proliferative papular lesions in the site of inoculation and in a few days secondary lesions appeared in the mouth, margins of the nostrils, on the muzzle, and sometimes in the esophagus and first 3 compartments of the stomach (7). Erosions and ulcerations were noted in secondary lesions of the experimentally produced disease (8,9) and in a naturally occurring case (6). BPS has been found in animals affected with cutaneous streptothricosis and in animals recovered from rinderpest (10). It has been speculated that proliferative stomatitis of cattle, infectious ulcerative stomatitis, and bovine papular stomatitis may be one disease (11) and that variation in lesions may be related to different viral strains, susceptibility of the host due to nutrition, prior exposure, or intercurrent infections (8). Electron microscopic studies in this case revealed that the ulcerative lesions contained pox-like virus in cytoplasmic inclusions which were morphologically similar to those previously described for BPS (12). In addition, tubular structures were noted in the nuclei of epithelial cells. The structures noted in the nuclei have not been previously reported in electron microscopic studies of Vaccinia, Ectromelia, Molluscum contagiosum (Pox viruses) (13), fowl pox (14, 15), swine pox (16), or BPS (12). Tubular or filamentous structures have been noted within nuclei of cells infected with Infectious Laryngotracheitis virus (17), the Virus of Epizootic Diarrhea or Infant Mice (18), Guinea Pig Cytomegalovirus (19), Polyoma Virus (20), and Parainfluenza Type 3 Virus (21).

The severity of the disease in the case described leads one to suspect that an intercurrent infection was present. It is tempting to speculate that the nuclear forms noted represented some stage of viral morphogenesis of a second virus infection, however, viral isolation and replication of similar forms in-vivo or in-vitro are lacking. Intercurrent infection was present in two additional, separate cases of severe BPS which were submitted to the diagnostic laboratory. In one case, Bovine Virus Diarrhea virus was isolated from mesenteric lymph nodes of the animal and in the second case severe lungworm infestation was present. Electron microscopic examination of inclusions from these cases did not reveal the tubular structures that were noted in the nuclei of affected epithelial cells in the first case.
SUMMARY AND CONCLUSIONS

High morbidity and mortality were noted in a group of beef animals. Ulcerative stomatitis, esophagitis, and abomasitis were the most significant lesions noted at necropsy. A pox-like virus was demonstrated by electron microscopy within the intracytoplasmic inclusions of epithelial cells located at the periphery of the ulcers. The nuclei of these cells contained long tubular structures which may have represented a second viral agent. Bovine Virus Diarrhea and Lungworm infestation have also been diagnosed concurrently in animals infected with Bovine Papular Stomatitis. These findings support the concept that the severity of Bovine Papular Stomatitis appears to be dependent upon intercurrent infection.

REFERENCES


LEGEND
Fig. 1. Gross photograph of esophagus containing numerous areas of necrosis and ulcerations.
Fig. 2. Gross photograph of abomasal folds showing marked edema and necrosis.

Fig. 3. Hydropic degeneration of the esophageal epithelium with necrosis (arrows) and ulceration present. X46.

Fig. 4. Intracytoplasmic inclusions (arrows) within the mucosa of the esophagus. X1250.
Fig. 5. Electron micrograph of an epithelial cell demonstrating numerous tubular structures within the nucleus and intracytoplasmic inclusion containing pox viruses (arrows). X7360

Fig. 6. Nucleus of an epithelial cell containing cross and longitudinal views of tubular structures. X19500

Fig. 7. Dense staining around to ovoid virus particles within a cytoplasmic inclusion (arrows). X21000
USE OF SUCKLING MICE FOR DETECTION OF ENTEROPATHOGENICITY OF ESCHERICHIA COLI ISOLATED FROM CALVES AND PIGS

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From the Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, S. Dak. 57006

INTRODUCTION

Methods for determining the enteropathogenicity of Escherichia coli isolates have been limited to oral exposure tests or intestinal loop exposure tests (1). Both tests are time-consuming and expensive and thus are not feasible for routine testing of E. coli isolated from cases of colibacillosis. Recently, a test to determine enteropathogenicity of human isolates of E. coli via intragastric inoculation of suckling mice was described (2). Studies were initiated to determine the applicability of the suckling mouse test by comparing results obtained with known enteropathogenic E. coli (EEC) of porcine and bovine origin to results obtained with isolates from cases of porcine and bovine colibacillosis submitted to the South Dakota Animal Disease Research and Diagnostic Laboratory.

MATERIALS AND METHODS

Strains of pathogenic E. coli used and their sources are indicated in Tables 1 and 2.

The procedures for producing enterotoxin and injecting mice were described by Dean et al. (2). Stains of E. coli were inoculated into 10 ml. Tryptic Soy Broth (Difco) in 250 ml. Erlenmeyer flasks, and incubated at 37 C on a rotary shaker at 200 rpm. Following overnight incubation, the cultures were centrifuged at 7500 g for 30 min. The cell-free supernatant fluid was withdrawn, divided into aliquots, and stored at -70C. Immediately prior to use, the supernatant fluids were thawed and 0.1 ml. was injected with a 30 ga needle directly into the stomach of each of at least 4 (four to six day old) suckling Webster Swiss mice.

Four hours post-injection, the mice were killed with choloroform. To evaluate enterosorption, the entire intestine was removed, the intestines and bodies of the mice were weighed separately, and the ratio of intestine weight to body weight was calculated. A ratio $\geq 0.0900$ was considered indicative of enteropathogenicity (positive), and a ratio $\leq 0.0799$ was considered negative. Ratios between 0.0800 and 0.0900 were considered equivocal and the test was repeated at least twice in these cases.

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RESULTS

Twenty *E. coli* known to be pathogenic for calves or pigs were tested, and 12 were found positive by the suckling mouse test. Three replicate experiments using separately prepared supernatant fluids were conducted with these strains and the results were consistent for each experiment. Negative controls (mice injected with sterile broth or supernatant broth from a known nonenteropathogenic *E. coli*) were always negative (Tables 1 and 2).

Fifty-six isolates from cases of colibacillosis submitted to the South Dakota Animal Disease Research and Diagnostic Laboratory were tested, and 10 of the isolates were positive by the suckling mouse test. Three replicate experiments, using all of the positive isolates and 10 of the negative isolates were conducted, and the results were consistent for each experiment. Negative controls were always negative. (Table 3).

DISCUSSION AND CONCLUSIONS

Nine of fourteen *E. coli* strains known to be enteropathogenic for pigs' were positive when assayed by the suckling mouse test (Table 1). Enteropathogenic *E. coli* may produce a heat stable enterotoxin (ST) and/or a heat labile enterotoxin (LT) (3,4). Three of the mouse negative strains, P307, Arnold, and Emery, are EEC which produce primarily LT (4,5). Therefore, it may be hypothesized that the suckling mouse test is most sensitive to the ST and relatively insensitive to LT. Studies to resolve this hypothesis are currently in progress. Another of the negative porcine strains, G205, was found to produce a low level of enterotoxin (6) and the other negative strain, E68II, was reported to be nonpathogenic when administered to suckling pigs (5). The enterotoxin production of these latter two strains by the cultural procedure employed in the present study may have been below the level necessary to be positive when assayed by the suckling mouse system.

Four of six bovine pathogenic *E. coli* were positive when assayed by the suckling mouse system (Table 2). The four positive strains have been reported positive when tested in ligated calf intestinal loops (7,8). The remaining strains, 219G and B788, were isolated from calves that had signs of septicemic colibacillosis as well as some calves with enteric-toxemic colibacillosis (9,10,11). It may be that these two strains do not produce enterotoxin, and therefore would not be positive when tested in either ligated calf intestinal loops or suckling mice.

The reasons that the supernatant fluids from the majority of the isolates from cases of enteric colibacillosis were negative in the suckling mouse test are not clear at this time. As stated above, it appears that the suckling mouse is more sensitive to ST than to LT. It is possible that most of the isolates tested produced relatively small amounts of ST and thus appeared negative when tested. Further studies, involving concentration of culture supernatants and determination of heat stability and heat lability of enterotoxins, are in progress and will be reported when complete. It appears at this time that the suckling mouse assay for enterotoxin may be applicable for routine use in diagnostic laboratories.
when the limitations and sensitivities of the test have been defined.

SUMMARY

Bacteria-free supernatant fluids from cultures of *E. coli* were tested by intragastric injection of suckling mice in an effort to determine enteropathogenicity.

Twenty *E. coli* strains known to be pathogenic for calves or pigs were tested, and 12 were found positive by the mouse test. Three replicate experiments using separately prepared supernatant fluids were conducted with these strains and the results were consistent in each experiment. Fifty-six isolates from cases of colibacillosis submitted to the South Dakota Animal Disease Research and Diagnostic Laboratory were tested, and 10 of the isolates were positive. Three replicate experiments, using all the positive isolates and 10 of the negative isolates, were conducted and the results were consistent in each experiment. Negative controls (mice injected with sterile broth or supernatant broth from a known nonenteropathogenic *E. coli*) were always negative.

It appears that the suckling mouse may prove to be a useful animal for determining the enteropathogenicity of *E. coli* isolates.

REFERENCES

# FOR DETECTION OF ENTEROPATHOGENICITY

Table 1. Suckling mouse assay of bacteria-free supernatant fluids of porcine enteropathogenic Escherichia coli

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Source</th>
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¹Three replicate experiments using separately prepared supernatant fluids were conducted, with at least 4 mice injected per test.

²Dr. E. M. Kohler, OARDC, Wooster, Ohio.

³Number of tests positive/total number of tests.

⁴Dr. D. A. Barnum, Univ. of Guelph, Guelph, Ontario.

⁵Dr. P. J. Glantz, Penn. State Univ., State College, Penn.
Table 2. Suckling mouse assay of bacteria-free supernatant fluids of bovine enteropathogenic *Escherichia coli*

<table>
<thead>
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<sup>1</sup>Three replicate experiments using separately prepared supernatant fluids were conducted, with at least 4 mice injected per test.

<sup>2</sup>Dr. P. J. Glantz, Penn. State Univ., State College, Penn.

<sup>3</sup>Number of tests positive/total number of tests.

<sup>4</sup>Dr. L. L. Myers, Mont. State Univ., Bozeman, Mont.
Table 3. Suckling mouse assay of bacteria-free supernatant fluids of *Escherichia coli* isolated from cases of bovine and porcine colibacillosis submitted to the South Dakota Animal Disease Research and Diagnostic Lab.

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¹Three replicate experiments using separately prepared supernatant fluids were conducted, with at least 4 mice injected per test.

²Number of tests positive/total number of tests.
A REVIEW OF THE DIAGNOSIS OF CASEOUS LYMPHADENITIS*

Leon W. Turner, D.V.M., M.S.*

INTRODUCTION
A complete description of caseous lymphadenitis in sheep in the United States was given by Nogaard and Mohler in 1899. Infection with *Corynebacterium pseudotuberculosis*, the causative agent of this disease, has been reported in deer, horses, cattle, and other warm blooded animals. The primary site of infection in most animals is the lymphatic system. Orchitis and abscessation of the lungs and liver of the guinea pig after condemnation at slaughter as the major economic loss caused by caseous lymphadenitis in sheep.

CASE REPORTS
A shipment of 400 goats purchased in Texas was condemned at a slaughter establishment in Nashville, Tennessee, due to abscess formation resulting from caseous lymphadenitis. A heard of milk goats kept for supplying milk for a family in the mountain region in Tennessee was examined. The heard had a history of chronic illness, reduced milk production, and death. Caseous lymphadenitis was diagnosed and it was recommended that all chronically ill animals be destroyed and milk from the herd be pasteurized before being used for food for the family.

At Mississippi State University, the sheep flock had two sister ewes to become emaciated. Necropsy examination revealed massive abscessation of the lungs and lymph nodes in both animals. *C. pseudotuberculosis* was isolated from the abscesses. A ram was purchased and placed in the flock. After breeding and settling approximately 15 ewes, the ram became infertile. Infertility followed an injury resulting from falling in a ditch. Immediately following the injury, the number of ewes giving birth to twins decreased and infertility occurred three weeks following the injury. One testicle was nodular and the other swollen. The ram was sacrificed to determine the cause of infertility. A composite of small abscesses typical of caseous lymphadenitis was found in the apical lobe of the right lung. The nodular testicle was abscessed and the testicular tissue was atrophied. Extensive fibrous tissue had replaced the normal testicular tissue. *C. pseudotuberculosis* was isolated from both the lung and testicular lesions. No inflammatory reaction was found in the swollen testicle but absence of spermatogenesis was observed microscopically in both testicles.

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** Associate Professor, Department of Veterinary Science, School of Agriculture, Mississippi State University, Mississippi State, Mississippi 39762

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A REVIEW OF THE DIAGNOSIS

DIAGNOSTIC PROCEDURES

Methods and Materials: For histopathologic examination typical lesions were chosen, fixed in buffered formalin and sections cut at a thickness of 6 μ. Each was stained with the hematoxylin and eosin stain and tissues with typical lesions were stained with Brown and Bren Gram's Stain.8

Bacteriologic examination was made by taking swabs from the necrotic tissue of the abscesses and streaking these on 5% bovine blood agar and tryptose agar enriched with 10% bovine serum. Colonies of gram-positive organisms were picked and media inoculated for determination of the biochemical characteristics.

LABORATORY FINDINGS

In most cases of caseous lymphadenitis the lymph nodes were enlarged to the point that detection of infection was not difficult (Fig. 2). Abscesses in the lung were obvious. In advanced cases gross examination of the thoracic cavity revealed adhesions of the lung serosa to the thoracic wall. The lymph nodes and lung abscesses were prominent due to the thick fibrous granulomatous-type lesion (Fig. 3). The incised lesion revealed a thick fibrous wall surrounding a caseous core. The core sometimes was laminated. The testicular lesion had a less caseous type core but was more liquefied in nature.

Microscopic examination revealed a caseous necrotic center surrounded by a layer of neutrophils and lymphocytes. Around this layer lymphocytes, plasma cells and epitheloid cells had infiltrated the fibrous connective tissue. The farther from the core the more dense the fibrous granulation tissue became (Fig. 4 and 5). Within the fibrous wall small deposits of neutrophils and lymphocytes were found. This indicated the beginning of multiple abscess formation. In the lymph nodes the entire tissue in some cases was replaced by the inflammatory process.

Using Brown and Bren Gram's stain clumps of gram-positive organisms were found in the necrotic core (Fig. 6 and 7). The organisms were pleomorphic rods or coccoid forms single or in short chains. The above findings are ample for a diagnosis of caseous lymphadenitis yet farther proof may be obtained by isolation of C. pseudotuberculosis.

Review of the literature will reveal conflicting reports concerning the biochemical reactions of C. pseudotuberculosis.9 Our findings are as follows. A Gram's strain of a smear made from the caseous core revealed pleomorphic gram-positive rods. Demonstration of small gram-positive rods with other coccoid forms will cause one to suspect a mixed infection yet culture from these areas usually results in a pure culture of the causative agent.

Incubation of blood and serum plates inoculated with swabs of the necrotic tissue resulted in growth in one to two days (Fig. 8). The colonies appeared as small white growths. A narrow zone of beta
hemolysis was found, however subcultures of this organism were not hemolytic. Biochemical reactions were as follows: acid was formed in dextrose, fructose, galactose, lactose, maltose, sorbitol, and sucrose. No acid was formed in dulcitol. Variable acid formation occurred in mannitol, raffinose, rhamnose and xylose. Gelatin was not liquified, no change occurred in litmus milk and nitrates were not produced. The organism is non-motile and does not produce hydrogen sulfide. It is an aerobe and grows best at 37°C. Simmons citrate did not support growth. Urease was not produced.

Two characteristics that will help identify the organism is its tendency to fall to the bottom in a broth culture leaving the broth clear; the organism also has a tendency to adhere to the side of the tube at the top of a broth culture. After a few day's growth and evaporation of the medium a rim of the culture on the side of the tube is evident.

SUMMARY AND CONCLUSIONS

Caseous lymphadenitis is a common disease in livestock, especially sheep and goats. Most reports indicate that the disease is only of concern as a cause of condemnation at slaughter. However, infertility, emaciation, poor feed conversion and contamination of milk should be included when establishing the impact of this disease upon the livestock industry.

*C. pseudotuberculosis* is a gram-positive pleomorphic rod with beta hemolysis. It may ferment most of the common sugars used in laboratory procedures with the exception of dulcitol. It causes a thick fibrous granulomatous reaction with the organism being found in the necrotic center.

This disease needs to be investigated more thoroughly for better determination of its true incidence in the livestock population of this country.

REFERENCES

Figure 1
Thick Fibrous Granulomatous Lesion Replacing Normal Testicular Tissue

Figure 2
Massive Enlargement of Mediastinal Lymph Node Due to Abscessation. Multiple Abscess Formation in Each Lung.
Figure 3
Typical granulomatous lesion of caseous lymphadenitis with central necrotic core surrounded by inflammatory reaction and fibrosis. A small abscess is seen within the large lesion wall. X5

Figure 4
Demonstration of typical lesion with central core and layer of inflammatory cells surrounded by dense fibrous capsule. X100
Figure 5
Higher magnification of granulomatous lesion demonstrating fibrous connective tissue infiltrated with lymphocytes, epitheloid cells and neutrophils. Note multiplication of capillaries. X1000

Figure 6
Demonstration of massive clumps of organisms within the necrotic core. X400
Figure 7
Higher magnification of organisms demonstrating pleomorphism of *Corynebacterium pseudotuberculosis*. Filaments, coccoid forms and pleomorphic rods are observed. X1000

Figure 8
Typical Colonial Morphology of *Corynebacterium pseudotuberculosis* on serum enriched tryphose agar.
78th ANNUAL MEETING
November 28-December 3, 1976
AMERICANA HOTEL at Bal Harbour
Miami Beach, Florida