PROCEEDINGS
SEVENTY-FIRST
ANNUAL MEETING
of the
UNITED STATES LIVESTOCK
SANITARY ASSOCIATION
WESTWARD-HO HOTEL
Phoenix, Arizona
October 16, 17, 18, 19, 20, 1967
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Dr. R. A. Bankowski, Davis, Calif.
Dr. E. H. Bohl, Wooster, Ohio
Dr. E. M. Dwyer, Boston, Massachusetts
Dr. James E. Fox, Ashland, Ohio
Dr. A. A. Erdmann, Madison, Wisc.
Dr. Robert Hall, Madison, Wisconsin
Dr. E. O. Haefterman, Lafayette, Ind.
Mr. J. Nance, Alamo, Tennessee
Dr. E. Pichard, Urbana, Illinois
Dr. J. D. Ray, Whitehall, Illinois
Dr. M. Ristic, Urbana, Illinois
Dr. N. E. Schulz, Hyattsville, Maryland
Dr. J. P. Torrey, Ames, Iowa
<table>
<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sept. 27-28, 1897†</td>
<td>Fort Worth, Texas</td>
<td>*Mr. C. P. Johnson, Springfield, Ill.</td>
<td>*Mr. D. O. Lively, Fort Worth, Texas</td>
</tr>
<tr>
<td>5. Oct. 8-9, 1901</td>
<td>Buffalo, N. Y.</td>
<td>*Dr. E. P. Niles, Virginia</td>
<td>*Dr. F. T. Eisenman, Louisville, Ky.</td>
</tr>
<tr>
<td>11. Sept. 16-17, 1907</td>
<td>Richmond, Va.</td>
<td>*Dr. D. F. Luckey, Columbia, Mo...</td>
<td>*Dr. C. E. Cotton, St. Paul, Minn.</td>
</tr>
<tr>
<td>26. Dec. 6-7-8, 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. 5-6-7, 1923</td>
<td>Chicago, Ill.</td>
<td>*Dr. W. J. Butler, Helena, Mont.</td>
<td>*Dr. Theo. A. Burnett, Columbus, Ohio</td>
</tr>
</tbody>
</table>
29. Dec. 2-3-4, 1925
31. Nov. 30-Dec. 1-2, 1927
32. Dec. 5-6-7, 1928
33. Dec. 4-5-6, 1929
34. Dec. 3-4-5, 1930
35. Dec. 2-3-4, 1931
36. Nov. 30-Dec. 1-2, 1932
37. Dec. 6-7-8, 1933
38. Dec. 5-6-7, 1934
39. Dec. 4-5-6, 1935
40. Dec. 2-3-4, 1936
42. Nov. 30-Dec. 1-2, 1938
43. Dec. 6-7-8, 1939
44. Dec. 4-5-6, 1940
45. Dec. 3-4-5, 1941
46. Dec. 2-3-4, 1942
47. Dec. 1-2-3, 1943
48. Dec. 6-7-8, 1944
49. Dec. 5-6-7, 1945
50. Dec. 4-5-6, 1946
51. Dec. 3-4-5, 1947
56. Oct. 29-30-31, 1952
57. Sept. 23-24-25, 1953
58. Nov. 10-11-12, 1954
59. Nov. 16-17-18, 1955
60. Nov. 28-29-30, 1956
61. Nov. 13-14-15, 1957

Chicago, Ill.  
*Dr. J. H. McNeil, Trenton, N. J.
*Dr. John R. Mohler, Washington, D. C.
*Dr. L. Van Es, Lincoln, Neb.
*Dr. C. A. Cary, Auburn, Ala.
*Dr. Chas. G. Lamb, Denver, Colo.
*Dr. A. E. Wight, Washington, D. C.
*Dr. J. W. Conaway, Columbia, Mo.
*Dr. Peter Malcolm, Des Moines, Iowa
*Dr. E. T. Faulder, Albany, N. Y.
*Dr. T. E. Robinson, Providence, R. I.
*Dr. Edward Records, Reno, Nev.
*Dr. Walter Wisnicky, Madison, Wis.
*Dr. R. W. Smith, Concord, N. H.
*Dr. D. E. Westmoreland, Frankfort, Ky.
*Dr. J. L. Axby, Indianapolis, Ind.
*Dr. H. D. Port, Cheyenne, Wyo.
*Dr. E. A. Crossman, Boston, Mass.
*Dr. I. S. McAdory, Auburn, Ala.
*Dr. W. H. Hendricks, Salt Lake City, Utah
*Dr. J. M. Sutton, Atlanta, Ga.
*Dr. C. U. Duckworth, Sacramento, Calif.
*Dr. William Moore, Raleigh, N. C.
*Mr. Will J. Miller, Topeka, Kan.
*Dr. Jean V. Knapp, Tallahassee, Fla.
*Dr. T. O. Brandenburg, Bismarck, N. D.
*Dr. C. P. Bishop, Harrisburg, Pa.
*Mr. F. E. Mollin, Denver, Colo.
*Dr. Ralph L. West, St. Paul, Minn.
*Dr. T. Childs, Ottawa, Canada.
*Dr. T. C. Green, Charleston, W. Va.
*Dr. H. F. Wilkins, Helena, Mont.
*Dr. A. L. Brueckner, Baltimore, Md.
*Dr. G. H. Good, Cheyenne, Wyo.
*Dr. O. E. Dyson, Wichita, Kan.
*Dr. O. E. Dyson, Wichita, Kan.
*Dr. O. E. Dyson, Wichita, Kan.
*Dr. O. E. Dyson, Wichita, Kan.
*Dr. O. E. Dyson, Wichita, Kan.
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*Dr. O. E. Dyson, Wichita, Kan.
*Dr. O. E. Dyson, Wichita, Kan.
*Dr. O. E. Dyson, Wichita, Kan.
*Dr. L. Enos Day, Chicago, Ill.
*Dr. L. Enos Day, Chicago, Ill.
*Dr. L. Enos Day, Chicago, Ill.
*Dr. L. Enos Day, Chicago, Ill.
Dr. Mark Welsh, College Park, Md.
Dr. Mark Welsh, College Park, Md.
Dr. Mark Welsh, College Park, Md.
Dr. R. A. Hendershott, Trenton, N. J.
Dr. R. A. Hendershott, Trenton, N. J.
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Dr. R. A. Hendershott, Trenton, N. J.
<table>
<thead>
<tr>
<th>Date</th>
<th>Place of Meeting</th>
<th>President</th>
<th>Secretary</th>
</tr>
</thead>
<tbody>
<tr>
<td>62. Nov. 4-5-6, 1958</td>
<td>Miami Beach, Fla.</td>
<td>Dr. John G. Milligan, Montgomery, Ala.</td>
<td>Dr. R. A. Hendershott, Trenton, N. J.</td>
</tr>
<tr>
<td>63. Dec. 15-16-17-18, 1959</td>
<td>San Francisco, Calif.</td>
<td>Mr. F. G. Buzzell, Augusta, Me.</td>
<td>Dr. R. A. Hendershott, Trenton, N. J.</td>
</tr>
<tr>
<td>67. Oct. 15-16-17-18, 1963</td>
<td>Albuquerque, N. M.</td>
<td>Dr. T. J. Grennan, Jr., Providence, R. I.</td>
<td>Dr. R. A. Hendershott, Trenton, N. J.</td>
</tr>
<tr>
<td>70. Oct. 10-11-12-13-14, 1966</td>
<td>Buffalo, N. Y.</td>
<td>Dr. C. L. Campbell, Tallahassee, Fla.</td>
<td>Dr. R. A. Hendershott, Trenton, N. J.</td>
</tr>
</tbody>
</table>

§This was the last meeting of the Interstate Association of Livestock Sanitary Boards.
INVOCATION

R. A. HENDERSHOTT

Almighty God, our Heavenly Father, we give Thee thanks that we are again privileged to meet and discuss our various problems and report upon the progress of research and diagnostic procedures developed and improved upon during the past year.

We thank Thee for a very fruitful year and implore Thy guidance and Blessing on our future endeavors.

Grant that what we do here during our Seventy-first Convention will be found pleasing to Thee and of value to our fellow-man to the end that we may assist in the production of wholesome and disease-free animal products to sustain our expanding population.

We beseech Thee to bless all those charged with the authority and responsibility at all levels of Government. Enlighten and guide them to the end that Thy will be done.

Amen
President Kalev, Distinguished Guests, Ladies and Gentlemen:

Each year the United States Livestock Association take 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:

**RICHARD M. CLARK**, JR. - Cornell - 1908; died March 23, 1967. Dr. Clark, a general practitioner, was a member of this association and the Orange County Veterinary Medical Association.

**R. M. GOW** - died October 18, 1965. Dr. Gow was formerly the State Veterinarian in Colorado.

**GEORGE HOPSON** - Cornell; died June 27, 1967. Dr. Hopson was employed by Borden Milk Co., later by DeLaval as an expert on mastitis control. He was a member of the New York Medical Milk Commission.

**CHARLES A. KNORTH** - C.V.C. - 1913; died December 30, 1966. Dr. Knorth had practiced in the Red Wing area about 50 years. Before moving to Red Wing, he practiced for several years in Prescott, Wisconsin.

**DELBERT A. McGIN** - W.S.U. - 1941; died December 22, 1966. Dr. McGill was supervisor of the Animal Industry Division of the Washington State Department of Agriculture. Prior to joining the department in 1960, he practiced in the Centralia area for many years. He was active in the Washington State Veterinary Medical Association, civic affairs, and was serving as chairman of USLSA Committee on Stockyards, Markets and Transportation at the time of his death.

**FRED C. MAU** - McK - 1918; died on June 5, 1967 following brain surgery. Dr. Mau had worked for the USDA for 42 years and had supervised meat and poultry inspection for the Illinois Department of Agriculture since 1960.

**WILLIAM T. OGLESBY** - died April 13, 1967 in Baton Rouge, Louisiana. Dr. Oglesby joined the faculty at Louisiana State University in 1934 and since 1937 was head of the Department of Veterinary Science. For many years he worked very effectively on committees of the AVMA
and USLSA. In 1965, Dr. Oglesby was the recipient of the National Award known as the AVMA Award given for “Meritorious Service to the Membership.”

CHARLES D. STUBBS - North Little Rock, Arkansas, died June 1, 1967. Dr. Stubbs was in charge of helping eliminate the Texas fever tick in Arkansas and in starting the tuberculosis and brucellosis testing of cattle in the state. From 1933 to 1941 he was state veterinarian.

PHILIP D. WITTLINGER - died May 3, 1967. Mr. Wittlinger was president of Wm. Cooper Nephews Co., Chicago, Illinois.

WILLIAM D. POUNDEN - died September 30, 1967. Chairman Veterinary Science Department, Ohio Research and Development Center, Wooster, Ohio. Dr. Pounden was born in Derbyshire, England, of Irish parents and was reared and educated in Ireland. He received his diploma in agriculture from Albert Agricultural College, Dublin. After completing his undergraduate work, Dr. Pounden came to the United States and worked for several years with some of the country’s finest dairy herds. Developing a great interest in animal health problems, he enrolled in veterinary school at Colorado A & M College and received his D.V.M. degree there in 1938. He then went to the University of Wisconsin, where he received his M.S. degree in bacteriology in 1940. Dr. Pounden served on the research staff at Wisconsin and in the Wisconsin State Diagnostic Laboratory before joining the Research Center staff in 1942.

L. M. HURT - former Los Angeles county veterinarian; former president AVMA and former president of the California Veterinary Medical Association.

HADLEIGH, MARSH - died September 3, 1967, in California. Internationally known research pathologist; retired 1950. Head of Montana Veterinary Research Laboratory; Professor Emeritus 1961; prepared over 100 technical papers. In 1965, Governor of Montana proclaimed November 12, “Hadleigh Marsh Day;” Member AVMA.

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 on and help complete the links of the chain in the ever moving progress in our chosen field.

Phoenix, Arizona
October 19, 1968
PRESIDENT'S MESSAGE

J. F. QUINN

USLA—October 1967

Phoenix, Arizona

Distinguished Guests, Ladies and Gentlemen . . . . .

It is indeed a very great honor to address this distinguished organization as President-Elect in its 71st year of existence. These 71 years have been marked by continued and consistent progress in the field of animal disease eradication—the basic reason for the foundation of the United States Livestock Sanitary Association.

It is most gratifying to one in the field of animal health to look about and observe and evaluate the progress that the State-Federal Cooperative Disease Eradication Programs have made throughout the years. It is further gratifying to realize the important position that the United States Livestock Sanitary Association has taken in these programs, for as you all know, primarily it is the recommendations coming forth from hard working committees that are followed by the USDA and their cooperative state components in the eradication of disease.

I would like to, at this time, pause briefly and commend the people of Arizona on their fine state and to thank the regulatory officials of Arizona and the City of Phoenix for playing host to our 1967 convention. As a northerner, and one who is probably more appropriately classified as a tenderfoot, it is awe inspiring to see and to visit this great state.

I would also like to very sincerely commend Dr. Grant Kaley for the fine job he has done as President of our organization in 1966-67. I too fully realize the precarious position I have been placed in for Dr. Kaley leaves a very large pair of shoes to fill.

This year, 1967, marks an epic milestone in our fine organization. Dr. Ralph Hendershott, Secretary-Treasurer of USLSA since December of 1943 is retiring. This marks 24 years of dedicated service, devoted entirely to the operation and welfare of the United States Livestock Sanitary Association. It is with mixed feelings that we are confronted with a change in the leadership and guidance of our organization. Ralph Hendershott has done more than any individual to further the integrity and reputation of USLSA. It has been under his guidance that the Proceedings Manual has attained such a lofty position among the scientific veterinary publications. I extend to Ralph my sincere wishes for a long and happy retirement and hope that he will continue active in the functions of this organization. It is my regret that Ralph's retirement coincides with my inauguration as President for I would have been both proud and happy to have served with him.

As time goes on, things change; new ideas are brought forth and older implemented ideas are subject to change. I feel that with the direct participation of state regulatory officials and their staffs in the functions of this association that in turn the latter should become publicly concerned with the welfare of those state officials.

xxi
The American Veterinary Medical Association is very interested in the salaries and conditions under which veterinarians work for the U.S. Government and they continually maintain communications with the divisions of the government who have to do with making the decisions pertaining to these. I feel USLSA should become actively interested in promoting these same conditions for state officials. Whether alone or in cooperation with other professional organization, this association should assume the role of an authority to recommend salaries and conditions under which professional regulatory people work.

There is complete lack of uniformity between states in regard to the position of state veterinarian. The office of state veterinarian is not consistently placed in one department in state governments. The large number are in the Department of Agriculture, within the state, whereas others are a separate state agency, known as the Livestock Sanitary Board. This, I don't think, is as important as the lack of uniformity as to the conditions and salaries under which state veterinarians are employed and carry out their responsibilities.

I have in my possession a letter from AVMA to USDA recommending increases in salaries of federally employed veterinarians. I am sure the federal government is very cognizant of the recommendations of that organization. I further feel that state governmental units, for example, Civil Service Departments, should be likewise cognizant of any recommendations passed on to them by USLSA in regard to salaries of their state livestock sanitary officials who are responsible in their respective states for the control and eradication of animal diseases therein. I would recommend that action be taken by this organization to implement an active interest in this field.

As time goes on, vast changes come about in the animal population of our country and we, as veterinarians, are directly involved, both by profession and responsibility, because of our positions in our respective states.

There is a general and alarming decline in the number of farms in the United States. It has been estimated recently that in the next three years one million farms will go out of existence in this country. The family-dominated small farm is on its way out and being replaced by fewer and much larger farming operations. This especially holds true in the production of livestock. These changing conditions do not reflect at all on the responsibility we have in the control of animal diseases. It only changes somewhat the manner in which we go about our regulatory activities.

However, from a regulatory standpoint, more species of animals heretofore given little attention by state regulatory agencies has come to the forefront. More and more attention by all concerned is being placed on the care and welfare of so-called small animals. In the past year, we have seen national legislation passed, which will regulate the care and treatment of dogs, cats and other primates being moved and exchanged for utilization in our laboratories and institutions of learning for research.

Some states have also passed similar type legislation for the control of these animals within their borders. While the passage of this federal legis-
islation does mark a milestone in increasing interest that the citizens of the United States are placing in the existence of small animals, I feel it is entirely inadequate in offering complete regulatory supervision in regard to the care and welfare of these small animals.

In the federal legislation and in the legislation which was recently passed in Michigan, the areas where the most brazen violations in the proper care and treatment of small animals, are exempted from the provisions of this legislation. In regard to this, I speak primarily of dog pounds, animal shelters and pet shops.

If Michigan is any criteria of the conditions that exist throughout the United States, there is a crying need for governmental intervention in these areas. In my state, at least, dog pounds are under the local supervision of municipalities, or counties or township governmental units. There is a complete lack of uniformity in the way these units are handled.

Some local areas have provided remarkable facilities for their functions while others have totally inadequate or no facilities for the care and disposal of dogs and cats. Likewise, pet shops are flagrant violators as to the control of disease and humane handling of animals confined within their premises.

I would recommend that our present existing rabies committee be given the responsibility of becoming involved in all aspects of disease eradication in regard to small animals. We have 27 million dogs in this great United States and God only knows how many cats. I, therefore, feel that it is the responsibility of this association to take an active interest in the disease problems of the small animal group such as they now do for our large animal species.

I offer these few suggestions with the sincere hope that all of you as members of USLSA will give serious thought to them.

PROPOSED AMENDMENT AND REVISION OF THE CONSTITUTION AND BY-LAWS OF THE ASSOCIATION

The undersigned, being member(s) in good standing of the United States Livestock Sanitary Association, in accordance with the constitution and by-laws of the said association do hereby purpose the following amendment to the constitution and by-laws, to wit:

Wherever the phrase, "United States Livestock Sanitary Association," appears in the Constitution and By-Laws that this be deleted and the following be substituted therefore, "The United States Animal Health Association."

Respectfully submitted:

John L. O'Harrar
W. L. Bendix
J. W. Safford
Mr. Chairman, Ladies and Gentlemen:

During the year starting on November 1, 1966 and terminating October 1, 1967, your secretary has attended the regional meeting of the Western States Animal Health Association, consisting of the official members of The United States Livestock Sanitary Association for the western states. This meeting is convened each year at the time and place of the Intermountain Veterinary Medical Association meeting held this year in the Flamingo Hotel at Las Vegas, Nevada, January 17-18, 1967. Doctor G. S. Kaley and I both were invited guests at this meeting and addressed the group on subject matter of concern to Veterinary Regulatory Officials. State-Federal Meat Inspection was one of the topics discussed.

In March I attended the State-Federal Relations Committee in Washington, D. C.

On April 4, 5, 6 I attended the Regional Meeting of the North Central States Regulatory Group at the Sherman House in Chicago, Illinois.

April 10 and 11 was spent attending the Northeastern States Regulatory Group Meeting, Albany, New York.

Both of these group meetings were well organized and many topics of interest to our Association were discussed. One was the selection of Philadelphia for our 1970 Annual Meeting, and another was a discussion of the new law on laboratory animals.

During the American Veterinary Medical Association Meeting at Dallas, Texas in July I arranged a breakfast meeting of the State-Federal Relations Committee at which time we were given a report of the status of the cooperative State-Federal animal disease programs. Doctor Kaley called a meeting of the Committee on local arrangements for the 71st Meeting.

In September on invitation from the National Academy of Science a meeting on morbidity and mortality.

A conflict in dates precluded an opportunity to meet with the Northeast Avian Pathologists formerly the Pullorum Conference.

I wish at this time to thank each chairman of the committees of this Association for the splendid cooperation this year. The excellent program to be presented at this our 71st Annual Meeting is in the main the result of their efforts. I also wish to express my thanks to the local committee and all who in any way contributed to the attendance and arrangements for this meeting. This is the first time we ever had available electronic machinery to reproduce committee reports so ably typed by the volunteer stenographic staff of the local disease control offices.

My thanks also to the Westward-Ho Management for their willing cooperation, and last but not least, to Mr. Norman Powers who has so ably assisted me at the registration desk during the past three years.

We are indebted to the National Band and Tag Company and the Haas Family for the social hour we will enjoy on Thursday.
I understand we have 467 men and some 70 ladies registered; this is another all-time high as is our individual membership of 1300.

Following is the Financial Statement:

**UNITED STATES LIVESTOCK SANITARY ASSOCIATION**

**STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS**

**FOR THE PERIOD FROM SEPTEMBER 16, 1966 TO SEPTEMBER 30, 1967**

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CASH BALANCE, October 1, 1966:</strong></td>
<td></td>
</tr>
<tr>
<td>First Trenton National Bank, Trenton, N. J.</td>
<td>63.18</td>
</tr>
<tr>
<td>Trevose Savings and Loan Association, Morrisville, Pa.</td>
<td>5,432.25</td>
</tr>
<tr>
<td>Sandia Savings and Loan Association, Albuquerque, N. M.</td>
<td>4,548.18</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10,043.61</td>
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<tr>
<td><strong>INCREASE BY CASH RECEIPTS:</strong></td>
<td></td>
</tr>
<tr>
<td>Individual Dues</td>
<td>6,832.64</td>
</tr>
<tr>
<td>Official Dues</td>
<td>5,425.00</td>
</tr>
<tr>
<td>Proceedings</td>
<td>2,349.89</td>
</tr>
<tr>
<td>Reprints</td>
<td>4,210.59</td>
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<tr>
<td>Registration Fees</td>
<td>4,760.00</td>
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<tr>
<td>Foreign Animal Disease Handbooks</td>
<td>645.25</td>
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<tr>
<td>Interest Income on Bonds and Savings Accounts</td>
<td>2,188.34</td>
</tr>
<tr>
<td><strong>Total Increase</strong></td>
<td>26,411.71</td>
</tr>
<tr>
<td><strong>DECREASED BY CASH EXPENDITURES:</strong></td>
<td></td>
</tr>
<tr>
<td>Meeting Expenses</td>
<td>1,280.84</td>
</tr>
<tr>
<td>Printing and Stationery</td>
<td>12,389.53</td>
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<td>Salary</td>
<td>7,500.00</td>
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<tr>
<td>Communications</td>
<td>1,199.17</td>
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<tr>
<td>Travel</td>
<td>2,139.13</td>
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<tr>
<td>Electric</td>
<td>104.49</td>
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<tr>
<td>Rent</td>
<td>480.00</td>
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<tr>
<td>Insurance</td>
<td>195.57</td>
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<tr>
<td>Miscellaneous</td>
<td>822.54</td>
</tr>
<tr>
<td>Bank Charges</td>
<td>66.75</td>
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<tr>
<td><strong>Total Decrease</strong></td>
<td>26,178.02</td>
</tr>
<tr>
<td><strong>CASH BALANCE, September 30, 1967:</strong></td>
<td></td>
</tr>
<tr>
<td>First Trenton National Bank, Trenton, N. J. (Checking)</td>
<td>748.61*</td>
</tr>
<tr>
<td>First Trenton National Bank, Trenton, N. J. (Savings)</td>
<td>11,024.91</td>
</tr>
<tr>
<td>Trevose Savings and Loan Association, Morrisville, Pa.</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10,277.30</td>
</tr>
</tbody>
</table>
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

SUMMARY OF OPERATIONS
FOR THE PERIOD FROM SEPTEMBER 16, 1966 TO SEPTEMBER 30, 1967

REVENUE:

Total Cash Receipts .................. $26,411.71
Increase in Accounts Receivable
Accounts Receivable Sept. 15, 1966 .. $1,308.24
Accounts Receivable Sept. 30, 1967 ... 1,308.25

Total Revenue (Accrual Basis) ... $26,411.72

EXPENDITURES .................. 26,178.02

NET REVENUE FROM OPERATIONS FOR FISCAL PERIOD .................. $ 233.70

NET WORTH—SEPTEMBER 30, 1967

Accounts Receivable .................. $ 1,308.25
Balance First Trenton National Bank, Trenton, N. J.
Checking Account .................. 748.61
Savings Account .................. 11,024.91
Balance, Trevose Savings and Loan Association, Morrisville, Pa. .................. 100
U. S. Treasury Bonds, 4% Due February 15, 1980 .................. 20,000.00
Furniture and Fixtures .................. 400.00

Net Worth, September 30, 1967 .................. $31,985.55

ANALYSIS OF CHANGE IN NET WORTH

Net Worth, September 15, 1966 .................. $31,751.85

INCREASED BY:

Net Revenue from Operations for Fiscal Period Ended September 30, 1967 .................. 233.70

Net Worth, September 30, 1967 .................. $31,985.55
I might state that in 1943 we had $4800.00 in 2\% \text{ Government Bonds and around } $2100.00 in the bank, or a total of about $7,000.

There are some items in the financial report I feel need explaining since they are up considerably from that of last year. One, the cost of printing to which there seems to be no ceiling. Some years ago we printed 2300 copies at a cost of $5000. It is now better than double and bids fare to reach $15,000.

Another item is Miscellaneous which is up somewhat over previous years due largely to the fact that a review of our Internal Revenue status resulted in a Classification of 50-5c which, while recognizing our non-profit status, requires us to pay social security taxes. This means that the salary of anyone employed by the Association must pay the tax and likewise the Association must pay its share of the tax. The amount paid by the Association, $82.50 per quarter, is reflected in the Miscellaneous item. Naturally I too paid the like amount out of my salary. On the other hand individual dues paid amounted to $6832.64—an all time high.

In conclusion I wish to express to you my pleasure in serving as your Secretary-Treasurer the past twenty-five years. It has been a rewarding experience to serve such a grand body of members. I am particularly pleased with the number of individual members. I would hope that we could encourage industry to participate more actively in our work; their views are needed. I am certain when they know that our committee and other meetings are open to them, and particularly when they realize that many of the things we discuss and adopt often are the rules they have to live by, they will take a more active interest in our Association. We should have thousands of farm members supporting our work to make their operations more profitable and healthful.

I thank you for your annual reappointment of me as your Secretary-Treasurer during the past twenty-five years. May God continue to guide your progress in disease control and eradication.
COMMITTEE ON ANIMAL VIRUS CHARACTERIZATION


The activities of the Committee's virus characterization data collection and processing center have continued at the Institute for Comparative Biology, San Diego, California, under the directorship of Dr. Charles York with the continued financial support of the National Institutes of Health.

After considerable evaluation the Termatrex system has been selected as the most suitable for processing the data being collected by the Committee. This equipment has been purchased and installed at the Center in San Diego. A standard nomenclature for coding of the virus characterization data has been developed and most of the accumulated information has been transferred into the Termatrex Retrieval System.

The revision of the virus characterization data collection questionnaire has been completed. These new "Investigator's Data Sheets" are now available to interested virologists and can be obtained by writing to: Dr. Charles J. York, Institute for Comparative Biology, Box 551, San Diego, California 92112.

The first editions of an Animal Virus Catalogue containing a list of 96 reference strains and an equal number of tentative working strains has been completed. This catalogue is now ready for distribution. The information embodied in the catalogue has been furnished to the American Type Culture Collection (ATCC) to serve as a guide for procurement of recognized animal virus strains. When available, the appropriate ATCC virus strain number is included in the catalogue. The Viral and Rickettsial Registry Committee of ATCC has welcomed this collaboration and has recommended that all future non-primate animal viral strains to be submitted to them for registrations, be first reviewed by the United States Livestock Sanitary Association Committee on Animal Virus Characterization.

At the request of the National Cancer Institute (NCI) the Committee has furnished its recommendations on reference viruses and procedures for production of reference reagents. These recommendations were accepted by the NCI for the production of certain viral reagents.

The Committee has agreed to add the non-primate laboratory and wild animal viruses to its program of virus characterization. Dr. John R. Gorham, Fur Bearing Animal Disease Laboratory, Washington State University, Pullman, Washington, has been appointed as Chairman of a sub-committee to initiate this new project.
A number of persons and groups have urged the Committee to also include the Simian (Primate) viruses in its program. It was, however, decided to postpone such a project until more information is available from research workers who are currently studying these viruses. Representatives of the primate virus research group will be invited to participate in the next workshop meeting to be held in San Diego in February, 1968.

Several members of the Committee attended a conference of the combined Western and Eastern Hemisphere virus characterization Committees held in Paris during the past summer. At this conference the members agreed on the desirability to establish international working sub-committees to deal with collection of data on the specific viral groups. This proposal is now under consideration by the Committee.
REPORT OF THE COMMITTEE ON DISEASES OF SHEEP AND GOATS

G. L. CRENSHAW, W. J. HADLOW, R. I. PORT, R. E. SIMMONS, O. H. TIMM,
W. VAN HORN, H. VERSLUS, J. L. HOURRIGAN.

Mr. Chairman, Members of the Association:

Your committee continued its review of diseases and current problems pertaining to sheep and goats. Particular attention was given to the following:

RAM EPIDIDYMISITIS

Ram epididymitis, (REO) causes considerable concern and loss to sheepmen. The disease is characterized in the ram by epididymitis, orchitis, and impaired fertility; in the ewe by placentitis and abortion; and in the lamb by septicemia and perinatal mortality.

Diagnostic aids include manual palpation of the ram, semen culture, and the complement fixation test. More needs to be known regarding the incidence of this disease in the United States and its transmission under natural conditions.

In an effort to obtain answers to problems of concern to veterinarians and sheepmen, the Idaho Sheep Commission and the Animal Husbandry and the Animal Health Divisions, ARS, USDA, have embarked on an REO transmission and vaccine evaluation study at the U. S. Sheep Experiment Station, Dubois, Idaho.

This study includes exposing known clean vaccinated rams (rams will be vaccinated twice at 60-day intervals using Ramedol) and nonvaccinated rams to known affected rams both venerally (ewes bred by infected rams and immediately being rebred by clean rams) and by direct contact exposure between the known affected rams and clean rams using a common pasture.

The known affected rams and the venerally or contact exposed rams will be examined by manual palpation approximately every 30 days for six months. Complement fixation tests and semen cultures will also be utilized. At the end of six months, all rams used in the study will be sent to market and tissues collected for culturing.

Your committee reviewed the progress of this project. It should provide an opportunity to observe the methods of natural transmission, the efficacy of the vaccine used, and a comparison and evaluation of the accuracy of the three procedures for diagnosis. It should also provide additional information for outlining effective control and eradication procedures insofar as farm or ranch flocks are concerned. Other aspects of the work at the National Animal Disease Laboratory include evaluation of the agglutination test, the gel diffusion test and improved semen culture media.

We also reviewed the ram epididymitis research which has been under-
way for several years in California. This work has shown that ram epididymitis vaccine is a very useful tool and can provide effective control of the disease in an infected flock. This work has also revealed that rams should receive their first REO vaccination at weaning (4-6 months of age) followed by a second dose 30-60 days later. Rams should also be given an annual booster vaccination one month before being used for breeding. This is a different procedure than presently being used and one that your committee endorses.

**SHEEP FOOT ROT**

Sheep foot rot is recognized by sheep farmers and ranchers and veterinarians in many States as being a major obstacle in the economical production of sheep. This disease has been an important factor in the continued decline of the number of farms on which sheep are kept and in the overall reduction in the number of sheep in the United States.

Your committee reviewed the Pilot Field Control Program which includes ten California flocks representing various geographical areas and management situations.

This work has shown that proper application of standard foot rot control measures such as accurate classification of individual animals, judicious culling, vigorous trimming, an effective treatment regimen and full segregation of affected and non-affected sheep can eradicate the disease from a well-managed flock.

In order to have effective control programs, extremely close supervision by management and veterinarians is required. It appears that until more is known about epidemiology and pathogenesis of foot rot the only way a program can succeed is to have intimate supervision by a veterinarian on each ranch where he has complete knowledge of the disposition of all sheep at all times, and is present during each trimming and inspection.

Also, chemicals superior to formaldehyde and copper sulfate should be developed. Currently a 2 hydroxymethyl 2 nitro, 1,3 propanediol solution and an Iodophor solution are being tested experimentally. Both appear promising.

The Committee on Diseases of Sheep and Goats recognizes the good work being done in California in their Pilot Field Foot Rot Control Program. We also commend the State of Idaho for their foot rot control program including inspection of sheep for the disease, and quarantines and required treatment of infected flocks and commend other states for their efforts to control the disease.

It is therefore recommended that State and Federal regulatory bodies give formal recognition and support, including the necessary financing, to carry such Pilot Projects into additional flocks, areas and states.

9 CFR, Part 71.3 provides that sheep found to have foot rot while in transit or upon arrival at a feed lot, stockyard or marketing center are allowed interstate movement under d(1) to immediate slaughter or d(4) to be quarantined. This places an unfair burden upon owners whose sheep are found to have foot rot or farms or ranches, therefore, it is recommended
that this association request that the subject regulations be amended so as to extend to such sheep the privilege of similar movement.

**SCRAPIE OUTBREAKS DURING FISCAL YEAR 1967**

Scrapie was reported in 11 flocks in six States during Fiscal Year 1967 as compared to nine outbreaks during the previous year. Ten outbreaks involved Suffolk sheep and one outbreak occurred in a Cheviot flock. The number of flocks under surveillance for scrapie has dropped to 418, the lowest since the beginning of the Scrapie Eradication Program in 1952.

The 11 outbreaks occurred in Tazewell County, Illinois; Fulton County, Indiana; Pratt County, Kansas; Johnson (2) and Jasper Counties, Missouri; Hancock County, Ohio; and Collin (2), Lampasas, and Limestone Counties, Texas.

Five of the 11 outbreaks were reported by veterinary practitioners; three were reported on routine inspection; and three were reported by sheep owners.

All bloodline sheep were either slaughtered or taken to Mission, Texas, for use in the Scrapie Field Trial. With the exception of the infected flocks in Illinois and Ohio where the nonbloodline exposed sheep are being held for 24 months, all exposed sheep in infected flocks have been slaughtered.

**SCRAPIE FIELD TRIAL—MISSION, TEXAS**

The Mission Field Trial has been underway since November 1964 (32 months); however, most of the animals taken to Mission for this study have been obtained since March 1965.

On infected premises No. 3, there are some 844 animals from 8 States.

The bloodline sheep brought into this flock are Cheviot, Hampshire, and Suffolk breeds and were purchased from flocks involved in 19 outbreaks in Indiana, Missouri, New York, Illinois, Texas, and Virginia. These sheep represent 16 bloodlines in which scrapie has been found in the United States.

The nonbloodline animals in the flock are Hampshire, Rambouillet, and Targhee sheep breeds, and Angora, Nubian, and Toggenburg goat breeds. These animals were purchased at six months of age or younger. Each animal is one of a twin pair or triplet from Oklahoma, Montana, and Texas flocks and represent 16 individual bloodlines. In each case, the other animals of the twin pair or two of the triplets are kept on the farm of origin as controls where they are inspected every six months for signs of scrapie. None of the nonbloodline, previously nonexposed, sheep or goats have shown any signs of scrapie. However, none of these animals have been subjected to scrapie exposure for longer than 24 months; and many were born in the spring of 1967.

Thirty-two cases of scrapie have been diagnosed on infected premises No. 3 from December 1964 through June 1967. These cases have occurred in the following categories:
A. Progeny of known affected ewes 6
B. Half-siblings to affected sheep via the sire 10
C. Full-siblings to affected sheep 4
D. Suspects held for observations 12

TOTAL 32

The Field Trial Study has demonstrated that if bloodline exposed sheep are held under observation, instead of being slaughtered, many of them will develop scrapie. Scrapie losses in these bloodlines have run as high as 10 percent per year and as high as 29 percent over a three-year period in the animals over 30 months of age.

The Field Trial Study has confirmed that losses do continue in exposed bloodline related animals; while in a small group of bloodline non-exposed animals at Mission, there has been no indication of scrapie to date.

It has further demonstrated that several animals, quite clearly positive on histopathological examination, did not exhibit a classical scrapie syndrome over an extended period of time. Also, a ewe of 12½ months of age, dying of other causes, had advance histopathology of scrapie but was never observed showing signs of scrapie.* There are some indications that the histopathology of scrapied sheep from one section of the country differ somewhat from the histopathology of scrapied sheep from other sections although both are clearly scrapie.

The Field Trial has provided a scrapie environment essential to natural transmission studies and sera and tissue from naturally affected sheep at various stages of the clinical disease for research purposes.

The Field Trial has also provided a flock ideally suited for studying internal parasitic and infectious disease conditions of interest to the sheepman and aids in evaluating treatment, control, and eradication procedures for these disease conditions.

The committee makes the following recommendation in view of the fact that the current program of scrapie eradication has been apparently successful in locating the disease of scrapie, controlling its spread in the affected sheep breeds, and preventing its spread to the nonaffected sheep and goat breeds of the United States, that this association and all regulatory officials oppose the passage of H. R. 10241, to amend the act of May 29, 1884, to strike “scrapie and” from this act.
REPORT OF COMMITTEE ON PROFESSIONAL EXTENSION AND EDUCATION


One of the functions of the committee co-chairmen during the past year was to represent the United States Livestock Sanitary Association at a meeting on the development of an animal disease reporting system at the National Academy of Science. This conference was called by the National Academy of Science Committee on Animal Health with representatives of Professional and Industry organizations concerned with livestock.

Organizations Invited to Conference on September 14, 1967

A. Professional Organizations:
   1. American Animal Hospital Association
   2. American Association of Equine Practitioners
   3. American Veterinary Medical Association
   4. Conference of Laboratory Diagnosticians
   5. Industrial Veterinarians Association
   6. Morris Animal Foundation
   7. National Association of State Departments of Agriculture

B. Industry Associations:
   1. American Feed Manufacturers Association
   2. Animal Health Institute
   3. American Meat Institute
   4. American National Cattlemen’s Association
   5. American Poultry and Hatchery Federation
   6. Associated Poultry and Egg Industries
   7. Certified Livestock Markets Association
   8. Livestock Conservation, Inc.
   9. National Broiler Council
   10. National Milk Producers Association
   11. National Pork Producers Council
   12. National Turkey Federation
   13. National Wool Growers Association
   14. Northeastern Poultry Producers Council
   15. Southeastern Poultry and Egg Association
   16. U. S. Livestock Sanitary Association

The NAS subcommittee on Morbidity and Mortality called attention to the studies conducted over a two-year series of meetings. All aspects of the problem were reviewed. It was concluded by all representatives
present that the best approach to insure the eventual development of a sound morbidity and mortality reporting system, was to organize a select group of specialists who would undertake in two years to develop the framework of a national system and to pilot test it in several types of situations. The proposed project by the Committee on Animal Health has been reviewed and approved by the Governing Board of the National Academy of Sciences-National Research Council.

Both the Committee on Animal Health, as a result of its evaluations, and the Governing Board of the National Academy of Sciences-National Research Council judged that the proposal under consideration had a reasonably good chance of success.

Following development of an acceptable national system through this pilot project there would be need to persuade the Department of Agriculture and the Bureau of the Budget to include in its budget the cost of establishing and operating the system, and to justify such expenses to the Congress. If this current pilot project proposal is financed in substantial fashion by the livestock interests, this fact would probably carry great weight with members of congressional committees.

**Total budgetary needs** for support of the project to develop a feasible national plan for reporting animal morbidity and mortality =

\[ \text{\$98,000 per year, for 2 years.} \]

A. Funds anticipated (now partly pledged) from all federal agencies concerned (U. S. Dept. of Agr., Public Health Service, National Institutes of Health, Food & Drug Administration, and Dept of Defense):

\[ \text{\$50,000 per year, for 2 years.} \]

B. Additional funds needed from *Professional Organizations* and *Industry Associations* concerned with the economic strength of the livestock industry:

\[ \text{\$48,000 per year, for 2 years.} \]

The Committee on Professional Education and Extension has submitted to the Committee on Resolutions the following:

Over the years a tremendous contribution to scientific literature has appeared in the proceedings of the United States Livestock Sanitary Association annual meetings. Much of this information appears in no other scientific publications but is limited in its usefulness because of lack of proper indexing. This committee discussed the feasibility of establishing a cumulative index of the United States Livestock Sanitary Association proceedings over the past twenty years. The committee unanimously recommends that the president appoint a subcommittee to investigate procedure and estimated cost for such an index.

The United States Livestock Sanitary Association represents a combined organization of professional and industry representatives. This committee recommends the establishment of a United States Livestock Sanitary Association newsletter outlining pertinent items of mutual interest to the entire membership. This sort of communique would serve a need to our profes-
sion and the industries represented in this organization not now being ful-
filled by any other means.

The committee discussed the need for long-term objectives and recom-
mends that the president define these objectives consistent with the long-
range purposes of the United States Livestock Association.

**RESOLUTION**

Committee on Professional Extension and Education  
Subject: Animal Disease Reporting System

**WHEREAS**, the livestock industry's need for a meaningful and feasible animal disease reporting system has been discussed for many years; and

**WHEREAS**, an effective reporting system is necessary for: (1) The development of a disease eradication program and the measurement of the progress of such a program; (2) sound epidemiology, which in turn, is the basis for the effective management of animal health programs; (3) providing a surveillance mechanism to alert the industry of new disease threats before they become catastrophic problems; (4) a guiding stimulus and source of epidemiological information to animal disease research agencies; (5) maintaining and/or increasing potential foreign markets; and

**WHEREAS**, the Animal Health Committee of the National Academy of Science proposes to establish a pilot test program to perfect a system for animal disease reporting estimated to require two years of study at a cost of $98,000 per year; and

**WHEREAS**, an amount of $50,000 per year for two years has already been pledged by the United States Department of Agriculture, the National Institute of Health, the Food and Drug Administration and the Department of Defense; therefore

BE IT RESOLVED, that the United States Livestock Sanitary Association endorse the need for a meaningful and reliable animal disease reporting system and contribute support to this project in every way possible.
COMMITTEE ON LAWS AND REGULATIONS

Dr. G. B. Rea, Salem, Oregon, Chairman; Dr. D. E. Flagg, Bismark, N. Dak.; Dr. J. F. Huddelson, Topeka, Kansas; Mr. A. G. Pickett, Topeka, Kansas; Dr. L. N. Butler, Jr., Phoenix, Ariz.; Dr. F. W. Hanson, Jr., Hyattsville, Md; Dr. T. A. Ladson, College Park, Md.; Dr. D. L. Smith, Indianapolis, Ind.

During the past year, the Committee on Laws and Regulations has considered eight major problems. Three are subjects previously discussed having to do with (1) Health Certificates and (2) Identification of Livestock in Interstate Trade, and (3) uniform Format for ARS-91-17-4. New topics for consideration have been:

(a) Threat of the Spread of Unknown Diseases by Virtue of Interstate Traffic in 4-D Meat.
(b) “Need to Know” Requirements for Federal Accreditation of Practicing Veterinarians.
(c) Affiliation of the National Association of State Departments of Agriculture (NASDA) with the USLSA.

Interstate Health Certificates

You will recall that President Campbell charged the committee with the responsibility of investigating new and different methods of satisfying the requirements of the several states as it pertains to the health of livestock in interstate trade, i.e., health certificates.

The lapse of time between the inspection of animals and the arrival of that information in the hands of the proper parties of the state of destination causes increased concern to many of us. This to such a degree as to cause one state official to request health certificates to be sent direct to his office without prior approval of the state of origin and has caused others to question seriously the value of this document in present day livestock traffic. At a recent hearing on the subject, livestock owners asserted that health certificates could be obtained throughout the state without the individual animal having been seen by a veterinarian. This was corroborated by the veterinarians themselves who stated “if we don’t give them one, they go down the road and get one from my colleague.”

Gentlemen, the time has come for us to quit looking with sanctimonious indignation at this situation and to develop a system which is workable and does not tempt industry or the profession to follow the line of least resistance. The whole health certificate system is built upon the integrity of the individual practitioner and when this breaks down the system has lost its value. Furthermore, livestock management today, under certain situations from the practicable point of view, does not lend itself to the individual examination of each animal involved. Small farm or purebred operations
offer no obstacles of merit against this practice, but when a thousand head of cows and calves are gathered in corrals for shipment to a neighboring state by truck, time and the general health of the animals concerned or the disease status of the community from which these animals come does not usually warrant individual inspection. An exception to this situation, of course, would be knowledge that certain diseases are prevalent in the area. If this is known to be true then we are justified in taking whatever steps necessary to prevent its spread. It would not surprise the members of this committee to hear that some states are revising their health requirements for livestock importation, making health certificates necessary only when certain situations are known to exist. The present permit requirement, as it pertains to some areas known to have scabies, is an example.

After discussing the wide area telephone system (WATS) with a representative of the Bell Telephone Company, the Committee decided that this system would not be adaptable to most states in meeting their intrastate communication requirements.

The committee continues to urge all regulatory officials to present ideas for improvement of interstate health surveillance regardless of how bizarre or unorthodox it may seem. The more ideas that are evaluated and discarded, the sooner we will find one that will work.

Livestock Identification in Interstate Trade

The committee has reviewed a proposal which would ask each state to promulgate laws and regulations requiring identification of individual animals in the channels of trade within a state.

We recommend that continued and where necessary—increased efforts be exerted to accomplish the backtagging and other positive identification of all cattle in intrastate traffic and that continued effort be made toward establishing satisfactory systems of individual identification of all livestock.

Interstate Movement of 4-D Animals, Carcasses or Parts

Present prohibition of interstate movement pertaining to the above subject is limited to diseased animals. The burden of proof that the animal has died of a contagious or infectious disease is on the Federal Government.

At first glance, regulatory people are apt to say “let’s change the law and stop the movement of all 4-D animals, carcasses or parts”. This may not be as easy or as practicable as it seems. We are rapidly approaching a protein deficient situation and to prevent the use of “safe” dead animal products could be a waste we cannot afford. Such material is used for pet food and if properly handled, moved under supervision, and sterilized, is perfectly safe. The control and assurance of maintaining the product in proper channels is a continuing regulatory problem.

This committee recommends that the individual states take cognizance of this situation and promulgate suitable laws and regulations to permit safe traffic in these products, and—that the several federal agencies with responsi-
bilities in this area, be exhorted to take all means available to them to afford adequate protection to the states.

**Requirements for Federal Accreditation of Veterinarians**

After considerable discussion of the proposed rule making concerning the “Requirements for Federal Accreditation of Veterinarians” the committee supports this action and recommends that after studying the proposal as published in the Federal Register, the regulatory officials of the several states submit their views, suggestions or pertinent data to the Director, Animal Health Division, Agricultural Research Service, U.S.D.A. Room 356, Federal Center Bldg., Hyattsville, Maryland 20782 within 45 days after publication.

**Affiliation of NASDA (National Association of State Departments of Agriculture) with the USLSA**

A resolution from one of the auxiliary groups of the USLSA proposed that in order to improve communications between these two organizations, both operating on the national level, that NASDA be asked to affiliate with our organization and be offered a seat on the executive board. After lengthy discussion it was the committee's opinion that this matter should receive further consideration by the board of directors.

**ARS-91-17-4**

Last year this committee recommended the adoption of a universal format for the publication of this book—commonly referred to as “Requirements Governing Interstate and International Movement of Livestock and Poultry”. The format was accepted and used in preparing the publication which is at this time in the hands of the Government printing office. We are advised that the majority of the states followed this recommendation and that the book most likely will be available sometime in November. The committee appreciates the cooperation of the several states and the Animal Health Division in this endeavor.

**Federally Approved Auction Yards**

This oft discussed subject was referred to this committee for recommendation. During our discussion it was learned that the Committee on Stockyards, Markets and Transportation had likewise considered the subject and had submitted a recommendation that was concurred in by the members of our Laws and Regulations committee. Hence we refer you to the Report of the Committee on Stockyards, Markets and Transportation for our joint recommendation.

**The Animal Welfare Act (PL-89-544)**

We were asked to review the above law with the thought in mind that this legislation needed to be called to the specific attention of some states
and needed concerted cooperation by all. A resolution to this effect was prepared and is herewith attached.

WHEREAS the Congress of the United States enacted P.L. 89-544, The Laboratory Animal Welfare Act, signed by President Lyndon B. Johnson on August 24, 1966, regulating the activities of laboratory animal dealers and research facilities using certain species of animals, and

WHEREAS Section 15(b) of the Act authorizes the Secretary of Agriculture to cooperate with the officials of the various states or political sub-divisions thereof in effectuating the purposes of the Act and any State, local, or municipal legislation or ordinance for the same subject,

NOW THEREFORE, BE IT RESOLVED that the USLSA go on record as strongly favoring the intent and purposes of P.L. 89-544, and

BE IT FURTHER RESOLVED that this organization recommend that the several States consider the problem stated herein and take such action as deemed necessary and appropriate in accordance with P.L. 89-544 now applicable to interstate commerce, and

BE IT FURTHER RESOLVED that the Congress be requested to provide such funds for the enforcement of the Act as are requested from time to time by the Secretary of Agriculture.
REPORT OF COMMITTEE ON PUBLIC RELATIONS AND LOCAL ARRANGEMENTS

L. N. Butler, Phoenix, Arizona, Co-Chairman; J. L. O'Harra, Reno, Nevada, Co-Chairman; M. Bay, Hyattsville, Maryland; R. A. Hendershott, Trenton, New Jersey; H. H. Hodges, Phoenix, Arizona; Ted Rea, Phoenix, Arizona; N. Powers, Lake Luzerne, New York; N. Rokey, Mesa, Arizona.

This committee, composed of new members with the exception of two, is completing a full year of intense work. The present 71st annual meeting is showing the benefits to be reaped from planning, experience and cooperation.

We have planned a good portion of our public relations program through the office of the Chief Animal Health Official of each state. These men have been very cooperative in forwarding mailings and carrying the message throughout their respective states in regard to the U.S.L.S.A. Without this help it would be impossible for this committee to adequately function with limited manpower and budget. The State Veterinarian remains the key man in each state for effective publicity. Most of them have given of their time and helped in many and varied ways.

This committee remains firm in the belief those in attendance at the annual meeting should be given the opportunity to learn about the area where the meeting is being held by the allocation of a few hours of time for a tour and so arranged in Phoenix. This tour with the western style dinner to follow was extremely well attended resulting, we believe, in a better knowledge of the area and a firmer relationship with our friends.

We believe the ladies deserve special consideration and organized activities were planned for their benefit.

Those in attendance at this meeting are probably more aware of the results produced by the activities of this committee than could be documented in this report.

The Committee recommends:

1. Appointment of new members as early as possible and a holdover of two key members each year for the benefit of experience.

2. A budget be allocated for necessary expenses of mailing, telephone and travel that cannot be readily accomplished through the Secretary's office.

3. Planning and action one to three years in advance at all times.

4. Preplanned press releases with the help of professional public relations men who are available without cost.

The Committee expresses untold thanks and gratitude to the State Veterinarians, Staff of the U. S. Department of Agriculture, members of the
Agricultural Extension Service, the many individuals too numerous to name, staff of the Westward Ho hotel, local and national firms and organizations, the many members of the press, radio and television and all agencies, organizations, affiliates and otherwise who have given of their time and resources to assist in the many preparations of this meeting. Let us all now continue our efforts to build to greater dimensions of value by our efforts.
REPORT OF COMMITTEE ON MEAT AND MILK HYGIENE


Your committee reviewed its report of the 70th Annual Meeting of the United States Livestock Sanitary Association, Buffalo, New York, October 1966. A continuing study of all major topics covered in that report has been conducted during the past year.

The committee recommends that it be designated as "The Committee on Meat, Poultry, and Milk Hygiene."

A subcommittee on the Diseases of Importance to Meat Inspection is being established in the committee.

Active liaison with the presently established United States Livestock Sanitary Association committees concerned with mastitis, public health, and salmonellosis is being maintained.

Each of the eleven subcommittees will be asked to meet the day prior to that designated for the full committee meeting at the subsequent annual meetings of the United States Livestock Sanitary Association.


The committee reviewed and accepted the following resolution adopted at the XVIII World Veterinary Congress in Paris, France, July 1967:

"In recent years there has been a strong pressure to establish microbiological standards for foods. The World Veterinary Association recommends that the following factors be considered before the development of a standard proceeds.

a. Technical: standard laboratory methodology, sampling, statistical interpretation, choice of numerical criteria and types of microorganisms, problems of food processing and microbial selection, and the training and uniform performance of the laboratory personnel.

b. Objectives: the standard should be valid relative to the purpose for which the standard is intended. The standard should be framed
in a flexible manner to allow change with new knowledge and developments.

c. Legal aspects.

d. Administrative and operational problems, including the cost, capability and practices of the regulatory authority in applying the standard.

e. Microbiological food standards should not be established unless being essential to consumer's protection."

The "abnormal milk" problem was studied. The committee decided that the interests of all concerned would be best served by coordinating its efforts in this area with those of the Committee on Mastitis of the United States Livestock Sanitary Association.

Your committee has reviewed the 1967 recommendations of the United States Public Health Service relating to the Grade A Pasteurized, Condensed, and Dry Milk Products Sanitation Ordinance. It finds that the principles are in compliance with sanitary standards to produce high-quality dried milk. The principles expressed in this code are approved by this committee.

The section devoted to animal health provides for bovine tuberculosis and brucellosis control and coordinates with the United States Department of Agriculture program for eradication of these diseases. It is the recommendation of this committee that a paragraph be added following brucellosis to provide for the development of mastitis surveillance program as recommended by the National Mastitis Council. It is urged that the above-mentioned code be published as soon as possible to ensure that high-quality Grade A dried milk will be available for individual consumers and the industry.

The committee has reviewed epidemics that have been associated with egg products. There is an urgent need for development of a code for processing frozen and dried products. The committee also noted with interest that definition does not include fowl ova.* It is recommended that the United States Public Health Service develop a code to meet the needs of the consumer and industry as rapidly as possible.

II. Control of Unwholesome, Condemned and Inedible Material

The committee supports Title II of HR-12144 (Purcell Bill) which is substantially the same as that included in the Report of this Committee prepared at the 70th Annual Meeting of the United States Livestock Sanitary Association in Buffalo, New York, October 1966.

Favorable comments on the committee's recommendations in the above-cited report have been received from many states and organizations concerned with the control of 4-D material. These comments have further indicated that these recommendations will be used as the basis for proposed legislation in many states.

*Fowl ova are those ova removed from mature fowl carcasses during eviscerating operations in a poultry slaughter and evisceration plant, which are salvaged and collected for use as a human food product. 
III. Model State Meat and Poultry Inspection Law

The committee accepted the revised version of the "SUGGESTED MEAT INSPECTION ACT" which has been prepared by the Federal Meat Inspection Program. If HR-12144 (Purcell Bill) is enacted into law, it is recommended that the revised version of the "Suggested Meat Inspection Act" be reviewed in consonance with the Purcell Bill, and that the same be submitted to the Council on State Governments to replace the model act currently being used by that Council.

IV. Toxic and Biologic Residues in Meat, Milk and Poultry Products

The following resolution was adopted at the XVIII World Veterinary Congress in Paris, France, in July 1967:

"Be it resolved that the World Veterinary Association recommends that the following three criteria be recognized for the use of a chemical or other added substance in the production and preparation of foods of animal origin:

a. a need has been demonstrated for the use of the chemical or other substance and such serves a useful purpose.

b. the chemical or other substance

1. does not lead to deception
2. has been proven to be not harmful
3. will not produce changes in these products which render these foods unsafe for consumption.

c. acceptable control procedures including analytical methods and residue maximums have been established."

In accordance with this action a resolution regulating the use of chemicals and other added substances in the production of food animals and the preparation of foods of animal origin was prepared and submitted for appropriate action of the Committee on Resolutions of the United States Livestock Sanitary Association at its 71st Annual Meeting in Phoenix, Arizona.

V. Bills to Amend the Federal Meat Inspection Act and to Extend Federal Meat Inspection

HR--12144 (Purcell Bill) along with House Report 653 was filed with the House Rules Committee on September 21, 1967.

HR-12144 is the "clean" bill which was developed from HR-6168 introduced by Congressman Smith of Iowa. HR-12144 is the administration-sponsored bill and is cited as the Wholesome Meat Act.

Action on the floor of the House can commence when the Rules Committee reports it out for placement on the agenda.

HR-12145 (the Smith-Foley Bill) is another bill that has been introduced into Congress. The significant difference in this bill and HR-12144
is the broader definition of (Interstate) commerce. This bill would extend Federal meat inspection to all intrastate plants doing a gross annual business in excess of $250,000.

Companion bills of HR-12144 and 12145 have been introduced into the Senate as well as others.

No action has been taken by the Senate at the time of this meeting.

Your committee fully recommends that HR-12144 be supported as necessary legislation to offer the meat consuming public the greatest amount of consumer protection.

In light of the public interest that has been generated in Federal Meat Inspection legislation, it is expected that there will be positive Congressional response.

As an aid to further extension of poultry inspection to assure the consuming public of a continuous supply of safe and wholesome poultry and poultry products, your committee recommends that an amendment be made to that section of the POULTRY PRODUCTS INSPECTION ACT regarding designation of major consuming areas. The present requirements call for the designation of major consuming areas. The present requirements call for the designation of such a declared area to be based on a request from the area and proof of economic burden imposed by the non-inspected product.

Your committee recommends that support be given for amending the present act to allow for the Secretary of Agriculture to designate an area and such designation would be based on public health needs.

VI. Federal-State Collaboration and Liaison

The committee continues to recommend that cooperation between Federal and State Meat and Poultry Inspection personnel be extended and further developed.

Due to continued growth and development in State Inspection programs the need for assistance in all areas is increasing; therefore, the committee recommends specifically that each Federal Meat Inspection District and each Federal Poultry Inspection Area take a more active part in cooperating with the State inspection officials and personnel of the States located in their respective District or Area.

This assistance is directed to the full spectrum of a comprehensive inspection system. This would include all aspects of training; e.g., technical, professional, administrative and managerial, as well as full program development and implementation.

VII. Continuing Evaluation of and Assistance to State Meat, Milk, and Poultry Inspection Programs.

As cooperative inspection programs are further and formally developed, it becomes apparent that the need increases for a practical system for evaluating State and Federal programs.

The committee further recognizes the necessity for such an evaluation
system of State Meat Inspection programs if the pending Federal Meat Inspection bill (HR-12144) is enacted, particularly in respect to Title III whereby a State may receive 50% financial assistance for the cost of the cooperative State program when certain requirements are met.

To determine if a given approved State continues to be eligible for federal financial assistance, an evaluation and review program must be initiated. Therefore, your committee recommends that the following evaluation and review program be established:

1. Establish an evaluation and review team for each participating state. This team will be composed of two members:
   a. A State Program official
   b. A Federal Program official from the appropriate district.
2. These persons will conduct random on-site plant reviews and submit their recommendations either singly or jointly to the Secretary of Agriculture’s State Advisory Committee.
3. The State Advisory Committee should consist of representatives from the following organizations:
   a. National Association of State Departments of Agriculture, Meat Inspection Committee
   b. Consumer organization
   c. State or local public health board
   d. State Agriculture Advisory Board.
4. A Federal agency representative will be designated to meet with the State Advisory Committee.
5. The Secretary of Agriculture should appoint a State Advisory Committee for each Meat Inspection District if needed. This committee will consider the evaluation and review team’s reports and submit to the Secretary of Agriculture their recommendation for final approval or disapproval for the State under consideration.

VIII. Public Information

Your committee has prepared appropriate releases for use in farm, meat industry, and veterinary periodicals and journals, as requested by the President of the United States Livestock Sanitary Association.

Your committee will continue to disseminate information on meat, milk, and poultry hygiene to all interested publics to include—but not limited to—the following:

1. Veterinary Colleges
2. Colleges of Agriculture
3. American Veterinary Medical Association
   a. Council on Education
   b. Council on Public Health and Regulatory Veterinary Medicine
IX. Veterinary Specialization in Food Inspection and Public Administration

It is recommended that the veterinarians presently engaged in the field of food inspection and public administration be encouraged to become diplomats in the presently established American Board of Veterinary Public Health. Also, that consideration be given to sectionalizing this Board to clearly define the area of particular interest in which the diplomat may be engaged. It was further considered that specific sections of specialization could be placed on the certificate issued to the diplomat.

X. Prerequisites, Selection, Training, Development, and Evaluation of Professional and Nonprofessional Personnel for Meat, Milk, and Poultry Inspection

Your committee has prepared a resolution for appropriate action of the United States Livestock Sanitary Association. This resolution relates to the instruction in Food Hygiene in both the veterinary professional course and the postgraduate courses and continuing education programs in the Colleges of Veterinary Medicine. If approved, the resolution will be submitted to the Association of American Colleges of Veterinary Medicine, the Council on Education and Council on Public Health and Regulatory Veterinary Medicine, both of the American Veterinary Medical Association.

It is recommended that the appropriate agencies of the Federal Government be requested to furnish assistance—financial, administrative, and instructional aid—to the Colleges of Veterinary Medicine in the development of their instructional programs in meat and poultry hygiene. Your committee further recommends that such assistance also be offered to states in the development of their inspection systems, with strong emphasis and active support in the area of training of their supervisory and inspectional personnel—professional and subprofessional.

The committee recommends that the prerequisites for employment of nonveterinary food inspection technicians be reviewed and steps be taken to strengthen such prerequisites to assure employment of better qualified technicians to assist in the food inspection areas.

In reference to the development of training veterinary inspection technicians it was established by the committee that a 2-year college curriculum be developed. The courses to be offered preferably in Colleges of Agri-
culture in conjunction with Colleges of Veterinary Medicine with all technical veterinary related material to be taught by veterinarians.

The committee recommends that the benefits of meat, poultry hygiene received by the consumer, the meat packer, and the livestock owner be extended to cover all meat and poultry slaughtered in the United States. These meat inspection programs must be adequately financed and located in that agency of government which can provide adequate funds to support and can enforce all aspects of a *Total* modern meat and poultry inspection program. Such Programs should be staffed with properly selected personnel, properly remunerated and free of conflict of interest and other influence.
RESOLUTION NO. 1

Meat and Dairy Hygiene

WHEREAS, the importance of meat and dairy hygiene has increased due to hazards involving the presence of residues and additives; and

WHEREAS, there is an ever present need for the control of domestic and exotic diseases transmissible to man; and

WHEREAS, this significant aspect of meat and dairy hygiene is of direct importance to the practicing veterinarian as well as to the veterinarian in public service, NOW, THEREFORE

BE IT RESOLVED That the United States Livestock Sanitary Association recommends to the Association of American Colleges of Veterinary Medicine that a study be made of the courses offered in meat hygiene to ensure that adequate content of offered in the undergraduate curriculum to properly prepare the veterinarian in the discharge of his professional responsibilities.

BE IT FURTHER RESOLVED That courses in the field of Veterinary Public Health, including meat and poultry hygiene, be offered to graduate veterinarians, and that the Colleges of Veterinary Medicine be instrumental in the establishment of Veterinary Public Health short courses and other continuing education programs in this area of veterinary professional endeavor.

RESOLUTION NO. 2

Laboratory Animal Welfare

WHEREAS the Congress of the United States enacted P.L. 89-544, The Laboratory Animal Welfare Act, signed by President Lyndon B. Johnson on August 24, 1966, regulating the activities of laboratory animal dealers and research facilities using certain species of animals, and

WHEREAS Section 15(b) of the Act authorizes the Secretary of Agriculture to cooperate with the officials of the various states or political subdivisions thereof in effectuating the purposes of the Act and any State, local, or municipal legislation or ordinance for the same subject,

NOW THEREFORE, BE IT RESOLVED that the USLSA go on record as strongly favoring the intent and purposes of P.L. 89-544, and

BE IT FURTHER RESOLVED that this organization recommend that the several States consider the problem stated herein and take such action as deemed necessary and appropriate in accordance with P.L. 89-544 now applicable to interstate commerce, and

BE IT FURTHER RESOLVED that the Congress be requested to
provide such funds for the enforcement of the Act as are requested from time to time by the Secretary of Agriculture.

The Committee on Salmonellosis

RESOLUTION NO. 3

*Salmonellosis*

As a result of information obtained from 1966 cooperative state-federal testing of feeds and feed ingredients, and in an effort to assist the state in controlling Salmonellosis, Be it resolved that the Animal Health Division, Agricultural Research Service, U. S. Department of Agriculture take the necessary steps to develop and put in effect minimum interstate requirements pertaining to pathogen contamination (especially Salmonella) of animal and marine protein intended for use in livestock and poultry feeds.

RESOLUTION NO. 4

*Animal Disease Reporting System*

WHEREAS, the livestock industry's need for a meaningful and feasible animal disease reporting system has been discussed for many years; and

WHEREAS, an effective reporting system is necessary for: (1) The development of a disease eradication program and the measurement of the progress of such a program; (2) sound epidemiology, which in turn, is the basis for the effective management of animal health programs; (3) providing a surveillance mechanism to alert the industry of new disease threats before they become catastrophic problems; (4) a guiding stimulus and source of epidemiological information to animal disease research agencies; (5) maintaining and/or increasing potential foreign markets; and

WHEREAS, the Animal Health Committee of the National Academy of Science proposes to establish a pilot test program to perfect a system for animal disease reporting estimated to require two years of study at a cost of $98,000 per year; and

WHEREAS, an amount of $50,000 per year for two years has already been pledged by the United States Department of Agriculture, the National Institute of Health, the Food and Drug Administration and the Department of Defense; therefore

BE IT RESOLVED, that the United States Livestock Sanitary Association endorse the need for a meaningful and reliable animal disease reporting system.
RESOLUTION NO. 5

Poultry Diseases

WHEREAS the United States Livestock Sanitary Association has been involved in formulating poultry disease programs for many years, and

WHEREAS the United States Livestock Sanitary Association has been active in developing uniform methods and rules for controlling, eradicating, and preventing poultry disease.

THEREFORE BE IT RESOLVED by the United States Livestock Sanitary Association assembled in convention this 20th day of October 1967, request the Animal Health Division of the United States Department of Agriculture give serious consideration to promulgating importation requirements involving poultry and poultry products from outside the United States.

RESOLUTION NO. 6

Poultry Diseases

WHEREAS, the United States Livestock Sanitary Association has been vitally interested in the eradication of Pullorum Disease and Fowl Typhoid in poultry for many years; and

WHEREAS, the United States Livestock Sanitary Association has endorsed uniform rules and methods for the eradication of Pullorum Disease and Fowl Typhoid; and

WHEREAS, the United States Livestock Sanitary Association adopted a five phase program for the eradication of Pullorum Disease and Fowl Typhoid; NOW THEREFORE

BE IT RESOLVED, that the United States Livestock Sanitary Association assembled in convention this 20th day of October, 1967 request that the Secretary of Agriculture provide official recognition of the five phase program for the eradication of Pullorum Disease and Fowl Typhoid in accordance with the delineations of the 1966 Report of the Committee on Transmissible Diseases of Poultry.

RESOLUTION NO. 7

Appreciation to Hosts

The Seventy-First Annual Meeting of the United States Livestock Sanitary Association has been one of the finest in the history of this organization. No small part of this recognition is due to the excellent pre-planning and diligent efforts of the Committee on Local Arrangements which has labored unceasingly to provide for the delegates and their wives most efficient accommodations and facilities during the business meetings and quite delightful entertainment in our moments of leisure.
In light of this, the United States Livestock Sanitary Association wishes to express its appreciation for a most inspiring meeting to the following:

Committee on Local Arrangements
Hotel Westward Ho
Arizona Cattle Feeders Association
Arizona Cattle Growers Association
Arizona Veterinary Medical Association
Arizona Veterinary Supply Company
Arizona Livestock Sanitary Board
Agricultural Research Office, Animal Health Division (Arizona Office)
Central Arizona Veterinary Medical Association
Phoenix Chamber of Commerce
Southern Arizona Veterinary Medical Association
University of Arizona, Pathology Division
Ketchum Manufacturing Company
National Band and Tag Company
REPORT OF THE COMMITTEE ON PARASITIC DISEASES AND PARASITICIDES

W. C. Tobin, Denver, Colorado; J. H. Brashear, Oklahoma City, Oklahoma; G. L. Crenshaw, Orland, California; J. H. Hourrigan, Hyattsville, Maryland; J. E. Kleck, Albuquerque, New Mexico; F. R. Koutz, Columbus, Ohio; H. B. McGrath, Kansas City, Missouri; M. D. Mitchell, Pierre, South Dakota; A. C. Newman, Jr., Opelika, Alabama; R. D. Radeleff, College Station, Texas; I. H. Roberts, Albuquerque, New Mexico.

The summaries of this fiscal year's activities in sheep scabies eradication, cattle scabies eradication, cattle fever tick eradication and the status of the screwworm eradication programs are being submitted for inclusion in the published proceedings of the United States Livestock Sanitary Association.

The committee reviewed the report submitted by Dr. L. N. Butler concerning the management of several outbreaks of Chorioptic Mange in bovine herds in Arizona during the past fiscal year. An additional report also concerning Chorioptic Mange in bovine herds in Arizona during the past fiscal year. An additional report also concerning Chorioptic Mange in bovine herds disclosed at the National Western Stockshow in Colorado was submitted to the committee. In view of the reports submitted, the committee recommends that added diligence by veterinarians and livestock interests be encouraged in order that Chorioptes Bovis be better controlled to prevent widespread losses to the livestock industry.

The committee recommends further that a standard procedure for inspection and management for external parasites of livestock at expositions and livestock shows be universally adopted. The following procedures are hereby suggested:

1. Careful inspection of all animals, by fully trained scabies inspectors immediately on arrival at the show—prior to allowing animals to mingle.
2. Upon diagnosis of scabies, animals be quarantined—including both infested and exposed.
3. Order and supervise treatment with approved products prior to showing.
4. Retreat both infested and exposed animals 10 to 14 days following initial treatment and prior to release from show.
5. Clean and disinfect vehicles used to transport infested or exposed animals.
6. Notify States of origin of animals found to be infested.

The committee further recommends that the proposed updating of Title 9, CFR, Parts 72, 73 and 74 be implemented at the earliest possible date, in order to allow more adequate control over scabies outbreaks, establishment and maintenance of quarantines, movements into and out of
quarantined areas, and reference to certain parasites not clearly defined in present regulations.

**CATTLE SCABIES ERADICATION**

During Fiscal Year 1967 increased efforts were made to locate any additional evidence of the disease. Approximately 24 million cattle were inspected on farms, 2 million more than the previous year. For the first time since 1963 psoroptic cattle scabies was not reported in the United States.

In May 1967 Federal quarantines, previously placed on one county in California and 8 counties in Texas, were removed. These quarantines had been placed in early 1966 when 7 infected lots of cattle were found in Texas and 4 infected lots found in California. All infected lots of cattle were dipped twice and exposed flocks were dipped once. In addition, active programs of inspections were carried out in both States.

**SHEEP SCABIES ERADICATION**

Program activities during the fiscal year were centered in Iowa where 32 infected flocks had been found during Fiscal Year 1966. During Fiscal Year 1967, two "down-the-road" inspections of all sheep in Iowa failed to disclose any scabies infection. Sheep scabies has not been reported in Iowa since May 9, 1966. Effective June 7, 1967, Part 74, Title 9, Code of Federal Regulations, was amended to remove Iowa from the list of States in the Sheep Scabies Infected-Eradication Area.

Major efforts in all other States involved "down-the-road" surveillance inspections and/or monitoring inspections at concentration points; i.e., premises of dealers; traders; feedlots; local fairs; shows; special sales; sales barns; public stockyards; and Federal, State, county and municipal slaughtering establishments. All inspection efforts were aimed at uncovering any foci of the disease which might have eluded previous efforts to find "that last infected flock."

Only two outbreaks of psoroptic sheep scabies were disclosed during Fiscal Year 1967 as compared to 49 outbreaks in Fiscal Year 1966. Both outbreaks during Fiscal Year 1967 were diagnosed at public stockyards on sheep that originated in Sheep Scabies Free Areas; one in Lake County, Illinois, and the other in Fillmore County, Minnesota. Effective December 22, 1966, Federal Regulations were amended placing Lake and DeKalb Counties, Illinois, in the Infected-Eradication Area. Four infected lots were found at public stockyards during Fiscal Year 1966 and six during the previous year. Also, in Fiscal Year 1967, 13,745,349 sheep were inspected on farms and 107,117 were dipped as compared to Fiscal Year 1966 when 20-010,807 sheep were inspected on farms and 405,629 were dipped.

**STATUS OF THE SCREWWMOR ERADICATION PROGRAM**

During fiscal year 1967, the barrier of sterile screwworm flies along the 2,000 mile U.S.-Mexican border continued to protect the United States from reinfestations of screwworms from Mexico. It was strengthened and the quality of sterile screwworm flies was improved.
During the fall of 1966, the Screwworm Eradication Program faced a strong challenge from screwworms. Due to ideal weather conditions favoring screwworms in Mexico and the Southwestern States, a large fall buildup of screwworm cases occurred from fly migrations through the barrier zone. In addition, there was complacency on the part of livestock owners, which resulted in less searching for infestations. This threat was met by producing and releasing greater numbers of screwworm flies. During fiscal year 1967, more than 6.5 billion sterile screwworm flies were released in the sterile screwworm barrier to protect areas of the United States already freed of the pests. This is an increase of 1.2 billion flies over fiscal year 1966. Seventy-three per cent of the flies were released over Mexico. Although more than 1,600 cases occurred in the United States, the pest did not gain a foothold and the United States remains free from established populations of screwworms.

A Mexican strain of flies has replaced the Florida strain which had been used to produce sterile flies since the beginning of the program at Mission. Program scientists believe that the Mexican strain is able to perform better in the field and should be more compatible with native Mexican screwworm flies. Further improvement in the quality of sterile screwworm flies released was made possible by the use of a new temperature control system on the larvae-rearing vats, enabling more uniform heating of the vats. Size of the sterile screwworm flies was increased by changing from a fluid media to a diet of animal protein mixed with non-fat dry milk. Research has shown that larger male screwworm flies are able to mate more efficiently.

During fiscal year 1967, there were 1,668 laboratory confirmed cases in the United States. One thousand three hundred and thirty-seven of these were inside the Barrier Zone and 331 outside. Texas had 1,138 cases in 82 counties; New Mexico 71 cases in 11 counties; Arizona 394 cases in 11 counties; and California 65 cases in 2 counties. There were 8,427 cases in the Mexico portion of the Barrier Zone.

Informal discussions have been held with Mexican government officials about the possibility of moving the barrier to the Isthmus of Tehuantepec, where it could be more economically operated.

CATTLE FEVER TICK ERADICATION

Prevention—keeping the ticks out of the United States—is a major part of the effort against cattle fever ticks. A quarantine zone is maintained along the international boundary and the lower Rio Grande River in eight Texas counties as adjacent areas in Mexico are infested. Cattle from Mexico are carefully inspected for ticks at the border. They must be free of ticks and must be given a precautionary dipping before they can be imported. Without these controls, cattle fever ticks would reinfest areas of the United State that have warm climates.

Active Program Continued in Texas

During Fiscal Year 1967 in the Buffer Zone, 156 livestock illegally
crossing the border were caught, of which 47 were tick-infested; 61 tick-infested Texas herds were found; and 58,871 lots of 1,672,247 livestock were inspected and 14,046 lots of 73,459 livestock were dipped.

**Tick Surveys and Exotic Ticks Found**

During Calendar Year 1966, 4,370 tick survey collections were made including 1,875 from cattle; 385 from dogs; 1,246 from horses or mules, 266 from zoo animals and miscellaneous; 461 from native wildlife; and 137 from animals offered for entry.

Exotic ticks collected from imported animals or materials or those offered for importation included *Amblyomma cajennense*, *A. dissimile*, *A. longirostre*, *A. rhinocerotic*, *A. varium*, *Dermacentor nitens*, *Rhipicephalus appendiculatus*, *R. evertsi*, *R. evertsi mimeticus*, *R. pulchellus*, *R. sanguineus*, and *Rhipicephalus sp.* These collections were made from a rhinoceros, a sloth, an anaconda, a porcupine, an eland, a giraffe, a sable antelope, horses, and zebras; Air Force personnel baggage from Viet Nam; frozen beef from Nicaragua; wool being imported from India; a partially dried spot of blood in the cargo compartment of a commercial plane which had made stops in 7 African countries; and in the cargo compartment of a commercial plane from Jamaica. These represent 21 separate collections, and they were made in the States of Alabama, California, Delaware, Florida, Illinois, Nevada, New Jersey, New York, and South Carolina.
The Committee on Regulatory Veterinary Medical Resources has been very active during the past year. Members of the Committee have visited other states and made extensive studies of all their programs and activities. All states have not been visited but the Committee did have complete studies of nineteen states at its disposal. Conclusions and recommendations in this report are based on the findings in those nineteen states.

The Committee did not find that there was any great amount of duplication of effort by state and federal forces. They also did not find any great amount of waste of manpower in carrying out programs.

The Committee did find that there was some minor duplications in some states particularly in the field of record keeping. Those states are making every effort to eliminate these duplications as soon as possible.

The Committee did not find that there were any misunderstandings or difficulties between state and federal officials in those states where the states have sufficient funds to carry their part of the disease control load. They did find some dissatisfaction in some states where state programs are underfinanced. Shortages of state funds causes increases in federal activities which leads to more authority being assumed by federal personnel. State officials may then become dissatisfied with these working arrangements. It is the opinion of the Committee that most of these misunderstandings involved personalities rather than policy differences.

The Committee found that there existed a good working relationship between state and federal personnel and in most states the two forces are so interwoven that they are melted into one working organization doing a real efficient job. We feel that these two agencies working together can be termed a check and balance between state and federal governments and for the better of all concerned.

The Committee recommends that all states make every effort to obtain sufficient funds to furnish at least 50% of the cost and at least half the manpower to carry on the cooperative programs. In no state where these facts were true did we encounter any misunderstanding or difficulties.

We recommend that all states take full advantage of all training programs so that state personnel are as fully trained as federal personnel.

We recommend that federal personnel be given the same authority as state personnel in handling all disease control matters within the state.

We would hope that this committee will be continued and that we may continue to visit other states and serve as a place where suggestions and complaints may be brought for study and action.
REPORT OF THE COMMITTEE ON SALMONELLOSIS

Salmonellosis is one of the major problems facing the livestock, food, and byproducts industries. Many agencies are conducting studies and reporting findings, yet a very real problem still exists. In spite of a need for regulatory control, however, the objective cannot be accomplished by this means alone. We must know more about the ecological aspects of salmonellae. If the organisms are to be effectively controlled, more information must be acquired on their organization and their relationship to our animal population and their environment. No one can question the merits of sanitation, but conversely, one cannot prescribe effective sanitation without a more complete knowledge of the organisms.

Reduction in the incidence of Salmonellae will require better animal practices, including such essential elements as improved animal husbandry, avoidance of overstocking and movement of very young stock, and the control of the bacteriological condition of mixed feeds. During the past year, most effort has been directed at breaking the feed transmission cycle.

The state-federal cooperative survey in livestock feeds included in last year’s committee report was completed. The incidence of salmonellae contamination in the 26 states was determined as:

1. Grain - 0.66 ± 0.19%
2. Cattle feed - 0.85 ± 0.22%
3. Oilseed meals - 2.28 ± 0.32%
4. Swine feed - 3.13 ± 0.58%
5. Fish meal - 4.72 ± 0.92%
6. Poultry feed - 5.23 ± 0.73%
7. Animal byproducts - 31.07 ± 2.18%

Subsequently, the state-federal voluntary salmonella control program, as outlined in the 1966 report, was initiated with the rendering industry in 21 states.

Minor changes in the program are suggested for FY 1968; e.g., three inspections per year and ten samples to be tested per inspection. This will intensify the surveillance and increase the probability of detecting problem plants.

Opportunity to participate in this program should be extended to all 50 states in FY 1968.

To facilitate USDA’s participation in the cooperative salmonella control program with the rendering industry, it is deemed desirable to have a federal regulation controlling the interstate shipment of animal byproducts. This proposal is being prepared as Part 84 of 9 CFR and will be published in the Federal Register in the very near future.

The committee has developed a proposed model for a state rendering plant law for the control of salmonella. (See attachment.) It is felt that uniform and adequate state laws will supplement the state-federal program.

As approximately one-half of the inedible rendering plants in the United States are in slaughtering establishments which are under the surveillance
of the Livestock Slaughter Inspection Division, C&MS, and as this division does not have specific authority for surveillance in the inedible rendering portion of slaughtering establishments, and in the interest of economy in the use of government personnel for rendering plant inspection for salmonella control, it is suggested that this committee give support to the LSID to obtain this added authority.

Many of the state diagnostic laboratories are finding it difficult to find a source of salmonella diagnostic antigens for regulatory programs. Because of this need, the committee makes the following recommendation:

Commercial laboratories be encouraged to produce these antigens and make them available for official testing programs. If this source is not available, then the Veterinary Biologics Division, ARS, USDA, be given the responsibility of seeing that diagnostic antigens are available for official testing programs.

The committee urges all laboratories conducting salmonella isolation and identification procedures to adopt the recommended procedures for the isolation of salmonella organisms from animal feeds and meat byproducts as set forth in ARS bulletin 91-36 and as revised by the committee on standard procedures for the isolation of salmonella by the Conference of Veterinary Laboratory Diagnosticians.

The committee has noted an increased incidence of salmonellosis in livestock as a result of surface water contamination. Investigations should be considered to acquire more information regarding potability of water supplies for livestock.

Although the veterinary profession is well qualified to handle outbreaks of salmonellosis as it occurs in rendering plant establishments, a need exists for training to be provided to regulatory veterinarians regarding the physical operations of a rendering plant. We urge the Animal Health Division of the Agricultural Research Service to investigate such training.

This committee urges the Federal-State Relations Committee of this association to fully support federal appropriation requests and urges the membership of this association to join in this effort.

(A suggested State Law Prepared for the Consideration of the Various States)

AN ACT

To Regulate Inedible Rendering Establishments

Be it enacted by the General Assembly of the State of ____________,

That as used in this Act unless the context indicates otherwise ______.

1. The term “Inedible Rendering Establishment,” means a place of business that deals in rendering material of animal origin and processes it into finished products in such a way that risk, damage, or
nuisance to animal or public health is avoided. Any person who receives from any other person the body of any dead animal for the purpose of obtaining the hide, skin, grease, meat, bones, or parts thereof from such animal for further processing to a finished form as described in section 5 is deemed to be engaged in the business of disposing and rendering of the bodies of dead animals or parts thereof.

2. The term “Rendering Material” means and includes any dead animal not slaughtered as food for animals or man, of if slaughtered for food, becomes unsuitable for such use, and includes all parts of dead animals and all inedible byproducts of animals slaughtered or processed as food.

3. The term “Animal” means any member of the animal kingdom such as fish, reptiles, birds, and mammals, etc.

4. The term “4-D Animals” means dead, dying, disabled, or diseased animals.

5. The term “Finished Products” means any product or material processed or manufactured from rendering material or from 4-D animals by an inedible rendering establishment or establishment processing 4-D animals such as bone meal, blood meal, meat meal, tankage, feather meal, tallow, etc., or fresh frozen, partially cooked, or cooked or canned pet, fur animal, or other animal feed.

6. The term “Establishments Processing 4-D Animals” means a place of business that processes the carcasses or any part of carcasses of 4-D animals to be used as feed for dogs, cats, fur-bearing or other animals.

7. The term “Inspector” means a state employee trained and assigned to inspect rendering plants and establishments processing 4-D animals.

8. The term “Department” means the State _________.*

9. The term “Laboratory Tests” means tests conducted as deemed necessary by the Department to ensure that the finished product meets required specifications for quality and safety (to include protein analysis, contaminating agents of disease, etc.); such laboratory tests to be performed in laboratories approved as provided in section 8, and on samples of finished products collected by the inspector.

10. The term “Licensing Authority” means the license require for an inedible rendering establishments and/or establishment processing 4-D animals to operate in the State of ___________; such license shall be issued to an establishment only if the following requirements are met:

(a) A fee of $ ______ for the issuance of a license shall be paid the State by the licensee, subject to renewal each year.

(b) Physical facilities. All rendering establishments and establishment processing 4-D animals are to be constructed in such a manner as to protect the finished product and to prevent pollution of surrounding environment or creation of a nuisance to the public. (Information and requirements on establishments, ________________________________.

*Department of Agriculture or other designated state authority.
location, construction, and sanitation requirements may be ob-
tained from ____________________.

(c) **Transporting all rendering material.** All rendering material
shall be transported to the rendering establishment in covered
and leak-proof vehicles, such vehicles to be used for this pur-
pose only and to be cleaned and disinfected after delivering each
load.

(d) **Heat treatment.** All rendering material shall be heated to a
sufficient temperature for a sufficient length of time to destroy
all pathogens, and processed under sanitary procedures that
prohibit the recontamination of the product after cooking.

(e) **Transporting finished products.** The finished product shall be
transported from the rendering establishment or the establish-
ment processing 4-D animals in a clean vehicle in such a manner
that will prevent contamination.

(f) **Inspection.** Rendering establishments and establishments proc-
essing 4-D animals may be inspected periodically by an inspec-
tor who may procure samples for laboratory testing.

11. **License.** No person shall engage in the business of collecting, dis-
posing, or rendering of the bodies of dead animals or parts thereof
without first obtaining a license for such purpose from the Depart-
ment.

12. **Application for license.**
(a) Application for license shall be made on forms provided by the
Department and shall be accompanied by an inspection fee of
$________. On receipt of such application, the Department shall
inspect the premises in which the applicant proposes to conduct
such business. No license shall be issued unless the Department
finds that the premises comply with the requirements thereof.
(b) If the Department finds that the applicant’s premises do not
comply with the requirements of this section or with the rules
of the Department, it shall notify the applicant wherein the
same fails to so comply. If within a reasonable time to be fixed
by the Department, but not more than 90 days thereafter, the
specified defects are remedied, the Department shall make a
second inspection and proceed therewith as in the case of an
original inspection.

13. **Revocation of license.** A license may be revoked if requested by
the operator of licensed establishment or if in the opinion of the
Licensing Authority, the establishment fails to meet the sanitation
or bacteriological standards required to effectuate the purposes of
this Act.

14. **Intrastate movement.** Finished products from only licensed inedible
rendering establishments or licensed establishments processing 4-D
animals will be allowed to move intrastate.

15. The authority conferred by this Act shall be in addition to authority
conferred by other statutes. Any provision of any other Act in-
consistent with the provisions of this Act is hereby repealed.
REPORT OF THE COMMITTEE ON FEDERAL-STATE RELATIONS


BRUCELLOSIS-TUBERCULOSIS

This committee is pleased to note the steady progress toward certification that is evident in most areas of the country. That there are some exceptions is certainly no fault of the Agricultural Research Service. Financial and technical assistance adequate to enable every state to achieve the modified-certified status has been made available. Failure of any state to show substantial progress toward certification can be explained only by inertia on the part of the state itself.

These states which are presently free or nearly free of brucellosis are already considering ways and means of reducing their programs to the surveillance level. One costly procedure—calfhood vaccination—will surely be drastically curtailed in state after state as it will be difficult to justify continued mass immunization against a disease which no longer exists in the area.

A reduced vaccination program will mean a rapid build-up of a susceptible cattle population. The states which move in this direction will have no choice but to adjust their import regulations to the situation as a matter of self defense. This would be unfortunate. There are already more than enough impediments to the free flow of livestock interstate.

If additional restrictions on movement are to be avoided, aggressive action in the lagging states is essential. Allocation of more funds and more manpower to these areas for the next couple of years would aid materially in getting them in line with the rest of the country. The so-called 60-40 rule is a definite handicap at this state and it is our hope that the Agricultural Research Service will make every effort to get this arrangement changed for the period immediately ahead.

There is another unfortunate suggestion which arises from time to time in connection with Brucellosis. This is the thought that control in the form of the modified status is sufficient. It is the feeling of this committee—and I think we can say of the vast majority of the members of the U.S.L.S.A.—that our objective must remain complete eradication. Nothing less is acceptable.

This includes brucella infection in swine. The need is obvious and the action of the West German government in excluding certain pork products originating in the United States emphasizes this need.

Last year we expressed the view that the expenditure of an additional $1,000.00 per year on Tuberculosis could shorten the eradication period by 20 years and that significant savings in the total cost of eradication would result.
Anticipated progress against brucellosis between now and July 1968 should permit transfer of this amount—or more—from the Brucellosis program without adversely affecting that program and without adding to the total allotted to the two diseases. It is urged that serious consideration be given to this means of accelerating the work of tuberculosis eradication.

**MISCELLANEOUS DISEASES**

Several diseases under the miscellaneous classification are worthy of special mention:

Trichinosis has become an increasingly important problem to swine producers. The incidence of this disease in swine has steadily declined since the inception of the Meat Inspection Law of 1906, and sharply declined since the introduction of Garbage Cooking Laws in 1953. Although the incidence in swine is low, the disease is still a threat to public health. Swine producers have come to the realization that much of the decline in per capita consumption of pork has resulted from adverse publicity surrounding the isolated cases of human trichinosis. Many other people decline to eat pork because they feel that in order for it to be safe, it has to be overcooked and therefore is not palatable.

The fact that we still have this disease in our swine population has also been a factor in the loss of foreign export markets for pork and pork products. An eradication program for trichinosis is essential to the survival of the swine industry. In order to establish such an eradication program, we must find the source of infection in our swine since it is now known that even grain fed swine are infected. Ways of identification for swine must be developed to make possible complete epidemiological investigations, particularly in cases which implicate humans.

We are pleased to learn that monies have been appropriated, both to ADP for research and to the Animal Health Division for studies of this disease and development of an eradication program. This committee recommends that these amounts be maintained or increased by 1969 if surveys and studies indicate the need for additional funds to initiate an eradication program.

Jowl abscesses is another malady of swine which has been a hazard to swine producers and meat packers. We feel this condition can be eliminated if sufficient funds are provided for research and for a survey by Animal Health to ascertain the cause of this condition.

We are all aware that there is growing emphasis for greater livestock production to feed our growing populations. In many sections of the country this increased production is expected on declining land areas. This calls for an increased efficiency in livestock production. One of the vital needs for increased efficiency is freedom from diseases and parasites. Those of us in regulatory veterinary medicine play a vital role in this increased efficiency. To fulfill our professional obligations we must have available laboratory facilities for quick, accurate and comprehensive diagnoses.

We urge the Department of Agriculture to study very seriously the
possibility of providing for the Animal Health Division adequate diagnostic laboratory services to supplement existing state, regional and private laboratories. The Animal Health Division should closely coordinate such a laboratory with all other agency laboratories within a given area to prevent duplication of expensive laboratory equipment and professional staff. Such a program would assure every segment of the livestock economy in all parts of our country, the availability of competent disease diagnostic service that they vitally need.

**IMPORT-EXPORT, INSPECTION AND QUARANTINE**

We are quite concerned for our ability to keep pace with the ever increasing potential for the invasion of our livestock industry by exotic diseases and parasites.

The criticism of certain elements of the livestock industry regarding the department's approval and support of the Canadian Grosse Ile quarantine station subsided greatly and in fact almost disappeared when it was brought to their attention that there were so many other avenues of ingress for foreign diseases to which and for which we had only a very minimum surveillance.

The present level of 36% boardings (first port of call) does not in our opinion provide needed protection.

We would feel much safer if this percentage could be raised to somewhere between 70 and 75 percent and be maintained. These boardings should not only be increased, but should be made within a very short time after the vessel has landed, whether it is surface or air.

The request for the 1968 budget year of an additional $387,000 will add 25 people. These people will be distributed to stations in 14 states. (Alabama, California, Florida, Georgia, Louisiana, Maryland, Massachusetts, New York, North Carolina, Oregon, Pennsylvania, Texas, Virginia, Washington.)

It is expected that these additional people will increase our percentage of boardings to about 57 percent.

**SUPERVISION OF INTERSTATE MOVEMENT OF LIVESTOCK**

We feel that there is a need for a continued support of this program at the present level.

This is one of the several programs needed to apprehend disease, to back up and support the inspection and quarantine program, and to act as a check point across all means and methods of interstate movement. This program also serves to maintain equality in state requirements by establishing minimum standards.

**LABORATORY ANIMAL WELFARE**

Legislation passed by the 89th Congress (PL 89-544), the Laboratory Animal Welfare Act, has given the Department of Agriculture the responsibility to prescribe and enforce standards and regulations for the
FEDERAL-STATE RELATIONS

humane care and treatment of dogs, cats, and other laboratory animals during transportation, sale, and handling by dealers and research facilities.

The Committee wishes to compliment the Department on the work it accomplished in prescribing the required standards, especially with the very meager factual information available and, also, on meeting its deadline of February 24th.

This program is extremely broad in the areas covered and the organizations involved. (Such groups as the Public Health Services, the National Society for Medical Research, Medical colleges, Medical and Scientific Research organizations, Biological and Pharmaceutical industries, Humane Societies, etc., are included.)

Fiscal Year 1967. To administer the law during the fiscal year 1967, the Committee understands that Congress provided an amount of only $300,000 and suggested that the needs of the Department for additional funds be considered during the 90th Congress.

The Committee made some inquiries regarding the adequacy of appropriations for the remainder of the fiscal year 1967, but it was not clear as to the means that the Department would employ to fund the program during the next four months.

Since the effective date for compliance by laboratory animal dealers is May 24, 1967, it would seem to be highly essential that this program be adequately funded during this initial period, so that the source of animals for research facilities not be interfered with, because of lack of funds within the U.S. Department of Agriculture. If the only available source of funds is through the request to the Congress for a supplemental appropriation for the remainder of fiscal year 1967, we should urge that necessary funds be requested at this time.

Fiscal Year 1968. The Committee was informed that the Department is requesting an increase of $1,200,000 for the purpose of administering PL 89-544 in the fiscal year 1968.

It is noted that this amount is required to assure compliance with the standards; however, it is not clear whether this appropriation is also intended to provide for research on environmental and behavioral factors influencing the health, comfort, and safety of animals being transported and maintained for research purposes.

The Committee wishes to point out that the state of the art of providing the optimum in laboratory animal welfare is not fully developed. It should be recognized that many of the values contained in the standards must necessarily be judgment estimates and are not, in fact, scientifically derived.

We urge that the Department undertake a comprehensive program of research to determine the effects on these animals of differing environmental conditions and that funds be provided for such studies.

Fiscal Year 1969. It is recognized that the Department has no base line of experience upon which to draw for budget estimates for 1969, since this is a new and unprecedented program. Since the values contained in the standards as finally promulgated must be subjected to constant critical
review and their validity firmly established through research and experience, we would urge that the Department, in its summation of request for 1969, point out that it is quite possible that requests for additional appropriations will be forthcoming as experience dictates, during the early part of the administration of the law.

State Cooperation. PL 89-544 authorized the Secretary to cooperate with the officials of the various States, in effectuating the purposes of the Act. Since a number of States are engaged in the administration of laws dealing with the procurement and supply of laboratory animals for research purposes, we believe it is highly essential that the Department undertake the task of surveying existing State laws that have a relationship to the enforcement of the Federal regulations.

The lack of veterinary experience in laboratory animal care and medicine provides one of the big problems in carrying out the Laboratory Animal Welfare Act, as developed by the 89th Congress. To minimize the problems on providing adequate veterinary care, there should be close cooperation between the USDA, the USLSA, the AVMA, the State Veterinary Medical Associations, all colleges of veterinary medicine, and Veterinary Departments of Land Grant Colleges and universities. There is a need for short courses, additional courses in curriculas of veterinary medicine for future graduates, and continuing educational programs to help prepare the practicing veterinarian for this new area of laboratory animal medicine and welfare.

SCREW WORM PROGRAM

We commend the progress and benefits that have been derived from the screw worm program. We realize that it must be a continuing program. In this area over a long-term evaluation, a stepped-up program that will push the barrier zone to the Tehuantepec peninsula is the acceptable one.

The additional expenditure of funds is urged to arrive at the 1.5 million dollar maintenance of the barrier zone that could be achieved at the end of the proposed 11 years. We feel the increased program would pay dividends beginning within 4 years—and after the completion of the 11 year projection would be a saving of 3.5 million yearly in actual expenditure of funds plus the savings derived from protection to the industry. The 50,000 square mile zone that could be plugged with few holes would offer security far greater than the 500,000 plus square miles that we are now maintaining. Of course we would welcome any cooperating funds that may come from industry and the Mexican Government.

In review it appears—The program, on the present level will cost 60 million dollars during the next 12 years. By stepping up the program—the overall cost would be 57 million dollars in 12 years and would lead to additional and more satisfactory protection.

CATTLE TICKS

Present appropriations must be shored up to compensate for attrition due to normal increases in cost of personnel and maintenance of present
minimum program. We cannot lower this maintenance below the present level because at this level tick carrying animals are penetrating the barrier. The U.S.L.S.A. recommends that an increased appropriation of $80,000 be granted to assure maintenance of the present level of protection.

SCABIES

Present appropriations appear adequate to accomplish the eradication of sheep scabies and to allow additional effort in the problem areas of cattle scabies that have developed due to the extended efforts expended on sheep scabies eradication in the immediate past.

BIOLOGICS

First of all I want to thank you for all past courtesies you have given members of this committee of the United States Livestock Sanitary Association. We represent among others, the states that are responsible for the control and eradication of diseases of livestock and poultry, throughout the nation. We are in the front line and answerable at the ground level. You are the cooperative, coordinating group at the top. Our goals of necessity must be the same. We look to you to provide, through your research ability, the answers we must have to questions in order to develop programs of control and eradication. We have worked together to carry out our responsibility to the livestock and poultry industries. We both can and should be proud of the accomplishment to date. I am confident the continuation of our cooperative effort in the future will provide us with other successes of note.

It has been my privilege to have been a member of this committee from its inception to the present. It has fallen to my lot today to present for your consideration some of the needs in the area of research in diseases of livestock and poultry, in the laboratory diagnostic facility and in the administration of the Serum Virus Toxin Act.

Needs as far as Research is Concerned

It will be remembered that when the Plum Island laboratory was constructed we were unable to provide all of the animal wings thought necessary. We now need more animal quarters. It is our judgment that these needs should be provided and request consideration be given by you to obtaining whatever funds are required to draw up plans for this need.

For some time we have presented to you the need for developing a parasite research facility at Beltsville. I understand that their necropsy incineration facility is inadequate and should be replaced with a modern one to meet all the needs of safety and in compliance with the air pollution control prevalent in the area.

Recently I understand we have found blue-tongue in our cattle population. This condition heretofore affecting sheep now poses a problem in cattle. We need to know much more about this virus and its possible vectors. Expanded facilities at the Denver Station should be provided so that
your research workers can develop facts concerning blue-tongue as it affects cattle as well as sheep.

I understand too, that funds required for salary increments are taken out of the money allocated for research. Provisions should be made to replace this.

The Licensing of Biologic Products

First, let me congratulate you on the improvement and effort you have put forth to endeavor to meet your responsibility in this area. However, this is no time to rest upon the progress made to date. Here are a few of the facts which reveal how much yet remains to be done to fulfill your responsibility. These are known to you but please let me review them. There are 239 kinds of products and of these and from these there are 1,202 licensed products. 50 percent of these are under testing surveillance. Of these 50 percent, only 30 percent of the lot are tested. Of the 30 percent, 6.6 percent were found unsatisfactory. 288 lots were found in this group of unsatisfactory products. These products which were unsatisfactory contained 244,000,000 doses. 166 were declared unsatisfactory for lack of potency, 122 because they were contaminated. Only in the instances of products made for brucellosis, tuberculosis, salmonella pullorum and PPLO were 100 percent of the products tested. We are impressed with the progress made. We shall not be satisfied until you have the facility and staff to provide that all products licensed are under surveillance and that the licensee will never know when his product may be picked up and subjected to test, to prove that it meets with the requirements of:

1. Of value for the condition for which it is produced and offered for sale.

2. Free from contamination.

3. Pure and

4. That it is safe to use.

We are impressed with those engaged in helping you discharge your responsibility in this most important area and we ask you to provide the physical facility and equipment and personnel needed to enable them to complete the task.
COMMITTEE ON STOCKYARDS, MARKETS AND TRANSPORTATION

DR. GEO. STILES, Chairman

The committee on Stockyards, Markets, and Transportation met in Great Falls, Montana, in June of this year in conjunction with the Livestock Marketing Congress. An open meeting of the committee, with assigned subjects for discussion was presented as the main feature of the Livestock Health Coordinating Conference. Health requirements as related to livestock movements, animal identification and market responsibilities were presented. The committee feels this meeting resulted in a better understanding of mutual problems and did much to strengthen industry relations.

After a lengthy discussion dealing with the need for specifically approved markets, inspection and releasing of livestock from federally inspected stockyards and the need for uniformity of requirements the committee requests the United States Livestock Sanitary Association make the following recommendations to the U.S. Department of Agriculture:

1. For animal health purposes, a single set of standards covering all species, for livestock markets be developed and the inspection of such markets be a joint cooperative State-Federal effort, and that prior to release all livestock meet the interstate and state of destination requirements.

   The above can best be handled by a Memorandum of Understanding between the State and Federal animal health officials and the livestock market management in the respective states. If the above recommendations are carried out, it will replace the need for specifically approved markets, and will place all livestock markets meeting the standards on an equal basis.

2. This committee, to more accurately reflect the present-day terminology in livestock marketing, changed its name to "Committee on Livestock Marketing and Transportation."
INDUSTRY’S ROLE IN DEVELOPMENT & MARKETING
BIOLOGICALS FOR ANIMALS*

LEWIS E. HARRIS, President
Norden Laboratories, Inc.
Lincoln, Nebraska 68501

Current policies and attitudes of various federal and state agencies may, if continued and expanded, cost the livestock and poultry industry and the general public millions of dollars, and may seriously curtail the food supply for our nation and the world.

Government agencies are always in the enviable position of being able to cite one or more cases, or to point with alarm to a situation which is considered serious and requires correction. Even though they represent only a very small segment, these incidents can be presented in a manner which may imply that the entire industry is equally suspect in the same area. Newspapers and trade magazines feature articles quoting such government spokesmen, regardless of the facts which may be available. When the industry tries to refute or answer such misleading publicity, it is looked upon with suspicion, or as being against “motherhood,” and any response usually gets a small article on page 19 of the same newspapers and trade magazines. Government spokesmen have developed this technique and use it frequently. This type activity must be curtailed, for it unnecessarily undermines public confidence.

The U. S. Livestock Sanitary Association is to be complimented for having provided an opportunity for both industry and government spokesmen to be heard on this program. For the past several years, members have heard only one evaluation of the matter of biologicals. It is my hope this year will mark the beginning of a policy which will enable spokesmen for both industry and government to appear on your program each year. This will enable members to better evaluate all aspects of each matter related to biologicals.

During the past two or three years we have observed an interesting trend in public relation activities maintained by the Veterinary Biologics Division, ARS, U.S. Department of Agriculture. There appears to be little thought given to whether releases will unfairly discredit the biological industry. On the other hand, such releases may picture the VBD as a knight in shining armor saving the livestock and poultry industry. We also find questionable tactics used to generate Congressional support for increased appropriations.

In my opinion, we have reached a point where it is most important that the many outstanding and continuing contributions of the biological industry be brought sharply into focus and presented to the U. S. Livestock Sanitary Association. I hope to present the positive side of this picture to

 enable a balance against some of the negatives which have been presented to you in past years and may be presented again this year by other speakers. Some of these contributions are:

1. Rabies vaccines—Modified live virus types, chick embryo and tissue culture origin.
2. Equine Encephalomyelitis Vaccine.
3. Blue Tongue Vaccine.
4. Hog Cholera Vaccines. While government agencies did develop the original virus-serum procedure for immunizing swine, it should be noted that had industry not proceeded with underwriting the cost of developing the modified live virus vaccines, it would have been impossible to reach the point where the present hog cholera eradication program could even have been considered.
5. Infectious Bovine Rhinotracheitis Vaccine. This disease, which was recognized in 1954, caused severe losses in cattle, and was a great threat to the cattle industry. Industry scientists, with cooperation from university scientists, developed an effective vaccine which was marketed in 1957. Government agencies made practically no contributions in this area. Incidentally, it is estimated that with present government regulations, it would have taken at least two additional years to get the product on the market. This would have meant two additional years of severe losses in cattle in areas affected.
6. Anthrax Vaccine.
7. Erysipelas Bacterin and Vaccine.
8. Enterotoxemia Toxoid and Antitoxin.
9. Poultry Vaccines—Industry developed vaccines which effectively controlled many serious poultry diseases. This is one of the most important factors in providing the opportunity for the poultry industry to grow and expand so rapidly.
10. Converted Brucella Abortus into a dependable vaccine through lyophilization to stable form.
11. Developed methods to stabilize live vaccines through use of appropriate stabilizers and lyophilization.
12. Developed adjuvants which have improved the immunizing capability of many biologicals.

It is interesting to note that federal government agencies have had little or nothing to do with the discovery or development of biological products since their early work with hog cholera and brucellosis which led to the development of the serum and virus method of immunization, the crystal violet hog cholera vaccine, and Brucella abortus Strain 19 vaccine. Indeed, industry has not only carried out the research and development phases, but also developed test procedures and supplied government with most of the few existing reference standards such as standard erysipelas bacterin, standard challenge for blackleg bacterin, standard perfringens toxin and antitoxin used in potency testing.
The biological industry originated the concept of developing "Standard Requirements" for various veterinary biological products. Industry conducted the tests to establish these standard requirements, cooperated with VBD in writing the standard requirements, and assisted in their revision as this became necessary. It should be stressed that industry urged government to set up standard requirements. This is hardly the type activity an industry would undertake if it were the "shady character" as implied by some government press releases.

But initiating these standard requirements was only one of the several steps taken by industry to assist in providing the highest quality biologicals for the livestock and poultry industry. The minutes of the March, 1954 Veterinary Biological Licensees Association describe contacts made with Dr. M. R. Clarkson, then associate director of the old Bureau of Animal Industry. The VBLA emphasized the need for a government laboratory to do spot checking of biologicals, and to develop new and improved testing procedures. Congressional support for appropriations to construct such a laboratory was strongly urged by industry, and this resulted in the construction of the well-equipped National Animal Disease Laboratory at Ames, Iowa in 1960. Again this initiative and continued effort by industry to improve quality is not compatible with the innuendo now used by some to picture industry as trying to market inferior products.

Incidentally, the March 1954 minutes of VBLA also outline industry proposals for standard requirements for nine small animal and seven large animal biologicals. Please note again that these proposals came from industry—not from government.

These examples should be sufficient to remind everyone of the contributions made by the biological industry, and to point out industry's continued efforts to establish standards to assure the availability of quality biological products. Now, let's move on to a discussion of other problems presently encountered.

Standard requirements for a given product usually include a potency level originally determined by industry for release and marketing of such product. This potency level is invariably many times greater than that required for immunization of the animal; however, to the disappointment of industry, and contrary to the original concept, there is usually no provision for the gradual decline of antigenic content, even though at expiration date the product will be more than adequately potent to immunize the animal. The current VBD position is that the product must pass the same potency test on expiration date as on the date originally released. This is unrealistic and results in abnormally high production costs and higher costs to the consumer. It also casts considerable doubt upon the real value to the livestock industry of the service performed by VBD at Ames. Products testing below standard levels, originally established for release, are being classified as unsatisfactory, and become a statistic in VBD's presentations, adding to the clamor for greater Congressional appropriations, at taxpayers' expense. In effect and in truth, these products may be and most likely would be perfectly safe and effective if used in the field.
Biological test procedures are complex and easily affected by a large number of controllable and noncontrollable factors. Industry has developed the majority of test methods currently used. Industry has scientists who are at least of equal competence to any scientists associated with government. However, we have frequent disagreement in test results on a specific product when tested by industry and government laboratories.

The director of VBD has stated they have an “open-door” policy, and will discuss and reconsider any case where there is a difference in test results. To the best of my knowledge, results of such discussions have generally been fruitless; the government laboratories at Ames maintain they cannot be wrong, and industry is left with no reasonable opportunity to appeal. This policy is unrealistic, unreasonable, and wholly unsatisfactory. It would appear that some government scientists really believe they are infallible! An “open-door” policy is useless when associated with a “closed mind.” This government policy must change if we are to maintain satisfactory relationships for developing and manufacturing the quantity of high quality biologicals required by the livestock and poultry industries.

Industry is aware of the nature of and need for regulation and inspection by VBD—it is an accepted fact of its business life. Should certain licensees fail to perform according to regulatory practices, then that licensee, not the entire industry, should be brought to task.

For the past two years, some individuals in VBD have been utilizing incomplete and/or misleading press releases, comments in both formal and informal speeches at livestock or poultry industry meetings, and closed hearing sessions with some Congressional committees to generate support for increased appropriations. They have used data from tests on products which were released for market weeks or months earlier, and these data have been presented in a manner which may result in the false impression that industry releases products which are substandard. Nothing could be farther from the truth. It must be re-emphasized that these products are selected for retest weeks or months after original release. Also, while they may not have the same potency as when originally released, there is no evidence that the product would not satisfactorily immunize the animal.

VBD has indicated a goal of testing every serial or lot of each biological product produced before it is released for market. And they contend this will greatly benefit the livestock and poultry industry by assuring quality; however, this program, vague and uncertain as it is, can be undertaken only if a very substantially increased appropriation is forthcoming.

The biological industry contends that such a test and release program under present conditions serves no useful purpose, it is unrealistic and misleading, and it will result in unnecessary increases in the cost of biological products. This industry has pointed out that not only is an increased appropriation unnecessary, but also the activities of VBD could be directed into far more productive areas to benefit the general public, the livestock industry, and the producer. In any event, if additional monies should be appropriated for VBD, they should be used for far more meaningful projects and programs. Specifically, they could be used for methods development,
improved analytical techniques, and the broad field of sophisticating our knowledge of animal and poultry immunology.

Industry’s position should not be construed as negative because we strongly oppose the concept of 100 percent test before release. Rather, our position is one of keen desire to continue to work with VBD to hopefully aid in the formulation of programs which will be truly meaningful and contribute to the animal industry and our economy.

When VBLA initiated steps which led to establishing NADL, it urged that this new laboratory devote substantial attention to the development of new standard requirements, new test methods and preparation of reliable reference standards. The original ideas of VBLA are as valid now as they were in 1960. The staff and facility of VBD at NADL are adequate for such programs, and no additional appropriation is required to initiate them. These activities would benefit all segments of industry and the public. Unfortunately, VBD has done little in these areas. Further, communications between VBD at NADL and VBD offices in Hyattsville appear to have never developed, and correlation of activities has left much to be desired.

It is interesting to note a strange line of thought developed by some individuals in government agencies. They suspect there is something basically dishonest about the owners and key personnel in any industry, they believe they will do anything to produce the poorest quality possible just to meet minimum requirements, and they assume anyone in business is making an exorbitant profit. These individuals have either ignored or have never been exposed to the plain economic facts of business life. Admittedly, there is, occasionally, a dishonest businessman, just as there is, occasionally, a dishonest government employee. But these are the exceptions. It would be utterly ridiculous for the established biological manufacturer to conduct tests dishonestly, to release products of questionable quality, or to knowingly jeopardize the image which has been created through long years of careful production and testing programs. A manufacturer increases his sales and improves his reputation only on the basis of producing quality products consistently.

As individuals or as representatives of industry or government, we tend to forget the “Benefit:Risk” ratio which exists in the development and use of biologicals, pharmaceuticals or pesticides for use by man or animals. While all of us would prefer to have this ratio developed on each product to a point where benefits are maximum and risks are zero, we must be realistic and recognize this is rarely possible. In our space program, billions of dollars are spent to make each step “fail-safe”; however, we still have failures and serious accidents. But these incidents do not discourage or deter continuation of the program, for the “Benefit:Risk” ratio is recognized.

It is important that we apply the “Benefit:Risk” ratio philosophy to biologicals. Regardless of the continued best efforts of industry and government we will have an occasional problem situation. A complete test and release program would not alter this appreciably. I have in my files two laboratory reports from NADL on the same production lot of a given biological. The first rejected the product, and the second indicated it was
satisfactory. This is not an isolated case. It is another reason for rejecting the value of complete test and release programs, and it punctures the myth of “infallibility” on the part of government scientists.

Government spokesmen have stated that any “test and release” program would be concerned with only spot-checking, or complete test and release, only until government was satisfied with the continued quality of a given product. This has a nice reassuring sound, but past experience with government agencies does not lend support to the credibility of such statements. Once any program is initiated, it invariably expands.

The biological manufacturers have made substantial contributions to the control of and elimination of disease in livestock and poultry, to the factors enabling rapid growth in the livestock and poultry industry and to the improvement in the quality and quantity of foods of animal origin. Their future contributions can be seriously curtailed and our food supply imperiled by unrealistic government controls. The U. S. Livestock Sanitary Association can play a most important role in this area through careful and thorough review of all aspects of each new State and Federal regulatory proposal.
CARRYING OUT THE RESPONSIBILITY TO INDUSTRY RELATIVE TO ANIMAL BIOLOGICS

JOHN M. HALE

The Veterinary Biologics Division of ARS has responsibility relative to the veterinary biologicals produced by the biological industry. We are responsible to the general public, including the people who use veterinary biologics. This responsibility was given USDA by Congress in 1913.

SLIDE #1: Our mission, under the Virus-Serum-Toxin Act, is to assure animal owners and veterinarians in the United States and other countries of the world that biologics produced here are pure, safe, and effective in the diagnosis, prevention, and treatment of animal diseases.

To help accomplish this mission, the Virus-Serum-Toxin Act requires that animal biologics distributed in interstate and foreign commerce be licensed by the Department of Agriculture—that inspections be made—that methods for testing and standards for producing these biologics be established, and that the products be examined to assure compliance with the standards. Two of these four basic requirements—the establishment of test methods and standards and the checking of products in our laboratories—have become the key factors in successful fulfillment of our responsibility.

To be given at 11:30 A.M., October 19, 1967 at the USLSA Meeting in Phoenix, Arizona.
of assuring that biologics are pure, safe, and effective. Yet, we do not have methods and standards for some biological products, and these are not being checked in our laboratories. Why is this so?

Slide #2: (1) Because resources and time are needed to do the job; the

VETERINARY BIOLOGICS
FISCAL YEAR 1967

57 LICENSED ESTABLISHMENTS
1,153 PRODUCT LICENSES
255 KINDS OF PRODUCTS
58 ANIMAL DISEASES
13,202 PRODUCTION LOTS
8.8 BIL. DOSES

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

scope and complexity of the products to be tested have increased tremendously in recent years. Over 250 different kinds of vaccines, bacterins, serums, toxoids, antitoxins, and diagnostics for 58 animal diseases are produced by 57 licensed establishments. There has been a growth of over 3,000% in the number of doses of biologics produced since 1945. The different kinds of biologics are produced according to over 1,100 different protocols. With few exceptions, production protocols for any given biologic vary between manufacturers producing it with respect to the use of seed cultures, media, stabilizers, and other essential ingredients. Accordingly, each protocol must be considered as an individual product and evaluated as such; (2) Because the Division must actively engage in developmental work that will help establish methods and standards. Such work requires laboratory support. The Division's Biologics Services Laboratory at NADL has been operational since 1962. Before then, many products were licensed by the Division without the means to develop and establish appropriate methods and standards. The Division now must take the lead in developing methods and standards for products licensed in the past and also keep pace with new products coming along; and (3) Because developing and establishing valid methods and standards require extensive animal experimentation. Protection tests in laboratory animals, guinea pigs, for example, will not assure the value of the product in host animals, unless guinea pig protection is
correlated with host animal protection. Such correlations are essential to the establishment of valid requirements.

Test reagents and their quality are important also to successful application of test methods. For example; challenge cultures used in animal protection tests must be standardized to assure uniformity in testing at different laboratories. The use of reference products of known effectiveness and quality used as a control for the “unknown” under test will further assure validity of testing techniques and methodology between laboratories. These reagents need to be developed and made available concurrently with establishment of test methods.

Each production lot of each licensed biologic must be tested for qualities of contamination, purity, safety, potency, and effectiveness. The Division has in effect 58 methods for testing some biological products for these qualities. Some of the methods in use have been proven valid; others need improvement; and, more important, still others must be developed.

Slide #3: Our goals are to develop and establish scientifically and stat-

![GOALS]

- DEVELOP AND ESTABLISH SCIENTIFICALLY VALID TESTING METHODS FOR ALL BIOLOGICS AND TO IMPROVE EXISTING ONES
- CONTINUALLY STRIVE TO BRING ABOUT IMPROVEMENT IN PRODUCT QUALITY

...tistically, valid testing methods for all biologics and to improve existing ones; and to continually strive to bring about improvement in product quality through the application of these test methods by licensed manufacturers and the Division. Time is too short to tell you those which need improvement and those which need development; however, I will give you the status of the Division’s standard requirements for potency and efficacy, since those for contamination are quite well defined.

Slide #4: This is a summary chart of the number of approved tests, those needing improvement, and those which need to be developed for test-
ing biologics used in the treatment of major diseases affecting various species of animals. Fifteen approved methods are now in use. Thirteen additional methods in use need further improvement or validation. Twenty-one methods must be developed.

The next seven slides chart a breakdown according to major diseases affecting each species of animals.

Show Slides 5, 6, 7, 8, 9, 10, and 11: We consider our needs for methods development and improvement as top priority in carrying out our responsibility to users of animal biologics and our relationship with manufacturers of biological products. Division requests for additional resources and an expanded animal biologics program have been directed toward overcoming these deficiencies.

Another important requirement under the Virus-Serum-Toxin Act is the examination of biologics by the Division. Testing in our laboratories has been at a monitoring level varying from 1 to 20% of the production lots for most products, and up to 100% for a select few which are used in national disease control and eradication programs, such as Brucella abortus vaccine, pullorum antigen, and tuberculin.

Few products are subjected to across-the-board testing for contamination, purity, safety, potency, or efficacy by the Division. Most are examined for only one of these factors. Tests for contamination now exceed those for potency or efficacy at a ratio of about 2 to 1. Safety tests have been conducted and some production cultures examined for purity, but the

### SUMMARY OF STATUS STANDARD METHODS—POTENCY & EFFICACY BIOLOGICS—ALL SPECIES

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>MAJOR DISEASES</th>
<th>STANDARD METHODS</th>
<th>TO BE DEVELOPED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APPROVED NUMBER</td>
<td>14</td>
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</tr>
<tr>
<td>BOVINE</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>OVINE</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PORCINE</td>
<td>8</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>POULTRY</td>
<td>5</td>
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<td>0</td>
</tr>
<tr>
<td>EQUINE</td>
<td>9</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>PETS AND FURBEARING</td>
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<td>0</td>
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<tr>
<td>TOTAL</td>
<td>47</td>
<td>15</td>
<td>13</td>
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</tbody>
</table>

FISCAL YEAR 1966
### Standard Methods—Potency & Efficacy

#### Bovine Biologics

<table>
<thead>
<tr>
<th>Major Diseases</th>
<th>Standard Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Approved</td>
</tr>
<tr>
<td>Blackleg</td>
<td>X</td>
</tr>
<tr>
<td>IBR</td>
<td>X</td>
</tr>
<tr>
<td>BVD</td>
<td>X</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>X</td>
</tr>
<tr>
<td>Anaplasmosis</td>
<td>X</td>
</tr>
<tr>
<td>Anthrax</td>
<td>X</td>
</tr>
<tr>
<td>BRUCELLOSIS</td>
<td>X</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>X</td>
</tr>
<tr>
<td>Malignant Edema</td>
<td></td>
</tr>
<tr>
<td>Infectious Necrotic Hepatitis</td>
<td>X</td>
</tr>
<tr>
<td>Pasteurellosis</td>
<td>X</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>X</td>
</tr>
<tr>
<td>Calf Scours</td>
<td>X</td>
</tr>
<tr>
<td>Red Water</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
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</tr>
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</table>

Fiscal Year 1966

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### Standard Methods—Potency & Efficacy

#### Ovine Biologics

<table>
<thead>
<tr>
<th>Major Diseases</th>
<th>Standard Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Approved</td>
</tr>
<tr>
<td>Blue Tongue</td>
<td></td>
</tr>
<tr>
<td>Ovine Ecthyma</td>
<td></td>
</tr>
<tr>
<td>Ram Epididymitis</td>
<td></td>
</tr>
<tr>
<td>Vibriosis</td>
<td></td>
</tr>
<tr>
<td>Enterotoxemia</td>
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<td><strong>Total</strong></td>
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Fiscal Year 1966
### Standard Methods—Potency & Efficacy

#### Porcine Biologics

<table>
<thead>
<tr>
<th>Major Diseases</th>
<th>Standard Methods</th>
<th>Approved</th>
<th>Need Improvement</th>
<th>To Be Developed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erysipelas</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hog Cholera</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.G.E.</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Salmonellosis</td>
<td></td>
<td></td>
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<tr>
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</tr>
</tbody>
</table>

**Fiscal Year 1966**

#### Poultry Biologics

<table>
<thead>
<tr>
<th>Major Diseases</th>
<th>Standard Methods</th>
<th>Approved</th>
<th>Need Improvement</th>
<th>To Be Developed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newcastle Disease</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchitis</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Fowl Laryngotracheitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fowl Pox</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Fowl Cholera</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Avian Encephalomyelitis</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>PPLO (Antigen)</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Pullorum (Antigen)</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
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</table>

**Fiscal Year 1966**
### Equine Biologics

<table>
<thead>
<tr>
<th>Major Diseases</th>
<th>Standard Methods</th>
</tr>
</thead>
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<td></td>
<td>Approved</td>
</tr>
<tr>
<td>Equine Encephalomyelitis</td>
<td>X</td>
</tr>
<tr>
<td>Equine Influenza</td>
<td></td>
</tr>
<tr>
<td>Equine Rhinopneumonitis</td>
<td></td>
</tr>
<tr>
<td>Strangles</td>
<td></td>
</tr>
<tr>
<td>Tetanus</td>
<td>X</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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</tbody>
</table>

**Fiscal Year 1966**

### Canine, Feline & Fur Bearing Biologics

<table>
<thead>
<tr>
<th>Major Diseases</th>
<th>Standard Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Approved</td>
</tr>
<tr>
<td>Canine Distemper</td>
<td>X</td>
</tr>
<tr>
<td>Canine Hepatitis</td>
<td>X</td>
</tr>
<tr>
<td>Rabies</td>
<td>X</td>
</tr>
<tr>
<td>Canine Leptospirosis</td>
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</tr>
<tr>
<td>Feline Panleukopenia</td>
<td></td>
</tr>
<tr>
<td>Feline Pneumonitis</td>
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</tr>
<tr>
<td>Mink Distemper</td>
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</tr>
<tr>
<td>Mink Enteritis</td>
<td></td>
</tr>
<tr>
<td>Botulism</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
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</table>

**Fiscal Year 1966**
numbers have been insignificant in relation to the numbers tested for contamination and potency.

During F. Y. 1966, about half of the biological products produced by licensed manufacturers were monitored in our Division laboratories. Four thousand one hundred and three production lots were tested or about 30% of the lots produced. Two hundred and sixty-seven lots were found unsatisfactory. In F. Y. 1967, 6,473 lots were tested and 250 were found unsatisfactory. Testing all lots of live virus poultry vaccines for Mycoplasma gallisepticum contamination accounted for the major part of the increase in the number of lots tested during F. Y. 1967.

The way in which we carry out our responsibility of monitoring commercial production of biologics depends on what kind of a production job the manufacturer does. If he doesn't do a good job, our job becomes difficult and it is necessary to place more testing emphasis on such manufacturers. If he does a good job, our job is less difficult and less testing of products of such manufacturers is necessary.

Slide #12: This is a summary of percent rejection of lots of biologics tested in our laboratories during F. Y. 1966. Again, time is too short to give you a complete breakdown of this summary, but the next three slides show selected examples of each of the 3 groups.

Show Slides #13, 14, and 15: These findings suggest that monitoring of commercial production of animal biologics is an important and necessary function.

Slide #16: Our goals are to examine and test all licensed veterinary
### VETERINARY BIOLOGICS DIVISION TESTING

#### PERCENT REJECTION RATE

<table>
<thead>
<tr>
<th>NUMBER LICENSEES</th>
<th>REJECTION RATE OF LOTS TESTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0%</td>
</tr>
<tr>
<td>6</td>
<td>UP TO 5%</td>
</tr>
<tr>
<td>18</td>
<td>OVER 5%</td>
</tr>
</tbody>
</table>

**FISCAL YEAR 1966**

### VETERINARY BIOLOGICS DIVISION TESTING

#### SELECTED EXAMPLES

<table>
<thead>
<tr>
<th>LICENSEE</th>
<th>PRODUCTION PRODUCTS</th>
<th>PRODUCTION SERIALS</th>
<th>VBD TESTING PRODUCTS</th>
<th>VBD TESTING SERIALS</th>
<th>UNSATISFACTORY PRODUCTS</th>
<th>UNSATISFACTORY SERIALS</th>
<th>RATIO: NO. SERIALS UNSAT. TO NO. TESTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>16</td>
<td>128</td>
<td>7</td>
<td>40</td>
<td>3</td>
<td>19</td>
<td>8/9,6/11,5/10</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>193</td>
<td>14</td>
<td>53</td>
<td>7</td>
<td>18</td>
<td>3/6,3/12,2/2,2/2,1/2,3,3,4/4</td>
</tr>
<tr>
<td>C</td>
<td>46</td>
<td>483</td>
<td>21</td>
<td>128</td>
<td>3</td>
<td>19</td>
<td>12/40,2/3,5/18</td>
</tr>
<tr>
<td>D</td>
<td>64</td>
<td>715</td>
<td>31</td>
<td>177</td>
<td>4</td>
<td>20</td>
<td>9/28,7/16,1/3,3/7</td>
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<tr>
<td>E</td>
<td>43</td>
<td>804</td>
<td>34</td>
<td>250</td>
<td>6</td>
<td>25</td>
<td>3/7,5/15,6/18,1/16,6/20,4/5</td>
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</table>

**FISCAL YEAR 1966**
### VETERINARY BIOLOGICS DIVISION TESTING
#### SELECTED EXAMPLES

<table>
<thead>
<tr>
<th>LICENSEE</th>
<th>PRODUCTION</th>
<th>VBD TESTING</th>
<th>UNSATISFACTORY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRODUCTS</td>
<td>SERIALS</td>
<td>PRODUCTS</td>
</tr>
<tr>
<td>F</td>
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<td>13</td>
</tr>
<tr>
<td>G</td>
<td>28</td>
<td>463</td>
<td>20</td>
</tr>
<tr>
<td>H</td>
<td>17</td>
<td>242</td>
<td>5</td>
</tr>
<tr>
<td>I</td>
<td>34</td>
<td>322</td>
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<td>J</td>
<td>34</td>
<td>432</td>
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<td>K</td>
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<td>6</td>
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**FISCAL YEAR 1966**

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### VETERINARY BIOLOGICS DIVISION TESTING
#### SELECTED EXAMPLES

<table>
<thead>
<tr>
<th>LICENSEE</th>
<th>PRODUCTION</th>
<th>VBD TESTING</th>
<th>UNSATISFACTORY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRODUCTS</td>
<td>SERIALS</td>
<td>PRODUCTS</td>
</tr>
<tr>
<td>L</td>
<td>31</td>
<td>584</td>
<td>11</td>
</tr>
<tr>
<td>M</td>
<td>9</td>
<td>52</td>
<td>8</td>
</tr>
<tr>
<td>N</td>
<td>23</td>
<td>335</td>
<td>12</td>
</tr>
<tr>
<td>O</td>
<td>10</td>
<td>185</td>
<td>7</td>
</tr>
<tr>
<td>P</td>
<td>24</td>
<td>544</td>
<td>18</td>
</tr>
</tbody>
</table>

**FISCAL YEAR 1966**
biologics at a statistically valid level of sampling that will assure with a high degree of confidence that every lot of every biological product is pure, safe, potent, and effective for the purpose intended. We obviously, are still reaching for this goal. In this regard, Biometrics Services of ARS is working with us to develop a statistically valid testing program. The objectives of

![Goals](image)

the program are to improve the statistical validity of our methods, to reduce to a minimum the testing of products produced by manufacturers who are doing a good job, and to increase testing and impart necessary penalties to those manufacturers who are not doing a good job.
REPORT OF THE COMMITTEE ON BIOLOGICS


In accordance with discussions of the committee last year in Buffalo two segments of the profession were added to the Committee: representatives from the Extension Veterinarians and a representative from the State Diagnostic Laboratories. These two segments of the profession are becoming increasingly interested in the problems related to biologics and their addition has added competence to the committee.

The current objectives of the committee are:

1. Bring about an improvement in the quality of biological products used in the diagnosis, treatment, and prevention of animal diseases.
2. Provide guidelines to make sure that biological products are safe and effective.
3. Keep abreast and inform the Association of new developments throughout the world in the field of biologics.
4. Encourage adequate diagnostic facilities throughout the United States so that the effects of biologics may be properly evaluated.

After considerable deliberation the committee recognizes the need for improved standards of evaluating biologics and encourages the development of these standards.

Effective January 1, 1968 the Veterinary Biologics Division will institute procedures of check-testing of production lots of veterinary biological products prior to the manufacturer's release for marketing. It has been the Division's practice in recent years to check-test production lots at 50% shelf life in order to gain information on product stability in addition to safety and efficacy. The past practice required "recall" of lots found unsatisfactory. The new procedure will prevent the release of unsatisfactory lots and reduce "recall" actions to a minimum.

Reports from the field indicate that disease problems have been associated with the use of certain biologics. Some problems are due to the incorrect use of the product in question, yet other disease outbreaks have occurred after their proper use. This committee suggests that better reporting of these cases be made by state level officials to the Veterinary Biologics Division. Following study of these cases the Veterinary Biologics Division will be in a better position to evaluate the products in question and take steps to prevent similar difficulties.

It was suggested last year that a model state law be proposed by the
committee to regulate biological products produced within the individual states. It was the feeling of your committee that the most effective handling of this problem, to avoid costly and in our minds unnecessary duplication of activity, would be to recommend that each state include in their laws or regulations a clause restricting the sale and use of veterinary biological products to those licensed by the Veterinary Biologics Division of the Agricultural Research Service, U.S.D.A. The state could also include in their laws or regulations restrictions on the sale or use of any such licensed products considered to be not needed or not worth the risk, if it is thought that some risk is involved in their use.

Reports were given on two conferences held during the year that were of interest to the committee's activities. The Bovine Respiratory Symposium, sponsored by the American Veterinary Medical Association and the Veterinary Biologics Division, will result in publication of improved procedures for respiratory diseases of cattle. The estimated annual loss in the United States due to bovine respiratory diseases is 275 million dollars.

The second conference was Preconditioning of Feed Lot Cattle held at Oklahoma State University. This conference was attended by representatives of State and Federal Governments and University scientists (in animal production, microbiology and veterinary medical disciplines). In addition there were representatives from the cattle industry, nutritionists, and several producers of veterinary biological products. A thorough discussion was held regarding needs for improved conditioning of cattle and methods of implementing a program to accomplish better control of disease losses during the critical and complex transition from pasture to feedlot. The needs for immunization and proper timing of vaccination procedures to develop needed immunity in such a program was an important part of the discussion. The proceedings are to be published and the problem is to be further explored.
ANAPLASMOSIS SOLUTIONS ON A HERD BY HERD BASIS

NORMAN L. GARLICK, B.S., D.V.M.

A wealth of research on anaplasmosis has been completed over the past 40 years, and an imposing volume of data has become available. Much of this research has been directed toward eliminating the disease, and rightly so.

Anaplasmosis is an enigma. It has plagued the cattle industry for more than a century. No one wants it that hasn't got it, but few want to get rid of it once they have it. Surely this must be "The Thing"—the subject of a very popular song of a few years ago.

Anaplasmosis has been a popular research problem, and many excellent trials and useful data have been produced over the years. Many useful tools have been placed in our hands to do something about it.

The Anaplasmosis Committee of this Association has consistently encouraged research trials and has faithfully and objectively evaluated reported results. Solutions to the anaplasmosis problem have been offered on a number of occasions. The Committee proposed last year that field trials should be established and/or increased in scope in key states to study the feasibility of control and eradication of anaplasmosis using the tools now available. New trials have been inaugurated, but they have been disappointingly few. It is my purpose to present several plans which have been successful in eliminating anaplasmosis. These plans are practical and low in cost. Others may occur to you as you review these. First it would be well to review the procedures available.

Ever since 1952 when Foote reported the successful elimination of the Anaplasma organism from infected cattle using chlortetracycline,1 trials have been underway to evaluate this method of control. Splatter reported similar success in 1953.2 The literature is abundant on successful trials. Failures are conspicuous by their relative absence.

At first, the trials were designed to find out how much antibiotic would be necessary to accomplish desired results.3,4,5,6,21 Prevention through antibiotic prophylaxis was also demonstrated.7 Later work established the efficacy of chlortetracycline in preventing and eliminating anaplasmosis beyond a reasonable doubt.17,22,23 Endorsement of manufacturers claims has been given by Federal Regulation.8 This Association has also recognized the value of these procedures on several occasions.9

Another direction taken in early research was to develop satisfactory tests to detect anaplasmosis. One of these tests is the Complement-Fixation Test (C-F). The high degree of accuracy of this test has been recognized by the U.S. Department of Agriculture, which also had a hand in its development. This test has been utilized for more than 12 years on a broad basis and its usefulness and efficacy are recognized by this Association and by many other groups. It has been demonstrated on numerous occasions that anaplasmosis-free herds can be established, based upon this test.18,19,20
Further proof of its accuracy and dependability has been established through the successful eradication of anaplasmosis in Hawaii. This test has also proved useful in assessing the results of chemotherapy to eliminate Anaplasma infection from individual animals and entire herds.

The C-F test is available, on request, through Animal Health Division Veterinarians in Charge in the various states. In addition, 22 independent laboratories either offer the service or have the capability of performing the test. These laboratories are listed in the 1967 Report of the Anaplasmosis Committee of this Association.

Another test which has been available for a number of years is the Capillary-Tube Agglutination Test (C-A). This is a product of research conducted at the University of Illinois, and the test is produced commercially. It is widely used by researchers working in the field of anaplasmosis, and has been recognized by this Association as having merit. Fundamental differences in these two tests, C-F and C-A, have established spheres of usefulness which are similar, but not identical. These tests, and perhaps others now being developed, are expected to play a vital role in the eventual conquest of anaplasmosis.

The particular role of the C-A test, as I see it, is to provide rapid screening of large numbers of blood serums for the detection of foci of Anaplasma infection. However, I will confine my remarks to herd situations and will not speak of the possibility of area eradication.

I think of the C-F test as the test of choice when you're having only one. This is my choice in determining the status of animals in preparation for a herd anaplasmosis extirpation attempt. It is also well designed for determining the anaplasmosis status of individual animals within infected dairy herds as they go dry. It is important to disclose early infection, and this test will serve the purpose.

Another tool which has been receiving favorable reports from the field is the inactivated vaccine now commercially available. The principle on which this vaccine is based was first explored about 20 years ago. Clinical cases of the disease were prevented in most instances, and infection was prevented in several of the challenged cattle. Research at the University of Nevada and Oklahoma State confirmed the early findings and led to a satisfactory product which confers a measurable degree of resistance to challenge under simulated field exposure. This vaccine has earned consideration in plans to eliminate and control anaplasmosis.

As far as costs are concerned, the vaccine is within reasonable limits. Recent quotations on chlortetracycline indicate that the drug can be fed to a successful conclusion at a cost of $7.50 to $9.00 per head based on high-level feeding at 5 mg. per pound body weight for 30 days.

Anaplasmosis extirpation on a herd-by-herd basis offers an excellent opportunity for private practitioner-client relationships which can be profitable to both. Such arrangements should be encouraged by animal health officials through advice and counsel, as well as laboratory support.

There is a feasible plan for the extirpation of anaplasmosis from almost every herd, but before discussing the details it would be well to define
"extirpation". The term comes from "ex", meaning "out", and "stirp", meaning "root". A good literal definition is "root out".

The compelling reasons for getting rid of anaplasmosis are the economic losses caused by the disease. These may be in the form of mortality, morbidity, weight loss, early weaned and orphaned calves, decreased milk yields, costs of drugs and vaccine, veterinary fees for treatment of clinical disease, diagnostic services, etc.

The man who owns an anaplasmosis-negative herd is entitled to purchase anaplasmosis-negative animals. Regions which are free of the disease should remain free. Animal health authorities have a responsibility in this respect which cannot be avoided. Basic State and Federal statutes prohibit traffic in diseased animals, and animals harboring Anaplasma organisms are diseased in the fullest sense of the word. However, applied sanctions must be accompanied by practical measures for relief and compliance.

Economic losses caused by anaplasmosis can be quickly and effectively controlled by testing and segregation of the affected animals. When the segregation takes place during the winter while vectors are inactive, chemotherapy can be administered and the herd reunited the following spring with little likelihood of further clinical cases. This is especially true when the negative animals are vaccinated at the same time. Vaccination is particularly recommended when extirpation programs are undertaken in certain tick-infested regions.

Vector control, a difficult task, also aids in anaplasmosis extirpation programs. There is no immediate hope of eradicating the vectors of anaplasmosis. Therefore, preoccupation with vectors must be avoided. The basic premise on which anaplasmosis extirpation is based is that the true reservoir of the disease is in infected cattle, not in the vectors (Figure 1). In the absence of an infected-animal reservoir, vectors fail to transmit the disease.

A feasible plan for range beef herds is presented in Figure 2. On a yearly cycle, this consists of a herd test in the fall, segregation, treatment of suspects and reactors, with optional vaccination of negatives. The herd is reunited in the spring. The cycle is repeated the following year. With reasonable good fortune there may be no reactors remaining to treat the second winter. The vaccination may be repeated for all negatives, and annual tests will assess the relative success of the program. All herds on common range should be similarly treated.

A similar plan is suggested for beef herds under fence (Figure 3). Here the success can be more immediately determined if segregation can be prolonged until the herd is proved free by tests at more frequent intervals. However, tests should be conducted no sooner than 90 days following treatment to give the antibody titers time to subside. Although the C-A test can be used here, the temptation to rush the program should be resisted.

In dairy herds the cycle is on a 12-month basis, but the determining factor is the lactation period, rather than the season of the year (Figure 4). Test the entire herd once to identify the carriers. This test should be con-
Anaplasmosis Extirpation

**BEEF HERDS**

(Fenced)

**FALL**

C-F TEST

SEGREGATE

HERD

**SUMMER**

P&S

RETEST

TREAT

P&S

RETEST

**WINTER**

STRENGTH

P&S

CULL

Figure 3

Anaplasmosis Extirpation

**DAIRY HERDS**

**YEAR 'ROUND**

VECTOR CONTROL

C-F TEST

SEGREGATE

N

(VACCINATE)

P&S

CULL

TREAT

N

RETEST

PARTURITION

DRIED PERIOD

LACTATION

P&S

RETEST

Figure 4
ducted during the late fall or early winter. Vaccinate all negatives. As the suspects and reactors go dry, put them on treatment of 5 mg. aureomycin per pound body weight for 30 days. Vaccinated animals can be tested as they go dry after a lapse of six months. If they are positive, treat them. If they are negative, mark them for a booster shot at the proper interval. Withhold milk from treated animals for at least ten days following treatment. This interval should be safe. In beef animals, do not slaughter within ten days after treatment.

The herd can be maintained in segregation, if you wish, in which case there would be less need to vaccinate. Merge the herd as former carriers test out negative. The best time for the test is at the beginning of the dry period. A complete herd test can be run for informational purposes at any time, but preferably in the winter.

The Anaplasmosis Committee urges that all trials continue for at least five years to be certain that the disease is eliminated.

There are some unknowns:

(1) How long will a tick-infested range be hazardous for unvaccinated susceptible cattle?
(2) Can infected deer serve as a reservoir on tick-infested ranges under natural conditions in the absence of infected cattle?
(3) What makes sagebrush ranges more prone to anaplasmosis?
(4) Why are deer in the South apparently free of anaplasmosis although they share pastures with infected herds?

When feeding aureomycin, be prepared for diminished appetites about the third day.

Don’t turn nonvaccinated negative cattle on recently depopulated tick-infested ranges where anaplasmosis was formerly prevalent.

The procedures I have related are not new. All of them have shown independent success. Combining these effective procedures will bring prompt relief at low cost. Start with herds with low rates of infection to gain experience. Take on the tougher problems as you become more confident.

I cannot possibly cover all herd situations in the time allotted. Therefore, if you have questions as to how to proceed, our office is at your service.

In closing, here is an example of success. A North Carolina beef herd had six years of trouble with anaplasmosis. The 155 animals were C-F tested, disclosing 32 reactors. The herd was segregated, but no vaccine was given. The reactors were treated with aureomycin. Six animals which were not yet C-F negative two months after treatment were retreated. One year later the herd was retested disclosing one suspect. The cost of the drug was $369—about the price of one good, but dead, cow. This livestock owner could not afford the luxury of an infected herd, nor can the majority of the owners of other infected herds. It is up to us to spread the
word. Keep in mind that the undesirable animals are the infected carriers which usually constitute a minority of the herd. It does not make sense to continue to expose the noninfected majority to this reservoir of disease.

Anaplasmosis is a slowly spreading, seldom explosive, insidious disease which permits profitable cattle husbandry in all parts of the so-called endemic areas except under exceptional temporary circumstances. If losses were greater there would be no question about initiating successful control efforts. The cattlemen would be the first to demand our assistance. Anaplasmosis will eventually be eradicated unless the cattle owners decide it just isn’t worth the effort. Our most important task is education. The best time to begin an extirpation program is when there are no clinical cases. Rapidly spreading infection requires more heroic efforts than were outlined in this presentation.

REFERENCES
REPORT OF THE
UNITED STATE LIVESTOCK SANITARY ASSOCIATION
ANAPLASMOSIS COMMITTEE

J. W. Safford, Chairman, Helena, Montana; J. F. Christensen, Davis, California; A. A. Cuthbertson, Elko, Nevada; G. T. Edds, Gainesville, Florida; L. E. Foote, Baton Rouge, Louisiana; N. Garlick, Washington D. C.; W. W. Hawkins, Jr., Bozeman, Montana; R. J. Hidalgo, College Station, Texas; R. B. Lank, Baton Rouge, Louisiana; J. O. Pearce, Jr., Okeechobee, Florida; C. C. Pearson, Stillwater, Oklahoma; R. I. Port, Cheyenne, Wyoming; G. B. Rea, Salem, Oregon; M. Ristic, Urbana, Illinois; T. O. Roby, Clarksville, Maryland; E. E. Saulmon, Hyattsville, Maryland; W. L. Sippel, Kissimmee, Florida; J. C. Trace, Fort Dodge, Iowa; S. B. Walker, Austin, Texas; C. J. Welter, Des Moines, Iowa; E. H. Willers, Honolulu, Hawaii; L. Williams, John Day, Oregon.

The Committee assigned specific tasks to four subcommittees which were completed, in part, during the year. The objectives were considered by the Committee as follows:

Objective No. 1: Economic Loss Survey

The survey of economic losses caused by anaplasmosis throughout the United States was initiated by one member of the Committee. Replies to his questionnaire were received from the State Veterinarians of all States except Arkansas, California, Colorado, Louisiana, New Mexico, and West Virginia. Most States declined to estimate the losses (Table VI), explaining that there was no way of knowing what they were, or that there were no data on which to base their estimates. The few estimates that were submitted ranged from zero to $10,000,000, the latter coming from Texas. The estimates totaled only $12,703,000, of which the Texas losses comprised 79 percent. It is obvious that the survey was unsuccessful. However, it may have served a useful purpose in that it brought out clearly how little is known about the actual incidence of anaplasmosis, its prevalence, morbidity, mortality and economic impact. Certainly there is not enough concrete information available on which to base realistic estimates of (1) the research which should be underway, either in public institutions or private industry; (2) estimates of potential markets for drugs and biologics; nor (3) to plan and carry out satisfactory community and/or State programs for its suppression, extirpation and control. The need for a comprehensive reporting system was never brought more acutely to our attention.

Objective No. 2: Reporting Serologic Test Results

It was recommended last year that data be assembled covering the ana-
### TABLE I

**Anaplasmosis—Herds Affected**

<table>
<thead>
<tr>
<th>Month</th>
<th>1964</th>
<th>1965</th>
<th>1966</th>
<th>1967</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>409</td>
<td>386</td>
<td>392</td>
<td>275</td>
</tr>
<tr>
<td>August</td>
<td>650</td>
<td>531</td>
<td>524</td>
<td>402</td>
</tr>
<tr>
<td>September</td>
<td>668</td>
<td>596</td>
<td>533</td>
<td>496</td>
</tr>
<tr>
<td>October</td>
<td>661</td>
<td>400</td>
<td>751</td>
<td>684</td>
</tr>
<tr>
<td>November</td>
<td>301</td>
<td>306</td>
<td>304</td>
<td>461</td>
</tr>
<tr>
<td>December</td>
<td>364</td>
<td>209</td>
<td>312</td>
<td>178</td>
</tr>
<tr>
<td>January</td>
<td>96</td>
<td>79</td>
<td>65</td>
<td>74</td>
</tr>
<tr>
<td>February</td>
<td>140</td>
<td>74</td>
<td>58</td>
<td>81</td>
</tr>
<tr>
<td>March</td>
<td>64</td>
<td>98</td>
<td>63</td>
<td>73</td>
</tr>
<tr>
<td>April</td>
<td>70</td>
<td>63</td>
<td>60</td>
<td>58</td>
</tr>
<tr>
<td>May</td>
<td>174</td>
<td>94</td>
<td>128</td>
<td>89</td>
</tr>
<tr>
<td>June</td>
<td>183</td>
<td>206</td>
<td>273</td>
<td>174</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3,789</td>
<td>3,042</td>
<td>3,471</td>
<td>2,994</td>
</tr>
</tbody>
</table>

### TABLE II

**Anaplasmosis—Reported Cases**

<table>
<thead>
<tr>
<th>Month</th>
<th>1964</th>
<th>1965</th>
<th>1966</th>
<th>1967</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>568</td>
<td>659</td>
<td>732</td>
<td>365</td>
</tr>
<tr>
<td>August</td>
<td>1,333</td>
<td>960</td>
<td>1,052</td>
<td>667</td>
</tr>
<tr>
<td>September</td>
<td>1,172</td>
<td>1,206</td>
<td>992</td>
<td>1,091</td>
</tr>
<tr>
<td>October</td>
<td>1,149</td>
<td>594</td>
<td>1,529</td>
<td>1,172</td>
</tr>
<tr>
<td>November</td>
<td>585</td>
<td>544</td>
<td>876</td>
<td>836</td>
</tr>
<tr>
<td>December</td>
<td>765</td>
<td>624</td>
<td>747</td>
<td>441</td>
</tr>
<tr>
<td>January</td>
<td>380</td>
<td>108</td>
<td>97</td>
<td>182</td>
</tr>
<tr>
<td>February</td>
<td>381</td>
<td>102</td>
<td>221</td>
<td>129</td>
</tr>
<tr>
<td>March</td>
<td>104</td>
<td>154</td>
<td>93</td>
<td>116</td>
</tr>
<tr>
<td>April</td>
<td>193</td>
<td>122</td>
<td>175</td>
<td>98</td>
</tr>
<tr>
<td>May</td>
<td>407</td>
<td>151</td>
<td>183</td>
<td>128</td>
</tr>
<tr>
<td>June</td>
<td>384</td>
<td>351</td>
<td>367</td>
<td>198</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>7,421</td>
<td>5,575</td>
<td>7,064</td>
<td>5,423</td>
</tr>
</tbody>
</table>

National Report of Animal Diseases, USDA, ARS.
plasmosis testing activities of all laboratories conducting tests for this disease, and that a uniform reporting system should be devised.

It is the feeling of the Committee that the reporting system should be as detailed as necessary to provide meaningful, statistically significant infor-
TABLE V

Anaplasmosis Cases, by State, 1962 to 1966; Average Number of Cattle Tested Annually; Remarks Concerning the Disease Situation

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>200-300</td>
<td>Never diagnosed in this state.</td>
</tr>
<tr>
<td>Alaska</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>1964 case—import from Texas.</td>
</tr>
<tr>
<td>Arizona</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arkansas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No evidence of this disease in state.</td>
</tr>
<tr>
<td>California</td>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>3</td>
<td>152</td>
<td>Mostly clinical cases. Few confirmed.</td>
</tr>
<tr>
<td>Colorado</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Carriers found in imported cattle.</td>
</tr>
<tr>
<td>Connecticut</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Biennium, July to July. 1964 to 1966 = 255 Reactors and 125 Suspects. 1962 to 1964 = 40 Reactors and Suspects.</td>
</tr>
<tr>
<td>Florida (CA)</td>
<td>21</td>
<td>152</td>
<td>17</td>
<td>58</td>
<td>436</td>
<td>1,041</td>
<td></td>
</tr>
<tr>
<td>(Acute)</td>
<td>34</td>
<td>5</td>
<td>13</td>
<td>28</td>
<td>106</td>
<td></td>
<td>Extremely small number—all imports.</td>
</tr>
<tr>
<td>Georgia (Herds)</td>
<td>94</td>
<td>158</td>
<td>49</td>
<td>115</td>
<td>96</td>
<td>Limited</td>
<td></td>
</tr>
<tr>
<td>Hawaii</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>28,000</td>
<td></td>
</tr>
<tr>
<td>Idaho</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>400-600</td>
<td></td>
</tr>
<tr>
<td>Illinois</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indiana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iowa</td>
<td>39</td>
<td>52</td>
<td>50</td>
<td></td>
<td></td>
<td>Incomplete</td>
<td>1,249 (1966)</td>
</tr>
<tr>
<td>Kentucky</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>Unkn.</td>
<td></td>
<td></td>
<td>Never had a reported case.</td>
</tr>
<tr>
<td>Louisiana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maine</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
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<tr>
<td>Maryland</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>11</td>
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<td></td>
</tr>
<tr>
<td>Massachusetts</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
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<tr>
<td>Michigan</td>
<td>0</td>
<td>0</td>
<td>46</td>
<td></td>
<td>839</td>
<td></td>
<td>Cases resulted from imported animals.</td>
</tr>
<tr>
<td>State</td>
<td>Herds</td>
<td>Reactors</td>
<td>(Suspects)</td>
<td>(CF+)</td>
<td>(6 mo., 1966)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-------</td>
<td>----------</td>
<td>------------</td>
<td>-------</td>
<td>---------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minnesota (Herds)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mississippi</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missouri</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>307</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montana</td>
<td>97</td>
<td>269</td>
<td>194</td>
<td>20</td>
<td>1,500-2,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nebraska</td>
<td>150</td>
<td>175</td>
<td>250(?)</td>
<td></td>
<td>150-1,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nevada</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Hampshire</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Jersey</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Mexico</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New York (Reactors)</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>12</td>
<td>9,991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Suspects)</td>
<td>19</td>
<td>43</td>
<td>19</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Carolina</td>
<td>162</td>
<td>153</td>
<td>117</td>
<td>77</td>
<td>3,404</td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Dakota</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Never diagnosed in this state.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohio</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oklahoma</td>
<td>187</td>
<td>275</td>
<td>Unkn.</td>
<td>Unkn.</td>
<td>3,045</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oregon</td>
<td>1,059</td>
<td>2,318</td>
<td>11,774</td>
<td>5,648</td>
<td>2,824</td>
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<td></td>
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<td>Pennsylvania</td>
<td>0</td>
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<td>0</td>
<td>On request</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Carolina (Herds)</td>
<td>32</td>
<td>56</td>
<td>32</td>
<td>23</td>
<td>97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Dakota</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Tennessee</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texas</td>
<td>1,141</td>
<td>608</td>
<td>337</td>
<td>488</td>
<td>1,238</td>
<td></td>
<td></td>
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*Not available.
†Cannot be determined. Not all cases are reported.
‡Estimated.

1964 data include slaughterhouse samples.
Last outbreak was in 1960, feeders from South.
Trend shows annual increase.
Seldom have acute form. Mostly chronic.
One large problem herd in Eastern Wash.
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Texas............. Some herds treat, some vaccinate ........ Very encouraging
Utah............. Test. Vaccinate negatives. Eliminate reactors when feasible .... Quite effective
Vermont........... Test; eliminate or treat reactors ........ Too limited to determine
Virginia.......... Surveys by serology .................. ........ ................
Washington........ .............................................
West Virginia...... .............................................
Wisconsin........... .............................................
Wyoming........... No program ....................................... .................

*Program will be altered to permit treatment of reactors.
†Vaccination not permitted.

TABLE VIII
LABORATORIES WITH CAPABILITY FOR PERFORMING ANAPLASMOSIS COMPLEMENT-FIXATION TESTS

Delaware State Board of Agriculture
Georgia Coastal Plains Experiment Station
Hawaii Veterinary Laboratory
Idaho Livestock Disease Control Laboratory
University of Illinois, College of Veterinary Medicine
Diamond Laboratories
Kansas State University, College of Veterinary Medicine
Diagnostic Laboratory, Baton Rouge, Louisiana
Maryland Livestock Sanitary Service
Michigan Department of Agriculture, Laboratory Division
Mississippi Livestock Sanitary Board
Montana Livestock Sanitary Board
State Department of Agriculture, Reno, Nevada
Oklahoma State University, Department of Veterinary Pathology
Central Plains Diagnostic Laboratory, Oklahoma
Oregon State University, Department of Veterinary Medicine
Texas A&M University, College of Veterinary Medicine
Branch Veterinary Laboratory, Provo, Utah
Regulatory Laboratory, Ivor, Virginia
Washington State Department of Agriculture, Division of Animal Industry
Central Animal Health Laboratory, Madison, Wisconsin
Wyoming State Veterinary Laboratory, Laramie, Wyoming

mation which will serve the purposes of all agencies, public and private, program and research.

The Committee recommends that a proposed form be adopted in principle by this Association to assemble the needed data and that the various States and the American National Cattlemen's Association should be solicited for support of the data-gathering effort. If major modifications of
<table>
<thead>
<tr>
<th>State</th>
<th>Total Tests</th>
<th>Positive</th>
<th></th>
<th>SUSPICIOUS</th>
<th></th>
<th>COMBINED POSITIVE AND SUSPICIOUS</th>
<th></th>
<th>NEGATIVE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>Alabama</td>
<td>33,785</td>
<td>473</td>
<td>1.4</td>
<td>448</td>
<td>1.3</td>
<td>921</td>
<td>2.7</td>
<td>32,864</td>
<td>97.3</td>
</tr>
<tr>
<td>California</td>
<td>12,733</td>
<td>1,636</td>
<td>12.9</td>
<td>448</td>
<td>3.5</td>
<td>2,084</td>
<td>16.4</td>
<td>10,649</td>
<td>83.6</td>
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<tr>
<td>Florida</td>
<td>12,923</td>
<td>1,476</td>
<td>11.4</td>
<td>423</td>
<td>3.3</td>
<td>1,899</td>
<td>14.7</td>
<td>11,024</td>
<td>85.3</td>
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<tr>
<td>Georgia</td>
<td>36,864</td>
<td>1,233</td>
<td>3.3</td>
<td>583</td>
<td>1.6</td>
<td>1,816</td>
<td>4.9</td>
<td>35,048</td>
<td>95.1</td>
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<tr>
<td>Maryland</td>
<td>4,851</td>
<td>22</td>
<td>0.5</td>
<td>12</td>
<td>0.2</td>
<td>34</td>
<td>0.7</td>
<td>4,817</td>
<td>99.3</td>
</tr>
<tr>
<td>New York</td>
<td>48,326</td>
<td>120</td>
<td>0.2</td>
<td>223</td>
<td>0.5</td>
<td>343</td>
<td>0.7</td>
<td>47,983</td>
<td>99.3</td>
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<td>North Dakota</td>
<td>897</td>
<td>4</td>
<td>0.5</td>
<td>3</td>
<td>0.3</td>
<td>7</td>
<td>0.8</td>
<td>890</td>
<td>99.2</td>
</tr>
<tr>
<td>South Carolina</td>
<td>3,980</td>
<td>625</td>
<td>15.7</td>
<td>188</td>
<td>4.7</td>
<td>813</td>
<td>20.4</td>
<td>3,167</td>
<td>79.6</td>
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<tr>
<td>Virginia</td>
<td>4,597</td>
<td>162</td>
<td>3.5</td>
<td>116</td>
<td>2.5</td>
<td>278</td>
<td>6.0</td>
<td>4,319</td>
<td>94.0</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>2,455</td>
<td>359</td>
<td>14.6</td>
<td>73</td>
<td>3.0</td>
<td>432</td>
<td>17.6</td>
<td>2,023</td>
<td>82.4</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>161,411</strong></td>
<td><strong>6,110</strong></td>
<td><strong>6.4</strong></td>
<td><strong>2,517</strong></td>
<td><strong>2.1</strong></td>
<td><strong>8,627</strong></td>
<td><strong>8.5</strong></td>
<td><strong>152,784</strong></td>
<td><strong>91.5</strong></td>
</tr>
</tbody>
</table>

*Animal Health Division, Agricultural Research Service, USDA.*
the proposed form were to be required, the amended form would be sub-
mitt ed to the Anaplasmosis Committee for acceptance.

A tabulation of clinical cases and test results submitted by the various
States in response to a questionnaire is attached (Table V). The evident
lack of uniformity in reporting demonstrates the difficulty which is en-
countered in attempting a summary. For instance, the States were asked
specifically for the number of cases of anaplasmosis (incidence), but many
States reported, instead, the results of serologic tests. What was intended
was an estimate of the number of clinical cases occurring in each State,
rather than an estimate of the number of anaplasmosis carriers as disclosed
by serology.

Tabulations of clinical cases of anaplasmosis reported to the U. S. De-
partment of Agriculture are attached (Tables I, II, III, and IV). These
cannot be considered as representing all clinical cases that occur. Their value
may lie, primarily, in establishing trends in incidence, year by year.

Testing activities of the Technical Services Laboratory of the Animal
Health Division relating to anaplasmosis are presented (Table IX). All
States having more than 1,000 tests during the period—1962 to 1967—are
included, as well as North Dakota which had 897. The data cannot be con-
sidered a cross-section of the population in each State, but are valuable as
indicators of the incidence of the disease. There were many reasons why
the tests were requested—interstate and international movement of live-
stock, casual incidence surveys, laboratory confirmation of clinical diagnoses,
etc.

Objective No. 3: Pilot Field Trials

This concerned establishing pilot field trials for the extirpation of ana-
plasmosis from infected herds. The replies to a questionnaire indicate that
a number of States have programs for infected herds, running through a
range of various procedures. A number of trials are underway. Recent trials
have been summarized in current scientific literature and are not repeated
here.

It is anticipated that this Committee will continue to obtain progress
reports on all current field trials. A number of verbal progress reports have
been received, but written summaries of these are not yet available. The cri-
teria established by the Anaplasmosis Committee in 1966 were that these
 trials should be continued for at least five years to determine whether or not
herds apparently freed of anaplasmosis continue to be free. Other factors
which can be learned from these trials are the frequency of reinfection of
herds, efficacy and acceptability of various promising chemotherapeutic
agents, best methods of administering antibiotics under various types of
animal husbandry, feasibility of large-scale screen-test programs, persistence
of test reactions following elimination of the infection, persistence of vac-
cine-stimulated antibodies, efficacy of various tests to disclose carrier animals,
efficacy of new procedures and products arising from current and future
research, etc.

New estimates of the cost of antibiotics (aureomycin) needed to effec-
tively treat carrier animals and eliminate *Anaplasma* infection are available. These range from $7.50 to $9.00, using “high level” feeding regimes for 30 days. Trials, past and present, indicate that this is a satisfactory approach, and one which can be applied in dairy herds. The proposed Monthly Anaplasmosis Report can be used to report results of such studies. Pilot studies are underway in North Carolina, Wyoming, and elsewhere, but the extent of these efforts is not known at this time. A call for specific reports will bring many more to our attention.

The States are urged to assume an active role in promoting field trials and to furnish testing services and advice as needed. The anaplasmosis testing facilities of the Beltsville Laboratory of the Animal Health Division are available on request through the usual channels.

Table VIII is a list of various laboratories with capabilities for performing anaplasmosis complement-fixation tests.

Objective Number 4: Development of Vaccines

The Committee was to report progress made the past year in the development of vaccines as directed by the Association last year. Available information on vaccines and current studies have been reviewed, but it is not considered sufficient at this time. An evaluation report is to be expected next year.

Objective Number 5: Standardized Challenge Dose

It was recommended last year that a study be made in relation to standardizing the challenge dose of infectious material to be used in immunization trials. The Committee presents the following background statement:

1. **The Organism:**

Anaplasmosis is an infectious and transmissible blood disease of cattle characterized by a progressive anemia and the appearance in the erythrocytes of marginal inclusions, termed *Anaplasma marginale*. Each *Anaplasma* inclusion may consist of at least one to eight, or more, infectious units known as initial bodies. Enough evidence has been produced to clearly demonstrate that the initial bodies may invade the bovine erythrocytes and multiply there by binary fission which leads to the formation of marginal *Anaplasma* inclusions. Thus, *A. marginale* is a characteristic form in the growth and development of initial bodies which primarily occurs during the acute phase of infection in a susceptible host. This growth form of *Anaplasma* is less pronounced in the convalescent and carrier phases of the disease.

There are apparently two major circumstances which impede the attempts to standardize the infectious dose of *Anaplasma* that can be effectively used to challenge acquired immunity to anaplasmosis in the respective bovine host. These two major handicaps are: (1) There is no practical way to determine the number of initial bodies or infectious
Anaplasma units in a given quantity of infected blood, (2) There is believed to be considerable geographic regional variation in the virulence of Anaplasma isolates, thus the challenge effect on a host can vary greatly.

There are other circumstances which have to be taken into consideration when selecting the challenge dose. For example, some Anaplasma researchers have noted that challenge blood from carrier animals, which had recovered from an acute infection several years earlier, may cause a relatively mild infection on the first subpassage if small blood volumes are used. Other researchers note that subpassing blood from animals recently treated with tetracycline compounds may also cause a relatively mild infection on the first subpassage.

There are still other variables that can influence the final effect of the first subpassage in a susceptible host.

II. The Host:

Natural resistance is the inherent property of certain susceptible animals, such as deer and calves, enabling them to cope with Anaplasma infection to the extent that no drastic clinical signs of the disease develop. The basis of this natural resistance in calves is not known.

In an effort to select an experimental bovine host which would be most sensitive to an Anaplasma infection, infectivity titration studies were conducted in intact and splenectomized calves and cows. The results indicated that the splenectomized cow was the most susceptible animal. Splenectomized calves and non-splenectomized cows were found to have lesser susceptibility, and the non-splenectomized calf was found to be the least susceptible animal.

Conclusions:

1. Due to the properties of the Anaplasma isolant such as observed regional variation in virulence and peculiar growth characteristics, standardization of an inoculum dose by means of the total volume or number of marginal bodies may be scientifically unrealistic.
2. Under field conditions, clinical anaplasmosis most frequently causes losses in cattle of two years and older and the sensitivity of clinical anaplasmosis in a mature intact cow is comparable to that of a splenectomized three to six month old calf.
3. The immunity vaccines can confer to a given host is evaluated by experimental and field challenges of vaccinated animals with a pathogen used to prepare a given vaccine.

Recommendations:

Recognizing the need for additional knowledge, in particular the development of Anaplasma in its arthropod vector, it is recommended that a challenge dose be used to demonstrate immunity to
anaplasmosis in vaccinated and control animals. The morbidity and/or mortality in splenectomized calves and/or non-splenectomized older susceptible cows should be used to ascertain the virulence of the challenge dose. The calves should be splenectomized at least two months prior to experimental use and such calves should be used no later than five months after splenectomy. It is mandatory that after splenectomy the calves do not develop Eperythrozoon, Haemobartonella, and other possible contaminating blood infections. The cows should be at least two years of age, and preferably older.

Objective Number 6: Chemotherapy of Anaplasmosis

Reports on chemotherapy of anaplasmosis published since the 1966 United States Livestock Sanitary Association Report have been reviewed. Additional information concerning the use of chlortetracycline for the treatment of clinical anaplasmosis or for elimination of the carrier state has not been reported.

A number of Alpha—dithiosemicarbazones was reported to be active against A. marginale in splenectomized calves when administered in three oral doses of 100 mg/kg each. A recent report on an attempt to prevent the development of anaplasmosis in 6 two-month-old, non-splenectomized calves by administration of a dithiosemicarbazone indicated that this compound was ineffective. The dithiosemicarbazone was administered as a single subcutaneous injection of 10 mg/kg. Calves were challenged 70 days after treatment with 0.1 ml of blood having 64% parasitized erythrocytes.

Objective Number 7: Wild Mammalian Hosts and Vectors

New information on these subjects was reviewed. An investigation on white tail deer (Odocoileus virginianus) as wild mammalian hosts of A. marginale is currently in progress at Texas A&M University. The objectives of the investigation are: (1) To determine whether virulence of the organism for white tail deer can be increased by serial passage, (2) to determine whether A. marginale that has been serially passaged in deer remains infectious for cattle. A preliminary report indicated that serial passage of the organism in splenectomized deer has increased the virulence. Deer infected with thirteenth and fourteenth passages of A. marginale were observed to have packed cell volumes of as low as 11% and as high as 95% of erythrocytes parasitized. Blood from these deer remained infectious for splenectomized calves.

The only recent report on vectors of anaplasmosis was concerned with the transmission of the disease from an infected calf to an adult cow by immediate transfer feedings of the horsefly, Tabanus fuscicostatus (Hine). The authors stated that this vector may be of considerable importance in transmission of anaplasmosis in Louisiana since it is the most abundant tabanid in many areas of that state. Attempts to transmit the disease with Tabanus nigrovittatus were unsuccessful.
ANAPLASMOSIS

REFERENCES

8. Report from Drs. L. E. Foote and R. B. Lank, Department of Veterinary Science, Louisiana State University, Baton Rouge, Louisiana.
9. Report from Dr. R. M. Robinson and Dr. K. L. Kuttler, College of Veterinary Medicine, Texas A&M University, College Station, Texas.
We are pleased to report that there is continuing progress in the eradication of brucellosis from the Nation's livestock population. There have been developments during the year which will shorten the time to achieve freedom from the disease. On close observation of the progress, however, it is apparent that the advances in the noncertified states were not as great as they might have been. We recognize that in some instances funds and manpower were factors in this delay and also that in some states, now moving at a faster pace, time was required to reach the higher level of activity.

The effort being made in the modified certified states is encouraging. Most of these states are actively striving for brucellosis-free status. The State of Washington was the only state which achieved free status during the year. Two hundred and seventeen counties achieved Certified Brucellosis-Free status in the fiscal year. Currently, there are 898 free counties, 27 percent of the counties in the Nation. Alaska was the only State to become modified certified since the last meeting. Eighty-nine counties achieved modified certified status, 11 more than the previous year. There are 2,005 counties (64%) modified certified. Over 91 percent of the 3,153 counties in the Nation are certified areas.

Of the remaining 11 noncertified states, work is underway in 158 counties; only 106 remain in which area work has not been inaugurated. (Figure 1)

Although the previously established goal of Modified Certified Brucellosis status for the Nation by 1965 was not attained, many of the states did exceed that goal. The states have provided plans which schedule all counties working by 1969, and the Nation modified certified by 1971. Projections based on previous experience show the entire Nation will be Certified Brucellosis-Free in 1975. (Figure 2) In order that this goal can be achieved it will be necessary that all of the present program procedures be used to the full extent of their effectiveness. The technical competence for eradication of brucellosis is available. This has been demonstrated in the eradication of brucellosis from states that in past years had a very high incidence of disease, such as the dairy areas where conditions are optimal for the spread of brucellosis.

Range areas, as well, have been freed of brucellosis. In these, the prob-

1 Senior Staff Veterinarian, Cattle Diseases, Animal Health Division, Agricultural Research Service, United States Department of Agriculture.
Brucellosis Eradication

COUNTY CERTIFICATION STATUS

June 30, 1966

Percent of U.S. Counties

Certified Free 67%
Modified Certified 22%
Area Work in Progress 4%
Individual Hard Participation 7%

June 30, 1967

Percent of U.S. Counties

Certified Free 64%
Modified Certified 27%
Area Work in Progress 5%
Individual Hard Participation 4%

Figure 1

BRUCELLOSIS ERADICATION GOALS

Status of County Certification

NUMBER OF COUNTIES

PROJECTIONS

1960 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75

ACHIEVEMENTS

1/Total Counties in U.S. = 3,153

FISCAL YEAR

Figure 2
Problem was more of handling the cattle than heavy exposure. The only problem that remains appears to be the recognition in a few areas that brucellosis can be eradicated.

This accomplishment is readily seen in the progress in the certified states. Last year there were only 79 infected herds found in the certified-free states. Most of these herds were infected on only a single test. This is not what would be expected with typical brucellosis. (Figure 3)

**BRUCELLOSIS INFECTED HERDS FOUND**

In Noncertified, Modified Certified and Certified-Free States

![Figure 3](image)

We do, however, expect that, until adequate protection is provided these areas from infection from noncertified areas, the disease will occasionally spread into free states causing unnecessary waste of funds available for brucellosis eradication.

**BRUCELLOSIS RING TEST**

There is a continuing reduction of infection in the dairy herds as indicated by the percentage of herds showing a suspicious ring test. For the fiscal year, 0.6 percent of the brucellosis ring tests were suspicious. As the number of infected herds in the Certified States is reduced, the states are able to increase the number of ring tests per year, enabling an earlier location and removal of the infected dairy animal. As the number of dairy herds decrease, the size of the herds is increasing. The modification of the BRT, using increased milk in the test sample, is improving the effectiveness and simplicity of the testing procedure. Field study results continue to
provide confidence that the BRT is very effective in locating infection in the dairy herd. (Figure 4)

![Figure 4](image)

**MARKET CATTLE TESTING**

Market Cattle Testing has been a recognized procedure in the eradication program since 1960. There has been an increasing number of cattle screened by this means each year, until this year when the number decreased to a total of 4,635,940 of which 2,890,724 were from backtagged cattle. There are two reasons for this decrease: One is that less samples are being collected from dairy cattle covered by the BRT; the other is that there was a 14 percent decrease in cows slaughtered during the year. (Figure 5)

Although the decrease in cattle tested can be accounted for, there should have been normal program growth to make up that loss. The present methods of brucellosis eradication are very dependent upon an effective market cattle testing program. One step taken this year to improve the efficiency of the program was the assignment of responsibilities for the Market Cattle Testing Program by the Secretary of Agriculture. According to the Secretary's memorandum, the Agricultural Research Service, which would be the Animal Health Division and its cooperators, would be responsible for the application of backtags and the Consumer & Marketing Service would be responsible for collection of blood samples from cattle.
bearing the standard backtag. This action should increase the rate of blood sample return from backtagged cattle slaughtered under Federal inspection.

Use of the yellow and white flip tag as a combination backtag-salestag was inaugurated during the year. The one tag identifies the animal to meet the auction market's needs and, also, provides animal and carcass identity

for use by the meat inspector at the packing plant. If the white face of the tag is out, a blood sample is collected and the carcass identity is maintained through inspection. If the yellow face is out, no blood sample is collected, but the carcass identity is maintained through inspection for tuberculosis.

Ten or more states are now using this tag in their auction markets. In some, the transition is complete; in others, some markets are still in the process of adopting the flip tag. Extension of the use of the flip tag as a backtag-salestag to its universal adoption is a goal to be attained.

The use of MCT results is accelerating the eradication of brucellosis. Although a MCT reactor frequently leads to a herd in which no additional infection is found, MCT reactors did lead to the testing of 5,186 infected herds, containing about 24 million cattle, of which 26,570 were reactors. It is unfortunate that 5,420 MCT reactors could not be identified to a herd of origin; this is an area that needs improvement. In one of the States using the flip tag as a backtag-salestag, the change resulted in an improvement from 13 percent to 3 percent of the reactors not identifiable with a herd.
VACCINATION

Strain 19 *Brucella abortus* vaccine has been a valuable aid in reducing the incidence of brucellosis in areas where the rate of infection had been high. In some areas, very little vaccine has ever been used; the incidence of the disease was low and remains low. Over 90 percent of the counties are now modified certified; almost 1/3 of the counties are Certified Brucellosis-Free.

Recognizing that in those areas where there is a high incidence of brucellosis, vaccination can be of value. It must also be recognized that Strain 19 vaccine can cause problems. There are, undoubtedly, many reactors removed from herds each year because they carry a residual titer resulting from vaccination. In the certified-free counties, 71 percent of the herds in which reactors have been disclosed have had a reactor on only a single test. (Figure 6) The herd had a history of being clean prior to dis-

**REACTOR HERDS FOUND**

In Certified Brucellosis-Free Counties

![Diagram showing the distribution of reactor herds: 63% have 1 reactor, 29% have multiple reactors, and 71% of reactor herds disclosed on only 1 test.]

71% of Reactor Herds Disclosed on ONLY 1 TEST

Prepared September 1967

U. S. DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE

Figure 6

closure of the reactor and has, subsequently, remained free of reactors. Some of these are no doubt infected animals, but experience in isolating *Brucella* from singleton reactors indicates that only a small percentage of these reactors are infected animals.

A more serious problem arises when the vaccinate fails to resist infection. In some instances, the animal may be shedding *Brucella* but still may have a less-than-reactor blood titer. This does occur in nonvaccinates; but
the problem is the vaccinated animal, where the titer is suspected as being the result of vaccination. (Figure 7)

Several years ago, the decision was made to eradicate brucellosis, not merely control the disease. It has now become evident that a few of the calves vaccinated with Strain 19 will not recover from the vaccination infection; they will remain infected. *Brucella abortus*, indistinguishable from Strain 19, has been isolated from official vaccinates as old as eight years of age. The number of these isolations is not great, but it is great enough to prevent the eradication of brucellosis from the cattle population.

Considering these facts, it becomes apparent that in most areas of the country the use of Strain 19 vaccine is now a costly hindrance to the eradication of brucellosis rather than the benefit it is in the few areas of high infection. Vaccination should be discontinued in the free areas. In the modified certified areas, vaccine should be limited to those areas where the exposure risk is high. In those noncertified states with heavy infection, vaccination should be continued; but the cattlemen should be aware of the necessity of stopping vaccination when the incidence of the disease has been reduced.

Continued use of vaccine in the absence of exposure is a waste of manpower and funds, whether it be at public or private expense.
BRUCELLOSIS CARD TEST

The brucellosis card test was approved as a means for screening cattle herds for brucellosis in 1965. During the following year, it was used in a few States, each card-test reactor being subsequently tested by the standard plate or tube test. In those areas where it was widely used, it was apparent that it would provide a means for achieving eradication more rapidly; and it was recommended that the test be given full recognition as a diagnostic test.

During 1966, there was an opportunity to conduct a controlled study, using the card test on a group of animals with known exposure and supported by bacteriologic findings. These results were reported to the Brucellosis Committee last year. There was some concern that the test would cause over condemnation of noninfected animals. However, the test was approved in 1966 as a diagnostic test. Since the last meeting of this organization, there has been ample opportunity to observe the results in the field to ascertain the extent of the over condemnation and weigh its advantages against its disadvantages. The States of Florida, Louisiana, Mississippi, Oklahoma and Texas have been using the card test extensively since the first of the year. In those States, approximately 1 3/4 million cattle have been tested by this means. Each of those States were requested to provide test results comparing the infection rate for six months in 1966, using the standard plate or tube test and the corresponding 6 months in 1967, using the card test. Most of this testing was done for initial certification; and in the judgment of the local State and Federal veterinarians, the incidence of brucellosis in these areas should have been very nearly the same. The results reveal that in the period the card test was used there was less than one additional animal per hundred tested removed as a positive than in the previous period before the card test was used. The rate of infection was 2.07 percent in the period the plate test was used and 2.84 percent in the period the card test was used.

In some of these States, little testing had been done prior to the area work. There has been extensive calf vaccination. Some of the herds have probably been infected for many years. It is quite likely that some of these old infected animals have a titer less than necessary to classify them as reactors. This has been bacteriologically confirmed in some herds. Removal of these animals by the card test has been quite advantageous as shown in comparison of the average number of tests necessary to free an infected herd of brucellosis. Florida reports that, when using the plate test, 3.46 tests per herd were necessary and, when using the card test, this was reduced to 2.18. In Mississippi, the records reveal 3.3 tests before and 1.6 after using the card test. Louisiana reports that when the plate test was used 55.2 percent of the herds were negative on the first retest and, in the period the card test has been used, 76.1 percent of the herds were negative on the first retest.

We have gone into detail in reporting the use of the card test because there have been questions and some skepticism relative to its use.

The card test is providing an efficient and economical means for the
eradication of brucellosis. It is not a panacea; there is not at this time any test which is 100 percent perfect.

SWINE BRUCELLOSIS

The recommendations made by this organization last year have not yet been implemented because of problems in identification of slaughter sows and blood sample collection. These appear to have been solved and plans for a pilot project are complete.

The incidence of brucellosis in swine appears to be low. A survey was conducted during December in which all swine slaughtered at Federal plants were statistically sampled. There were 23,738 animals tested. Of the 13,333 market-weight animals slaughtered, 3.4 percent were reactors. There were 10,405 sows and boars tested of which 4.2 percent were reactors.

There were a total of 61,372 swine tested during the fiscal year; 2.4 percent were reactors. This includes the testing which has been done to maintain the status of the 137 validated counties and approximately 2,500 validated herds. (Figure 8)

The individual States have been encouraged to develop market swine testing programs to meet their needs. Nine States, Puerto Rico and the Virgin Islands now have programs underway. Five additional states have
conducted surveys other than that conducted nationwide last winter. (Figure 9) They support the belief that the incidence of brucellosis in swine is low. Oregon, for example, has collected over 3,000 samples from slaughter sows, identifiable to the herd of origin; and no reactors have as yet been found in the slaughter samples.

**Brucellosis Eradication**

**STATES ENGAGED IN MARKET SWINE TESTING ACTIVITY**

Nevada, Utah, Vermont and the Virgin Islands are Validated Brucellosis-Free.

In closing, it must again be pointed out that it is urgent that brucellosis eradication activities be accelerated in the noncertified states and in those few modified certified states which are not progressing as rapidly as expected. We face a drastic reduction of Federal funds. It would be unfortunate if it became necessary to use these funds for controlling movements from noncertified states to protect the certified states rather than eradicate the disease where it exists, providing a greater protection to clean herds and areas wherever they may be. Information provided by the Market Cattle Testing Program must be more effectively used. Infection must be contained in those herds in which it is disclosed. Vaccine should be used judiciously. Application of these procedures will insure eradication of brucellosis by 1975.
RECOVERY OF *BRUCELLA ABORTUS*, STRAIN 19 FROM IMMUNIZED CATTLE

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University of California, Davis, California
and
C. J. NELSON, D.V.M., M.P.H.
United States Department of Agriculture
Agricultural Research Service, Sacramento, California

In 1948, the state of California initiated a compulsory program to eradicate brucellosis from dairy cattle by making mandatory calfhood immunization, periodic blood testing to detect infected animals, and disposal of diseased animals by slaughter. The success of the program can be measured by the fact that in August 1962, California qualified for status as a Modified Certified Brucellosis Free State and, also, that by January 1967, 31 of 58 counties within the state had qualified as Certified Brucellosis Free Counties. However, within both the modified and certified free areas, many dairy herds that have been Brucella Milk Ring Test (BRT) negative for several years have reverted to displaying a suspicious BRT reaction, indicating reinfection with, or at the least reexposure to, *Brucella* organisms. Since serum testing individual animals within these herds usually failed to reveal agglutination titers sufficiently high to classify any animal as a reactor, the herds were designated problem herds and subjected to additional testing procedures to ascertain the cause of the reversions in reactions to the BRT test. This paper is a report on the findings that accrued from the investigations of these herds.

**Herd Histories**

The time spanned by this investigation was 37 months—from December, 1963 through June, 1967. During the course of this time 21 herds came under surveillance by virtue of being located within either a Modified or Certified Brucellosis Free county and of reverting from a negative to a suspicious BRT test. Twenty of the herds were within 5 Certified Brucellosis Free counties and one herd was in a Modified, Certified County. Many of the herds had had consistently negative BRT tests for as long as 5 years and one herd (no. 21), which reverted in April, 1967, had been continuously negative since January, 1958.

The size of the herds ranged from 100 to 425 lactating animals.

**Detecting Infected Animals in Herds Displaying a Suspicious BRT Reaction**

The herd that initiated this project (no. 1) reverted to a suspicious

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BRT reaction in December 1963. Segmented BRT testing revealed one suspicious herd segment. Serum titers of the animals in this segment ranged from negative to 1:200 as determined by the standard tube agglutination test. Removal of the two serum reactors failed to restore the BRT to negative. It was evident that the animal(s) responsible for maintenance of the antibody content in the pooled milk were not serum reactors and that more discriminating methods would have to be used to detect these animals. Since the change in herd status from negative to suspicious of harboring infection was effected by finding *Brucella* antibody in pooled milk, the source of this antibody was pursued by testing individual milk samples using the whey plate agglutination test. One animal (P-4) was positive and all others were completely negative. The serum agglutination titer of P-4 was 1:50. Rivanol was acidified plate antigen were used for supplemental testing of the serum. Both tests were positive in this animal and negative in the remainder of the segment. The removal of the animal P-4 from the herd restored the BRT to negative.

**Culturing of the milk**

The milk of the three animals removed from this herd was cultured on tryprose-serum agar plates containing antibiotics. *Brucella* organisms were recovered from each of the samples. Detailed examination of the isolates revealed that each was *Brucella abortus*, type 1. However, the isolates from the two serum reactors required CO₂ to initiate growth on primary isolation while the isolate from animal P-4 (Table 1) grew luxuriantly in an air atmosphere. Manometric determination of the oxidative metabolic patterns revealed that strains from the reactors had metabolic patterns identical to all other strains of *B. abortus*, but P-4 displayed the pattern of oxidation that is unique to strain 19 in that strain 19 is the only strain found thus far in this genus that does not oxidize i-erythritol.

Each of these organisms then was tested for its virulence for guinea pigs according to the method detailed by Meyer. At 21 days post-inoculation the guinea pigs that were given the two strains requiring CO₂ all had greatly enlarged spleens many had numerous pinpoint size abscesses of the liver, and masses of colonies too numerous to count were recovered from cultures and masses of colonies too numerous to count were recovered from cultures of the spleens. At 42 days post-inoculation, the results on these two strains were essentially the same as at the 21 day interval. This was true for the pigs that were given 10⁸ organisms as well as those that were given 10⁹ organisms. These results were the same as obtained with the virulent controls, strains 2308 and p-26.

The guinea pigs that were given strain 19 (controls) and isolate P-4 evidenced but little tissue response to the organisms. At 21 days postinoculation the spleens were only moderately friable and were somewhat darker than normal. However, neither strain 19 nor P-4 caused splenic enlargement or any grossly observable lesions. The organisms were cleared from the tissues, as determined by culture of the spleen, by 42 days post-inoculation.

On the basis of both the conventional and manometric determinative
```
<table>
<thead>
<tr>
<th>Herd Number</th>
<th>Animal Number</th>
<th>Breed</th>
<th>Date of Isolation</th>
<th>Age of Cow at Time of Isolation</th>
<th>Infected Animal</th>
<th>Standard Tube Test</th>
<th>APA Rivanol</th>
<th>Whey</th>
<th>Source of Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P-4</td>
<td>Jersey</td>
<td>12-17-63</td>
<td>2 1/2 yrs.</td>
<td>S</td>
<td>i:1:50</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td></td>
<td>P-16</td>
<td>Jersey</td>
<td>1-28-65</td>
<td>2 yrs.</td>
<td>S</td>
<td>i:1:50</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td></td>
<td>P-42</td>
<td>Jersey</td>
<td>1-4-67</td>
<td>3 yrs.</td>
<td>S</td>
<td>+1:100</td>
<td>0*</td>
<td>0</td>
<td>+4</td>
</tr>
<tr>
<td></td>
<td>P-43</td>
<td>Jersey</td>
<td>1-26-67</td>
<td>3 1/2 yrs.</td>
<td>S</td>
<td>i:1:50</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td>2</td>
<td>P-7</td>
<td>Jersey</td>
<td>4-14-64</td>
<td>2 yrs.</td>
<td>S</td>
<td>i:1:100</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td></td>
<td>P-12</td>
<td>Jersey</td>
<td>10-18-64</td>
<td>1 1/2 yrs.</td>
<td>S</td>
<td>i:1:200</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td></td>
<td>P-14</td>
<td>Holstein</td>
<td>12-18-64</td>
<td>7 yrs.</td>
<td>S</td>
<td>+1:50</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td></td>
<td>P-17</td>
<td>Holstein</td>
<td>2-24-65</td>
<td>3 yrs.</td>
<td>S</td>
<td>+1:50</td>
<td>N</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td></td>
<td>P-18</td>
<td>Jersey</td>
<td>4-5-65</td>
<td>3 yrs.</td>
<td>S</td>
<td>i:1:100</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>P-19</td>
<td>Holstein</td>
<td>4-5-65</td>
<td>2 yrs.</td>
<td>S</td>
<td>i:1:50</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td>7</td>
<td>S-28</td>
<td>Holstein</td>
<td>11-2-65</td>
<td>4 1/2 yrs.</td>
<td>S</td>
<td>+1:200</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>P-29</td>
<td>Crossbreed</td>
<td>12-13-65</td>
<td>3 yrs.</td>
<td>S</td>
<td>i:1:50</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td>9</td>
<td>P-30</td>
<td>Guernsey</td>
<td>12-29-65</td>
<td>3 yrs.</td>
<td>S</td>
<td>+1:25</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td>10</td>
<td>P-31</td>
<td>Jersey</td>
<td>12-29-65</td>
<td>2 1/2 yrs.</td>
<td>S</td>
<td>i:1:200</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td>11</td>
<td>S-32</td>
<td>Jersey</td>
<td>2-4-66</td>
<td>2 yrs.</td>
<td>S</td>
<td>i:1:100</td>
<td>0</td>
<td>0</td>
<td>+4</td>
</tr>
<tr>
<td>12</td>
<td>P-33</td>
<td>Holstein</td>
<td>4-4-66</td>
<td>4 yrs.</td>
<td>S</td>
<td>i:1:100</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td>13</td>
<td>P-35</td>
<td>Jersey</td>
<td>6-26-66</td>
<td>2 1/2 yrs.</td>
<td>S</td>
<td>i:1:200</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
</tbody>
</table>
```
<table>
<thead>
<tr>
<th>No.</th>
<th>Breed</th>
<th>Age 1</th>
<th>Age 2</th>
<th>Age 3</th>
<th>Age 4</th>
<th>Age 5</th>
<th>Age 6</th>
<th>Age 7</th>
<th>Age 8</th>
<th>Age 9</th>
<th>Age 10</th>
<th>Test</th>
<th>Test 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>P-38 Jersey</td>
<td>8-3-66</td>
<td>Aged</td>
<td>S</td>
<td>+1:200</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>P-39 Jersey</td>
<td>8-2-66</td>
<td>4 yrs.</td>
<td>S</td>
<td>i1:100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Milk</td>
<td>Milk</td>
</tr>
<tr>
<td>16</td>
<td>P-40 Jersey</td>
<td>12-18-66</td>
<td>2½ yrs.</td>
<td>S</td>
<td>i1:100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Milk</td>
<td>Milk</td>
</tr>
<tr>
<td>17</td>
<td>P-45 Jersey</td>
<td>3-3-67</td>
<td>2½ yrs.</td>
<td>S</td>
<td>i1:50</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Milk</td>
<td>Milk</td>
</tr>
<tr>
<td>18</td>
<td>P-46 Jersey</td>
<td>3-20-67</td>
<td>3 yrs.</td>
<td>S</td>
<td>i1:50</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Milk</td>
<td>Milk</td>
</tr>
<tr>
<td>19</td>
<td>P-47 Jersey</td>
<td>3-31-67</td>
<td>4 yrs.</td>
<td>S</td>
<td>i1:50</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Milk</td>
<td>Milk</td>
</tr>
<tr>
<td>20</td>
<td>P-48 Crossbreed</td>
<td>5-16-67</td>
<td>5 yrs.</td>
<td>S</td>
<td>i1:50</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Milk</td>
<td>Milk</td>
</tr>
<tr>
<td>21</td>
<td>P-51 Crossbreed</td>
<td>6-30-67</td>
<td>3 yrs.</td>
<td>S</td>
<td>i1:100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Milk</td>
<td>Milk</td>
</tr>
</tbody>
</table>

*0 = test not done.
tests and of the tissue response in guinea pigs, it was concluded that the two strains (from the reactors) were virulent field strains, but that strain P-4 evidently was *B. abortus*, strain 19.

Since December, 1963, 21 herds located within Modified or Certified Brucellosis Free Counties have reverted from a negative to a suspicious BRT test. In each instance the procedure found effective in detecting the infected animal was:

1. The herd was designated as a problem herd
2. The herd was segmented and each segment BRT tested
3. Each animal in the suspicious segment then was examined by the (standard Serum tube agglutination,) whey-plate agglutination test, Rivanol, and acidified plate antigen. The results of this testing procedure is shown in Table 1, where it can be seen that 1) a total of 26 animals have been removed from these 21 herds; 2) only 3 of the animals had serum agglutination titers sufficiently high to enable their mandatory removal as reactors; 3) the remaining 25 animals were removed from the herds by virtue of the fact that each was positive to the whey test and simultaneously positive on Rivanol and acidified plate antigen; 4) in each instance, removal of the single infected animal restored the herd BRT negative.

Since a culture indistinguishable from, and presumptively identified as, strain 19 was isolated from an animal in herd no. 1, and since this animal had been responsible for the retention of the suspicious BRT test, in all the herds subsequently examined, the animals displaying positive serologic tests were cultured. Quarter milk samples were collected from lactating animals and udder secretions obtained from animals that had ceased to lactate during the interim between the initial BRT test and collecting for culturing. Supramammary lymph nodes were salvaged at slaughter from four animals removed from the herds by virtue of being serum reactors. All 29 of the animals removed from these 21 herds yielded positive cultures. Three of the strains (two isolated from herd 1 and one from herd 2) required carbon dioxide to initiate growth, oxidized i-erythritol, and were highly virulent for guinea pigs. The remaining 26 isolates did not require carbon dioxide to initiate growth on primary isolation, did not oxidize i-erythritol, and none of them incited tissue response in guinea pigs. They also were similar to strain 19 and different from other members of the species *B. abortus* in that their growth was inhibited by both thionin blue and penicillin. The conclusion is inescapable that these 26 isolates are the vaccine strain, that it is shed in milk, and that it was responsible for these 21 herds in essentially brucellosis free areas to lose, at least temporarily, their infection free status.

An examination of the data on Table 1 reveals some other rather interesting features about these herds in areas that are essentially free of brucellosis.

1. Among the 21 herds in the Modified Free and Certified Free Counties that have reverted from a negative to a suspicious BRT, there was only one offending animal at a time in each herd, although in herds
1, 2, and 14, 2 to 4 animals were removed over the 3½ yrs. spanned by this investigation.

2. In only two herds, number 1 and 2, were both strain 19 and a virulent field strain isolated during these 37 months. Most of the herds included in this investigation are closed and raise their own replacements. The two herds in which virulent organisms were isolated were the only herds with a history of recent introduction into the herd of animals raised outside the brucellosis free areas.

3. Of the 26 animals found infected with strain 19, 17 were of the Jersey breed. Sexual maturity and the initiation of pregnancy, which occurs at a younger age in Jerseys than in other breeds, apparently enhances the possibility for localization of strain 19 in the tissues.

Ovage immunization also could account for persistent infection rather than immunity, but neither early maturity nor delayed immunization accounts for all the strain 19 infections found among these herds. Infection with strain 19 so far has been encountered in areas essentially free of virulent field strains i.e. New York, Wisconsin, and parts of California. During the course of the many years required to achieve eradication of brucellosis in these areas, the composition of the cattle population slowly has changed from a mixture of infected, susceptible and immune to a population that is entirely susceptible and unexposed until strain 19 is administered as a live immunizing agent. Inevitably, some of the animals have become infected rather than immunized. While the known number of animals within which strain 19 has localized is few compared to the number immunized, it none-the-less has caused suspicion to be cast upon the status of the herds where infection has occurred. In areas now free of bovine brucellosis and where control can be exercised over the ingress of animals into the area, perhaps it would be wise to effect a moratorium on the use of a live immunizing agent and let the course of events in these areas determine future policy on the continued use of strain 19.

REFERENCES

SITES OF LOCALIZATION OF BRUCELLA SUIS IN SWINE

B. L. DeYoe, D.V.M., M.S.
and
C. A. Manthei, D.V.M.

INTRODUCTION

In swine brucellosis, there are few detailed reports which compare different sites for recovery of *Brucella suis*. More knowledge of the most suitable tissues for attempted isolation of brucella organisms is necessary for studies on: (1) epidemiology, (2) evaluation of diagnostic tests, (3) pathogenesis, and (4) public health aspects of the disease. Early reports on bacteriologic examination of naturally infected swine listed the spleen as the most frequent source of *B. suis*, with various lymph nodes, liver, and kidneys secondary. Conversely, Hutchings indicated that *B. suis* could be isolated most often from lymph nodes and genital organs. The liver, spleen, and kidneys, as well as numerous other tissues were of lesser importance. However, Hutchings' report contained no details regarding the manner in which the swine were exposed or the postexposure interval when necropsy was performed.

This report compares the frequency of recovery of *B. suis* from various tissues of 147 swine in which infection persisted until necropsy. These swine were all experimentally infected and represented a diversity of ages, routes of exposure, and postexposure intervals.

MATERIALS AND METHODS

The 147 swine represented in this report consisted of 96 females and 51 males. The ages at exposure, routes and amount of exposure, and exposure to necropsy intervals were as follows:

(a) Fourteen were exposed as suckling pigs (0-28 days), 10 at 4 months, 64 at 6-12 months, and 59 at 1-3 years of age.

(b) Of the females, 5 were exposed by parenteral inoculation (intravenous or intradermal) and 91 by natural routes (intravaginal, intracervical, conjunctival, oral, or breeding to an infected boar). Fourteen of the boars were exposed by parenteral injection (intradermal, intravenous, intraepidymal, or intratesticular) and 37 were exposed by natural routes (conjunctival, oral, or breeding to infected sows).

(c) Exposure doses ranged from approximately $6 \times 10^6$ to $1 \times 10^{11}$ viable *B. suis* organisms. Twenty-six of the swine were exposed to strains of *B. suis*, type 3 and the remaining 121 to strains of *B. suis*, type 1.

(d) The time of necropsy ranged from 4 days to 52 months postexposure. The number of swine necropsied at various intervals were: 38 at less than 1 month, 42 at 1 to 3 months, 27 at 3 to 6 months, and 40 at 6 to 52 months postexposure. Other data concerning some of the swine included in this report were reported previously, but not as a detailed comparison of sites of isolation of *B. suis*.

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Bacteriologic examination of most tissues was performed by immersing the tissue in alcohol, flaming it dry, mincing a cut surface with a sterile scalpel and smearing the minced tissue on the surface of solid medium. Fluids were applied to media with either a cotton swab or by pipetting, then spreading with a triangular wire spreader. Either tryptose-serum or potato-serum agar and, sometimes, a selective medium were used for recovery of \textit{B. suis}.

The main tissues which were cultured are tabulated in the results. In addition to those tissues listed, various striated muscles, thymus gland, peritoneal fluid, pancreas, thyroid gland, and Peyer’s patches of the duodenum were cultured from 10 to 14 swine.

**RESULTS AND DISCUSSION**

The frequency of isolation of \textit{B. suis} from most sites seemed to be related more to postexposure interval than to any other factor. Usually the frequency of isolations and number of organisms recovered from a given location (excluding male genital organs) decreased with increasing time postexposure. Recoveries of \textit{B. suis} from genital organs was noticeably affected by sex, age, and route of exposure of the swine. Otherwise, no difference in the frequency of isolations due to sex, age, or route and amount of exposure was observed.

The lymph nodes were the principal location for \textit{B. suis} in swine (Table I). When large numbers of swine were examined over all the various postexposure intervals, the genital organs, spleen, liver, and blood also proved to be important sources of \textit{B. suis}. The lymph nodes are listed (Table II) in the order of their frequency in yielding \textit{B. suis} from 58 swine in which at least 15 different lymph nodes from each were cultured and direct comparison was possible. The mandibular lymph nodes were clearly the best source of \textit{B. suis}. Hutchings also found the mandibular nodes to be infected more often than other tissues.
There was little overall difference in the incidence of infection among the different organs of the male genital system (Table III), but this was affected by the route of exposure. Isolations were made from the genital system of 12 boars exposed by intratesticular or intraepididymal injection approximately twice as often as from a similar group of 12 boars exposed by other routes. The testes and epididymides of those parenterally injected boars yielded $B. \text{suis}$ about 1½ times as often as the seminal vesicles, prostate, and bulbourethral glands. The opposite was true of boars exposed by natural routes, where isolations were made more frequently from accessory genital glands, particularly the seminal vesicles. Nevertheless, most boars exposed by injection in the testes or epididymides were killed later than 3 months postexposure and the persistence of the organism at the sites of inoculation for that length of time demonstrates the affinity of $B. \text{suis}$ for the male genital organs of swine.

Infection was bilateral in the testes of 56%, epididymides of 32%, seminal vesicles of 74%, and bulbourethral glands of 50% of the boars infected in those sites.

Urine is listed under genital organs in Table III because the presence of brucellae in the bladder urine at necropsy was nearly always associated

### Table II

**Frequency of Recovery of Brucella suis from Lymph Nodes of Infected Swine**

<table>
<thead>
<tr>
<th>Lymph Node</th>
<th>0-1</th>
<th>1-3</th>
<th>3-6</th>
<th>6-52</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandibular</td>
<td>32/37*</td>
<td>34/42</td>
<td>20/27</td>
<td>23/39</td>
<td>109/145</td>
</tr>
<tr>
<td>Gastrohepatic</td>
<td>26/37</td>
<td>34/41</td>
<td>11/27</td>
<td>11/39</td>
<td>82/144</td>
</tr>
<tr>
<td>Internal iliac</td>
<td>29/37</td>
<td>32/41</td>
<td>12/27</td>
<td>8/39</td>
<td>81/144</td>
</tr>
<tr>
<td>Suprapharyngeal</td>
<td>26/37</td>
<td>29/42</td>
<td>8/27</td>
<td>7/39</td>
<td>70/145</td>
</tr>
<tr>
<td>Parotid</td>
<td>25/37</td>
<td>27/40</td>
<td>2/24</td>
<td>8/39</td>
<td>62/140</td>
</tr>
<tr>
<td>Sternal</td>
<td>17/32</td>
<td>22/29</td>
<td>2/6</td>
<td>†</td>
<td>41/67</td>
</tr>
<tr>
<td>Bronchial</td>
<td>19/37</td>
<td>25/41</td>
<td>6/26</td>
<td>6/39</td>
<td>56/143</td>
</tr>
<tr>
<td>Sup. inguinal</td>
<td>18/26</td>
<td>23/42</td>
<td>8/27</td>
<td>4/39</td>
<td>53/134</td>
</tr>
<tr>
<td>Cervical</td>
<td>19/33</td>
<td>16/29</td>
<td>2/6</td>
<td></td>
<td>37/68</td>
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<tr>
<td>Prefemoral</td>
<td>14/23</td>
<td>16/29</td>
<td>1/6</td>
<td></td>
<td>31/58</td>
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<tr>
<td>Renal</td>
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<td>15/28</td>
<td>1/5</td>
<td></td>
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<td>Mediastinal</td>
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<td>15/29</td>
<td>0/6</td>
<td></td>
<td>28/58</td>
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<tr>
<td>Popliteal</td>
<td>10/20</td>
<td>13/27</td>
<td>2/6</td>
<td></td>
<td>25/53</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>16/23</td>
<td>10/30</td>
<td>1/6</td>
<td></td>
<td>27/59</td>
</tr>
<tr>
<td>Prescapular</td>
<td>11/22</td>
<td>13/28</td>
<td>1/6</td>
<td></td>
<td>25/56</td>
</tr>
<tr>
<td>Splenic</td>
<td>12/22</td>
<td>7/29</td>
<td>3/5</td>
<td></td>
<td>22/56</td>
</tr>
</tbody>
</table>

*Number of swine which had $B. \text{suis}$ in this site/number of swine examined.
†None examined.
with genital infection; seldom with renal infection. In only one instance (a case of purulent urocystitis) was *B. suis* recovered from urine at necropsy without concomitant, and usually severe, infection in the genital system. Twenty-one of the 25 isolations from urine occurred in boars.

### TABLE III

**Frequency of Recovery of *Brucella suis* from Genital Organs**

<table>
<thead>
<tr>
<th>Organ</th>
<th>0-1</th>
<th>1-3</th>
<th>3-6</th>
<th>6-52</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus...</td>
<td>11/24*</td>
<td>8/24</td>
<td>2/13</td>
<td>5/20</td>
<td>26/90</td>
</tr>
<tr>
<td>Cervix...</td>
<td>9/24</td>
<td>6/23</td>
<td>1/13</td>
<td>5/28</td>
<td>21/88</td>
</tr>
<tr>
<td>Vagina...</td>
<td>8/24</td>
<td>6/23</td>
<td>1/13</td>
<td>5/28</td>
<td>20/88</td>
</tr>
<tr>
<td>Ovaries...</td>
<td>6/24</td>
<td>3/23</td>
<td>1/13</td>
<td>1/28</td>
<td>11/88</td>
</tr>
<tr>
<td>Mammary glands...</td>
<td>2/2</td>
<td>2/6</td>
<td>0/1</td>
<td>....</td>
<td>4/9</td>
</tr>
<tr>
<td>Fetuses (in utero)...</td>
<td>0/1</td>
<td>0/9</td>
<td>0/2</td>
<td>0/1</td>
<td>0/13</td>
</tr>
<tr>
<td>Testes...</td>
<td>2/13</td>
<td>4/14</td>
<td>6/13</td>
<td>4/11</td>
<td>16/51</td>
</tr>
<tr>
<td>Epididymides...</td>
<td>4/13</td>
<td>4/14</td>
<td>6/13</td>
<td>5/11</td>
<td>19/51</td>
</tr>
<tr>
<td>Seminal vesicos....</td>
<td>3/13</td>
<td>7/14</td>
<td>4/13</td>
<td>5/11</td>
<td>19/51</td>
</tr>
<tr>
<td>Prostate gland...</td>
<td>4/13</td>
<td>8/13</td>
<td>2/8</td>
<td>2/10</td>
<td>16/44</td>
</tr>
<tr>
<td>Bulbourethral glands..</td>
<td>3/13</td>
<td>3/14</td>
<td>3/13</td>
<td>3/10</td>
<td>12/50</td>
</tr>
<tr>
<td>Urine...</td>
<td>5/19</td>
<td>8/38</td>
<td>8/25</td>
<td>4/38</td>
<td>25/120</td>
</tr>
</tbody>
</table>

*Number of swine which had *B. suis* in this site/number of swine examined.

The frequency of isolations from female genital tracts was higher in gilts that were exposed by intravaginal or intracervical routes and necropsied at 2 months or less postexposure than in other gilts or sows. Sexually immature gilts exposed conjunctivally did not develop genital infection. Ovarian infection was bilateral in 5 swine and unilateral in 6. Since glandular tissue is sparse during non-lactating periods, mammary glands were usually cultured only when females were lactating or had gross mastitis. Four gilts had purulent mastitis and every gland examined yielded large numbers of *B. suis*. In contrast to the low incidence of mammary infection in non-lactating swine in this study, *B. suis* was recovered from the milk of 45 of 99 infected sows at the termination of pregnancy during other studies in our laboratory. Thirteen females were pregnant at necropsy and an average of 4 fetuses from each was cultured. *Brucella suis* was not isolated from any of the fetuses although isolations were frequently made from the uterus of the dam.

Tissues other than lymph nodes and genital organs are listed in order of the number of swine examined (Table IV). Many of these tissues were frequent sources of *B. suis*, especially spleen, liver, blood and lungs. However, the number of organisms recovered was usually quite low. When media plates were heavily inoculated with minced, undiluted tissue from
those specimens, the resulting number of brucella colonies was usually less than 5 per plate. During bacteremic periods, *B. suis* could probably be recovered from many organs, just by virtue of the residual blood in the tissues.

Twenty-four percent of 88 swine whose joints were cultured had brucellae in one or more joints. But, of 928 joints examined from those

### TABLE IV

**Frequency of Recovery of *Brucella suis* from Sources Other Than Lymph Nodes and Genital Organs**

<table>
<thead>
<tr>
<th>Tissue or Fluid</th>
<th>0-1</th>
<th>1-3</th>
<th>3-6</th>
<th>6-52</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen...</td>
<td>18/37</td>
<td>20/42</td>
<td>8/27</td>
<td>10/39</td>
<td>56/145</td>
</tr>
<tr>
<td>Liver...</td>
<td>18/37</td>
<td>16/42</td>
<td>4/27</td>
<td>8/39</td>
<td>46/145</td>
</tr>
<tr>
<td>Kidneys...</td>
<td>8/37</td>
<td>7/42</td>
<td>4/27</td>
<td>2/39</td>
<td>21/145</td>
</tr>
<tr>
<td>Blood...</td>
<td>21/36</td>
<td>13/37</td>
<td>5/25</td>
<td>1/35</td>
<td>40/133</td>
</tr>
<tr>
<td>Joints...</td>
<td>5/23</td>
<td>12/37</td>
<td>3/14</td>
<td>1/14</td>
<td>21/88</td>
</tr>
<tr>
<td>Brain...</td>
<td>2/33</td>
<td>3/28</td>
<td>0/6</td>
<td></td>
<td>5/67</td>
</tr>
<tr>
<td>Adrenal glands...</td>
<td>5/29</td>
<td>2/25</td>
<td>0/6</td>
<td></td>
<td>7/60</td>
</tr>
<tr>
<td>Lungs...</td>
<td>11/22</td>
<td>9/29</td>
<td>1/6</td>
<td></td>
<td>21/57</td>
</tr>
<tr>
<td>Tonsils...</td>
<td>2/25</td>
<td>6/26</td>
<td>0/3</td>
<td></td>
<td>8/54</td>
</tr>
<tr>
<td>Salivary gland...</td>
<td>2/18</td>
<td>3/23</td>
<td></td>
<td></td>
<td>5/41</td>
</tr>
<tr>
<td>Cerebrospinal fluid...</td>
<td>0/14</td>
<td>0/22</td>
<td>0/5</td>
<td></td>
<td>0/41</td>
</tr>
<tr>
<td>Bone marrow...</td>
<td>3/17</td>
<td>7/23</td>
<td></td>
<td></td>
<td>10/40</td>
</tr>
<tr>
<td>Pericardial fluid...</td>
<td>0/10</td>
<td>1/27</td>
<td>0/1</td>
<td></td>
<td>1/38</td>
</tr>
<tr>
<td>Vertebral lesions...</td>
<td>0/1</td>
<td>6/8</td>
<td>2/8</td>
<td>3/13</td>
<td>11/30</td>
</tr>
<tr>
<td>Ileocecal valve...</td>
<td>1/19</td>
<td>0/5</td>
<td>0/3</td>
<td></td>
<td>1/27</td>
</tr>
<tr>
<td>Subcut. abscesses...</td>
<td>0/8</td>
<td>3/5</td>
<td>0/3</td>
<td>4/4</td>
<td>7/20</td>
</tr>
</tbody>
</table>

*Number of swine which had *B. suis* in this site/number of swine examined.

88 swine, *B. suis* was recovered from only 34 (3.7%) of the joints and usually in small numbers. Very seldom were gross lesions observed in these joints.

Bone marrow is usually assumed to be a common seat of brucella infection. In an effort to evaluate the importance of bone marrow, samples from 8-10 long bones of each of 29 infected swine were cultured. *Brucella suis* was isolated from only 20 of 252 marrow samples.

*Brucella suis* was isolated once from the thymus of a boar killed during the first month postexposure, and also from ham, loin, and shoulder muscles of two sows which were inoculated intravenously and necropsied at 19 days postinoculation.

The presence of *B. suis* in many tissues of swine appears to be closely associated with brucellemia, *Brucella suis* was recovered from 2 or more
lymph nodes of 95%, from the genital system of 54%, and from one or more other organs of 86% of the swine which had brucellemia at the time of necropsy. The same groups of tissues from swine without demonstrable brucellemia contained _B. suis_ 67%, 47%, and 33% of the time. The differences in percentages when bacteremia was present and when not present indicates that there is some correlation between the degree of lymph node infection and bacteremia, although it is unclear which contributes more to the other. There seems to be little relationship between the persistence of genital infection and brucellemia. However, infection in organs other than the lymphatic and genital systems seems to be closely correlated with brucellemia.

The embargoes placed on pork products, mainly livers and kidneys, originating from the U.S. causes one to reconsider the importance of those organs as a source of brucellae. In this study, kidneys appeared to be unimportant as a source of _B. suis_, since organisms were recovered from the kidneys of only 14% of the swine and from only 10% of the individual kidneys. Livers present a more serious problem, since _B. suis_ was recovered from 32% of them. The seriousness is further increased because the gastrohepatic lymph nodes are generally left attached to the liver in slaughtering procedures. _Brucella suis_ was isolated from the liver and/or gastrohepatic nodes of 62% of the swine, which is 30% more than from livers only.

It must be emphasized, however, that the livers and kidneys for export are derived from market swine. The frequency of isolation of _B. suis_ from livers, gastrohepatic lymph nodes, and kidneys would most likely be low in market swine, since most of them would have become infected during the suckling or early weanling periods (3 to 5 months before market weight is reached). Epizootiologic evidence indicates that a high percentage of swine infected early in life recover within a relatively short period of time. Furthermore, in swine which remain infected, the frequency of recovery of _B. suis_ from the above organs is reduced to about ½ after 3 months postexposure (Tables II and IV).

Because of the widespread use of serologic methods for diagnosis of brucellosis, the results of standard agglutination tests on serums from the swine in this report should be of interest. Seventy-one percent of these swine had agglutinin titers 1:100 or higher at the time of necropsy; 21% had titers 1:25 or 1:50; and 8% had titers less than complete at 1:25. Almost identical results were obtained with serums from the swine killed at 1 year or more after initial exposure. Most of the serologically "negative" swine were infected as suckling pigs and many never developed significant agglutinin titers. Failure to develop diagnostic titers after exposure may be the cause of "negative" seroagglutinin titers in infected swine more frequently than recession of titers below diagnostic levels, especially in swine infected at young ages.

In situations where bacteriologic surveys of considerable numbers of swine are necessary, selection and examination of a minimum number of tissues to identify a reasonable proportion of the infected swine would be
desirable. \textit{Brucella suis} would have been isolated from 91\% of the swine in this study by culturing the mandibular, suprapharyngeal, gastrohepatic, and internal iliac lymph nodes. To isolate from 95\%, it would have been necessary to culture the genital organs also.

**SUMMARY**

The most common sites for recovery of \textit{Brucella suis} were determined by bacteriologic examination of 147 infected swine. The swine were exposed to \textit{B. suis} by various routes and at different ages, and the intervals from exposure to necropsy varied from 4 days to 52 months.

Lymph nodes were the most frequent source of \textit{B. suis}. Genital organs, spleen, liver, and blood were also frequent sites for recovery of the organism. Except for genital organs, the frequency of isolation and the number of organisms recovered usually decreased with an increase in postexposure interval. The number and variety of locations from which organisms were recovered indicated the widespread distribution of \textit{B. suis} in infected swine. However, 91\% of the swine would have been identified as infected by bacteriologic examination of the mandibular, suprapharyngeal, gastrohepatic, and internal iliac lymph nodes only.

From the National Animal Disease Laboratory, U. S. Department of Agriculture, Agricultural Research Service, Animal Disease and Parasite Research Division, Ames, Iowa 50010.

**REFERENCES**

REPORT OF THE COMMITTEE ON BRUCELLOSIS


In presenting this report of the Brucellosis Committee, may I first express my sincere thanks to the members of this committee who participated in discussions Monday afternoon, Tuesday morning, and Wednesday. These open meetings were well attended by livestock personnel and regulatory officials. As in the past, every one who desired to discuss a problem was given the opportunity to express himself to the fullest extent. The discussions have been most helpful to the Committee in preparing this report. The Committee trusts that this interest will continue. With your advice and recommendations, the goal of eradicating brucellosis by 1975 will be attained.

Since our last meeting, 180 counties have qualified as Certified Brucellosis-Free areas. One state, Washington, attained a Certified Brucellosis-Free status, and another state, Alaska, achieved a Modified Certified Brucellosis status. At this time 39 states have qualified as Modified Certified Brucellosis areas. Of these, 10 states and the Virgin Island have gone on to reach the ultimate goal—a Brucellosis-Free status. There are now a total of 898 free counties. Nationally, 92 percent of the counties are now Modified Certified; 28.4 percent have achieved a Certified Brucellosis-Free status. Only 106 counties do not have an organized program underway to eradicate brucellosis.

REVISION OF UNIFORM METHODS AND RULES

The Committee discussed the problem of handing suspect animals in clean herds in Modified Certified Brucellosis areas and Certified Brucellosis-Free areas.

The status of animals classified as suspects by the tube or plate agglutination tests should be determined by use of a supplemental test such as the card test, rivanol test, mercaptoethanol tests, or others that have proven reliable.

Suspects to the tube and/or plate agglutination tests in herds that are otherwise negative will not be required to be retested for certification purposes providing the various supplemental tests are conducted on the blood sample, and it has been determined that infection does not exist.

In order to alleviate some of the problems associated with vaccination, the use of Brucella abortus Strain 19 vaccine should be limited to female
calves from 3 to 8 months of age of either dairy or beef breeds. The definition of official vaccinate will be changed accordingly.

TESTING OF OFFICIAL VACCINATES

Reconsideration was given to the age that official vaccinates should be subject to test. Based on effective procedures for preventing spread of brucellosis by infected vaccinates, it was recommended that official vaccinates be subject to test at 18 to 20 months of age, effective January 1, 1970. This ensures that the status of most infected vaccinates would be determined before they aborted or calved at full term.

MARKET CATTLE TESTING RESULTS

Blood samples tested within the Market Cattle Testing program showing a titer to the standard plate or tube test should be subjected to supplemental testing as prescribed for suspect animals and the information provided to the state of origin of the animal tested.

SOURCE OF BRUCELLA ANTIGEN

The Committee reaffirms its previous recommendations that all antigen used in the cooperative program be prepared and provided by the United States Department of Agriculture, and that states take the necessary steps to limit the use of commercial antigen within their states.

SWINE BRUCELLOSIS ERADICATION

As stressed in last year's report, the Committee urges that the United States Department of Agriculture move as rapidly as possible in the development of a market swine testing program and of regulations requiring that all breeding swine moved interstate be from Validated Brucellosis-Free herds or areas.

ANIMAL IDENTIFICATION

The Committee urges the implementation of an animal identification procedure by the Federal Government requiring the backtagging of all female slaughter-type cattle over 2 years of age moving interstate. The backtag would be applied at the first concentration point.

INTERSTATE MOVEMENT OF CATTLE

The Committee reconfirms its recommendation that effective January 1, 1968 cattle moving into Modified Certified Brucellosis areas and Certified Brucellosis-free areas must originate from Modified Certified Brucellosis areas or Certified Brucellosis-Free areas. The Committee also recommends that the proposed revisions to Part 78 Code of Federal Regulations be adopted in its entirety as originally printed thus affording industry an opportunity to review and conform to the proposed revisions.
EOSINOPHILIC MYOSITIS IN CATTLE—PATHOLOGY AND INCIDENCE

George D. Imes, Jr., D.V.M., M.S. and George Migaki, D.V.M., Pathology Group, Laboratory Branch, Technical Services Division, Consumer and Marketing Services, United States Department of Agriculture, Beltsville, Maryland 20705.

Eosinophilic Myositis (EM) is an important economic disease to the cattle industry. The estimated annual loss due to carcass condemnation alone is over $650,000.00. EM is an asymptomatic and a specific inflammatory disease entity involving the heart and skeletal musculature in which the principal cellular infiltrate is large accumulations of mature eosinophils. The cause is unknown and no etiologic agent has been positively identified. Migaki and Brandly (13) offered an explanation for this condition formerly being called sarcosporidiosis or “sarco”. Kennedy (11) attempted to determine the cause by infecting cattle with Trichinella spiralis. An inflammatory process was produced in the skeletal muscles but the lesions were not comparable to those seen in EM. Davis (5) believed that the disease was due to some undetermined parasite and not due to Sarcocystis sp. We have microscopically examined over 300 cases of EM specimens submitted by federal veterinary meat inspectors (VAI) from throughout the United States and have never been able to associate the presence of Sarcocystis sp. with the inflammatory process. The presence of Sarcocystis sp. within individual striated muscle fibers and Purkinje fibers of the heart without any evidence of inflammation or degeneration has been a common microscopic finding in otherwise normal striated muscles. We also conducted histologic studies for the presence of Sarcocystis sp. in 25 normal esophagi and 25 affected esophagi from cattle originating from the same feedlot. Sarcocystis sp. were found equally from both groups. Jacobs (8) conducted digestion studies on the number of Sarcocystis sp. in normal heart and EM affected heart specimens and found no significant difference. Afifi (1) indicated that a relationship between Sarcocystis sp. and EM exists, but he offered no conclusive proof. Huchet (7) reported that EM was related to the leukemic diseases. Tengerdy and Imes (21) reported that there was no significant differences in the disk electrophoretic patterns in the serum proteins and muscle proteins from EM affected cattle and normal cattle.

EM is principally a disease of cattle, although we have occasionally seen it in swine and sheep. As no clinical signs are associated with EM, practically all cases are seen on postmortem examination of the carcass at the time of slaughter or when the carcass is cut up into primal cuts. Savage et al. (20) in Canada and Robertson and Hamilton (18) in Scotland reported on individual cases of EM detected at the time of boning of the carcasses which had passed inspection. Individual cases of EM were also described by European investigators (2,12,16).

As in any disease process, the stage of EM at the time of slaughter of the affected cattle varied from acute, subacute, granulomatous, chronic
with fibrosis to eventual replacement of the fibrous tissue with adipose tissue. Also, the size, distribution and number of lesions in the striated musculature varied from small and slight to large and numerous lesions in the carcass.

We have established that there are two distinct lesions of EM based upon gross and microscopic morphology. Similar observations have been made by others (3,4,6,9,10,19). First, the most common lesion is the multiple focal type which is characterized by multiple spindle-shaped to ovoid measuring in length from .5 cm to 1.5 cm with the diameter varying from 1 mm. to 3 mm. The lesion in the early stage of EM is green due to massive accumulations of eosinophils in the musculature. As the disease progresses, with atrophy and necrosis of the muscle fibers and degeneration of the eosinophils and fibrosis, the lesions vary in color from greenish-yellow to yellowish-white. The yellow is due to dead and degenerated tissue and the white due to fibrous tissue. There is considerable variation among the affected carcasses as to the degree of involvement. Generally, if only a few lesions are found in the carcass, the most active muscles, such as the tongue, masseter, heart and diaphragmatic muscles are found affected; however, in such carcasses the muscles of the shoulder, loin and rump may sometimes also be affected. In severe cases, all of the striated muscles of the body are affected, including the muscles of the eyeball and larynx. Smooth muscle fibers are not affected. Outstanding features of this disease are no apparent systemic effect on the general health of the animal and no involvement of the visceral organs, namely, the liver, kidney and spleen. In some cases the skeletal lymph nodes are green due to the eosinophil accumulations in the lymph sinuses. Microscopically, the incipient stages are characterized by large accumulations of mature eosinophils between muscle fibers and muscle bundles. As the disease progresses, atrophy and necrosis of the muscle fibers and degeneration of the eosinophils occur, resulting in granulomatous abscesses. The inflammatory process takes the path of least resistance which would be between the muscle fibers, thus resulting in the characteristic spindle-shaped lesions. The necrotic centers in these granulomas are surrounded by a wide zone of giant cells, epithelioid cells, eosinophils, lymphocytes and fibrocytes. It is within these granulomatous abscesses that we have attempted to demonstrate an etiologic agent but have so far been unsuccessful. In the later stage of EM, resolution occurs with proliferation of collagenous connective tissue and the predominant cellular response is mononuclear leukocytes rather than eosinophils. One must be cognizant that EM is a continuous progressive inflammatory process and the various stages may be seen within a single microscopic section.

The second type of lesion in EM, which is less commonly seen but is more spectacular when encountered, is the multiple diffuse. Grossly, this type is manifested by large, discrete, bright greenish lesions measuring from 2.5 cm. to 10 cm. in diameter and extending longitudinally for about 12.5 cm. to 15 cm. The green color is due to the presence of large numbers of eosinophils massed together which have almost completely replaced the muscle fibers. In the early stage of EM the lesions may appear greyish in
color due to many of the muscle fibers remaining intact. Microscopically, large numbers of mature eosinophils are massed together and have replaced the muscle fibers. The interstitial connective tissue stroma remains and this may, in part, explain the absence of clinical signs in those cases where the principal muscles of locomotion are extensively affected. It is possible that the retention of the endomysium, perimysium and epimysium is sufficient to compensate for the damaged and destroyed muscle fibers. Visceral organs are not affected. Interestingly, carcasses which are extensively affected are graded the same as the unaffected carcasses originating from the same lot. Generally, these lesions of multiple diffuse EM are not detected on postmortem inspection but rather at the time the carcass is cut into primal cuts or when the carcass is quartered.

Federal Meat Inspection regulations concerning the disposition of such affected carcasses are to remove the lesions before passing the unaffected parts. However, if the lesions are numerous and widely distributed throughout the musculature and removal of the lesions is impractical or uncertainly accomplished, the entire carcass is condemned.

In order to ascertain the incidence of EM in cattle, Nelson and Albritton (15) conducted a survey in 1964. This involved a one month (June) study at 75 stations throughout the United States in which 2,221,958 cattle were examined. 293 cattle or 13.2/100,000 were affected with EM. Reiten et al. (17) reported on a 3-year study in cattle slaughtered at Greeley, Colorado, in which there was 1 case /3,500 or 28.6/100,000. In a one-month survey (November, 1964) reported by Moody (14) the finding of EM was 92/100,000 in the slaughtering establishments in and around Denver, Colorado.

In 1965 the Meat Inspection Division initiated epizootologic and etiologic studies at Denver, Colorado.

Materials and Methods

From May, 1965 through June, 1966, all cattle carcasses showing lesions of EM were classified as to sex, breed, lesion character and extent, age, condition, and feedlot or ranch origin.

Hides were retained when retrievable for brand identification by state brand inspectors.

Personal interviews and questionnaire forms were used to obtain feedlot and ranch information from owners and managers. Personal interviews were also used to observe environmental conditions of feedlots. Information requested was ration, feed supplements, health and vaccination history, pesticides used, percent breeds fed, percent sex fed, number of animals fed annually and cattle breeder origin.

In compiling data for crude and specific attack rates the population at risk was considered to be animals slaughtered and not animals on feed during the survey period. The significance of breed incidence findings were related back to feedlot information because records of breeds slaughtered are not maintained.
Results

Attack Rates.—A summary of totals of each class of animal slaughtered and their attack rates are found in Table I.

### TABLE I

**ANIMALS SLAUGHTERED, CASES OF EOSINOPHILIC MYOSITIS AND ATTACK RATES**  
**MAY 1965 THROUGH JUNE 1966**

<table>
<thead>
<tr>
<th>Types</th>
<th>Slaughtered</th>
<th>Eosinophilic Myositis</th>
<th>Sex Specific Attack Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heifers</td>
<td>728,675</td>
<td>627</td>
<td>86/100,000</td>
</tr>
<tr>
<td>Steers</td>
<td>758,128</td>
<td>284</td>
<td>39/100,000</td>
</tr>
<tr>
<td>Cows</td>
<td>123,680</td>
<td>58</td>
<td>47/100,000</td>
</tr>
<tr>
<td>Bulls</td>
<td>11,919</td>
<td>5</td>
<td>42/100,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,622,402</strong></td>
<td><strong>974</strong></td>
<td><strong>Crude Attack Rate 60/100,000</strong></td>
</tr>
</tbody>
</table>

Monthly sex specific attack rates for heifers and steers are found in Figure 1 and monthly crude attack rates in Figure 2.

Comparison of the incidence of EM in cattle slaughtered on a nationwide basis and those slaughtered in Colorado are summarized in Table II.

Breed Affected.—Incidence of breeds affected at slaughter and percentage of different breeds in feedlots as reported by operators are summarized in Table III. Only a few purebred cattle were found in the breeds listed. Black white face cattle were assumed to be Hereford-Angus cross. The three cattle listed as “other” were an aged Holstein cow, a Shorthorn cow and a Guernsey cow. Most of the unknown cases were reported from slaughtering establishments using an “on the rail” system where hides leave the kill floor before inspection is completed and cannot be successfully identified or retrieved because of the method of hide processing.

Animal Age and Condition:—Average age of heifers and steers was estimated at 18 to 24 months with most heifers listed in the lower age group and steers in the upper age group. The age of bulls ranged from 18 months to aged with two reported as 5 years old. Cows varied from 3 years to aged. Heifers and steers were classed as being in good to very good condition with most being listed as very good. Cows and bulls were classed as fair to good condition with most listed as good.

Lesion Character:—In all cases except one, the lesion character was of the multiple focal type. It was interesting to note that the one case of diffuse EM was in a lot of 88 heifers and two other heifers in this lot had multiple focal EM.

Brand Identification:—Traceback of cattle by brands, except for a few exceptions, was not attempted because many of the imported feeder cattle originated from states without brand laws. Repeat brands were found but in most instances these were single letters, numbers or configurations which
might be used by anyone. Repeat brands were identified in a few instances which belonged to a single source. The cattle involved had usually been purchased at the same time by the feedlot operator.

Feedlot Environmental Conditions:—Eastern Colorado is subject to wind, low precipitation and low humidity; consequently dusty conditions in feed-
lots are prevalent throughout most of the year. Clouds of dust are particularly noted to hang over feedlots at dusk when young cattle are most prone
to exercise. Many respiratory ailments suffered by feedlot cattle are attributed to dust and affected cattle are referred to as "wheezers" or as having "dust pneumonia". Sprinkler systems have been installed by some operators to aid in overcoming the problem.

TABLE II

<table>
<thead>
<tr>
<th></th>
<th>National</th>
<th>Colorado</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Slaughter</td>
<td>27,294,382</td>
<td>1,401,215</td>
</tr>
<tr>
<td>Total Cases</td>
<td>3,587</td>
<td>871 or 24.5%</td>
</tr>
<tr>
<td>Crude Attack Rate</td>
<td>13/100,000</td>
<td>62/100,000</td>
</tr>
<tr>
<td>BREED</td>
<td>Number of Cases</td>
<td>Percent Total Cases</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------</td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hereford</td>
<td>863</td>
<td>88 64</td>
</tr>
<tr>
<td>Angus</td>
<td>5</td>
<td>51 54</td>
</tr>
<tr>
<td>Hereford-Angus Cross</td>
<td>16</td>
<td>1 64</td>
</tr>
<tr>
<td>Angus Cross</td>
<td>8</td>
<td>82 89</td>
</tr>
<tr>
<td>Hereford Cross Other than with Angus</td>
<td>2</td>
<td>20 22</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>30 33</td>
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<tr>
<td>Unknown</td>
<td>77</td>
<td>7 90</td>
</tr>
<tr>
<td>Total</td>
<td>974</td>
<td></td>
</tr>
</tbody>
</table>
Feedlots are completely cleaned annually or semi-annually and during the interim period, manure, and other materials are pushed into heaps in the center of corrals. When wet weather occurs cattle use these to lie on because the heaps remain drier than the rest of the lot. Some cattle lick holes (licks) in these manure heaps and also in other portions of the feedlot.

Feed is kept before animals at all times but not to the extent that it accumulates and spoils. Only during wet weather does feed accumulate and become unpalatable. When this occurs feed troughs are cleaned out. Troughs are located outside coral fences along driveways which facilitates filling and cleaning and also prevents urine and fecal contamination. Water supply is nearly all from wells.

Ration:—Full feed rations consisted of corn, green chop, silage, barley, milo, wheat, dehydrated alfalfa pellets or alfalfa hay and dehydrated beet pellets. These were used in various combinations generally depending on relative market price and seasonal availability. Supplements when used were the commercially available protein supplements which may or may not contain diethylstilbestrol and/or an antibiotic.

Pesticides:—Most often used pesticides were the systemic organo-phosphorus compounds. Of 145 feedlots reporting the use of pesticides 99 used this type and 75 of the 99 used the product of one agricultural chemical company.

Immunization and Health History:—Immunization against infectious bovine rhinotracheitis and leptospirosis was a consistent finding.

Other immunizations commonly administered were for hemorrhagic septicemia and black leg. All these biologics were given to cattle soon after they entered the feedlot.

Most often mentioned disease problems occurring in feedlots were respiratory in nature. Except for two cases no history of illness could be definitely associated with affected animals. These two exceptions consisted of a Hereford heifer and a 24 month old purebred Hereford bull. The heifer had a history of chronic bloating throughout the feeding period. The Hereford bull bloated some when he was first placed on feed. He was taken off feed and put on native grass where he improved. He was put back on feed to be fitted for sale and again developed bloating problems. He again was turned out to grass but did respond as he had previously so was shipped for slaughter. All striated muscles were found extensively affected with multiple focal lesions of EM.

States of Origin:—Feedlot operators gave numerous states as the origin of their cattle. Only rarely could they say what state the affected cattle came from because many of the cattle they buy are from auction sales and companies or individuals who mass cattle from many sources. In addition to Colorado, states given as sources of feeder cattle were Alabama, Mississippi, Louisiana, Missouri, Kansas, Iowa, Minnesota, Nebraska, Oklahoma, Texas, New Mexico, Montana, North and South Dakota and Oregon. Most of the imported cattle came from Texas.

DISCUSSION

Nine hundred and forty-seven cattle were found affected with EM
during a 14-month survey period extending from May, 1965 through June, 1966. Of these, 911 were feedlot cattle. Carcass finish was listed as good to very good and health history of groups of cattle in which affected animals were found did not reveal any evidence of disease problems. Comments similar to the following were heard frequently in conversations with cattle feeders: “The cattle were an extremely vigorous healthy group and gained 2.8 pounds daily for the fattening period.” “These were about the finest feeder cattle I ever fed. It seems strange that there would be an affected animal.” Exceptions to this typical history were two animals with chronic bloat problems. If the bloating was associated with EM we would assume it was a result of the muscle lesions, and not produced by the cause of the disease.

Of the 947 cases only one was the multiple diffuse type and it is considered uncommon to detect these at the time of postmortem examination. Muscle distribution in diffuse EM is usually of such nature that it is only after primal and wholesale cuts are removed that the condition is discovered, and this often occurs several hundred miles from the slaughter point. The single diffuse case encountered on postmortem had a 2 cm. in diameter lesion in one external masseter muscle and further incisions of carcass musculature revealed extensive lesions of multiple diffuse EM.

Because of difference in muscle distribution and difference in gross and histologic morphology, it has generally been considered that a different etiologic agent was responsible for the two forms of EM. The finding of two other heifers affected with extensive multiple focal lesions of EM in the same lot as the heifer with diffuse EM is suggestive of the same cause. Data accumulated for specific attack rates statistically showed a highly significant difference in the incidence of EM in fattened heifers as compared to cows, bulls and fattened steers. This agrees with the findings of previous investigators (14,17) except that Reiten, et al. (17), reported cows also had an incidence similar to heifers and suggested estrogens as an etiologic factor. Statistically, our findings did not reveal any significant difference between cows, steers and bulls (Table I) suggesting that if estrogens are involved it is only in the young animal. When heifers come into feedlots they vary in sexual development from just beginning estrus cycles to being pregnant. Upon entering the feedlot, many are given 5 cc. of diethylstilbestrol as an abortifacient and throughout their feeding period heifers are noted to show signs of estrus. Thus they are potentially affected by two sex-related factors to which the steer is not subject.

From July, 1965 through June, 1966 cattle slaughtered in Colorado showed an incidence of 62/100,000 or nearly 5 times the national incidence for the same period (fiscal year 1966), with the national incidence being 13/100,000 (22). The incidence in non-feedlot animals (cows and bulls) slaughtered in Colorado compared with national incidence illustrates the importance of geographic location of this disease. Animals of this type slaughtered in the Colorado markets are native to the Western Plain States. Results of this comparison showed a significant difference with cows and bulls slaughtered in Colorado having an incidence of 44/100,000, nearly 3.5
times greater than national incidence. This indicates that an agent, or combination of agents, present in the Western Plains States, not confined to feedlots, are responsible for producing EM.

Annual recurrence of EM in feedlots was studied by Reiten, et al. (17) and their findings were suggestive of a transmissible agent or vector which does not remain viable from year to year. They further found incidence higher in the summer than in winter. Our studies showed the highest incidence in the fall and early winter months, with a decrease in late winter and spring. However, weather conditions during the period of our survey may have had some bearing on the monthly and seasonal incidence we observed. June, 1965 was the month of the severe rain and flooding in eastern Colorado and July was also an abnormally wet month. The entire summer of 1965 was one of the coolest on record. October was warm and November, 1965 was one of the warmer Novembers recorded in Colorado. Consistent sub freezing temperatures started occurring the latter part of November and continued on a daily basis until March, 1966 (23).

These observations are all suggestive of an agent which continually builds up during the months with temperatures above freezing and then is rapidly decreased when prolonged freezing temperatures occur.

Other important changes in environmental conditions which occur during the late months of winter may also be contributory in decreasing incidence. One is that feedlots are continually wet at this time, and this not only solves dust problems, but also decreases the palatability or desirability of material in feedlot sites where cattle like to lick (licks) and they would not be expected to indulge in this habit during these periods. The other change is that this is when many lots are given their annual or semi-annual cleaning and manure heaps and feedlot debris are removed to fields prior to spring plowing.

Feedlot practices and husbandry could not be properly evaluated because we had no unaffected feedlots (controls) for comparison. Attempts to establish control lots failed chiefly because the ones we selected eventually had cases of EM. The number of cattle fed was an important factor as to how often cases of EM would recur in a given feedlot. Perhaps a method of establishing control lots on a statistical basis could be devised but because of differences in husbandry practices found in affected lots, ranging from one extreme to the other, we did not feel this would be a productive undertaking.

Findings and questions brought about by this investigation show the need for a continuing collection of data concerning age, sex, and breed incidence, seasonal incidence and corresponding climatologic data. This should be carried on concurrently in geographic areas other than the Western Plains region.

Considerable progress in understanding EM could probably be made by isolation of affected live animals for observation and clinical studies. Results of this and previous investigations showed the highest incidence in heifers (14,17) and a high percentage of cases occurring in Hereford or
Hereford-cross cattle (14). Attempts to isolate live animals should be limited to this type of cattle.

SUMMARY

Eosinophilic myositis is an asymptomatic and a specific inflammatory disease entity involving the heart and skeletal musculature in which the principal cellular infiltrate is large accumulations of eosinophils. The cause is unknown. The estimated annual loss due to carcass condemnation alone is over $650,000.00. The disease is manifested by two types of lesions, the multiple focal, which is more commonly found, and the multiple diffuse. Epizootologic and etiologic studies were conducted in Denver, Colorado. 974 cases of EM were found on postmortem examination in a 14 months period giving a crude attack rate of 60/100,000. Heifers had an attack rate of 86/100,000 and steers had an attack rate of 39/100,000. Cows and bulls had attack rates of 47/100,000 and 39/100,000, respectively. Feedlot practices and environmental conditions were investigated in an attempt to determine the cause of EM but no relationships were found.

BIBLIOGRAPHY

SUGGESTED REGULATIONS ON INTERSTATE MOVEMENT OF FEEDER CATTLE

DR. JOHN B. HERRICK
and
DR. RICHARD BRISTOL

Ames, Iowa

Cattle feeders throughout the nation are aware of the problems of moving and adapting cattle to feedlot confinement where they are then forwarded to market. Death losses from several studies indicated 1.5 to 2.0 percent calves and 0.5 to 1.5 percent death loss in yearlings. Feed utilization, shrink, rate of gain and death loss due to stress and disease have been estimated to cost between $10 to $20 for every feeder animal that moves into a feedlot in the United States. This monumental loss can be attributed to several different factors. One of the factors is the stress due to change of nutrition and environment plus the fear and anxiety of weaning and handling. Another factor is the stress an animal undergoes in movement due to long travel time and inadequate care during this traveling period. Further, losses can be attributed to the movement of cattle through facilities where cattle are assembled thus contacting and spreading diseases peculiar to cattle.

The biggest stumbling block to date has been attributed to the lack of identification of the animals after they leave their point of origin. Various surveys indicate that less than 20 percent of the animals that move into feedlots can be identified and/or traced back to their point of origin. National cattle identification is of top priority from the aspects of epidemiology, that is, disease trace backs and also to aid in the pinpointing of suitable genetic material for the feedlot. The Infectious Diseases of Cattle Committee of the United States Livestock Sanitary Association in their committee report in 1966 recommended a uniform method of cattle identification for the following purposes:

1. To expedite trace back of diseased cattle
2. To expedite morbidity and mortality reporting
3. To provide a livestock owner with information regarding the origin of cattle as a basis for economical evaluation
4. To assist in the ascertainment of preconditioning procedures applied to feeder cattle, specifically referring to immunization and grub control.

To date there has been little, if any, action from the disease regulatory level in this particular program. There is interest evidenced within the various segments of the livestock industry to encourage development of a national identification system for cattle. Therefore, again it is urged that the United States Livestock Sanitary Association initiate a program to achieve this end.

Suggested regulations pertaining to the interstate movement of cattle are as follows:

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1. Animals that are subject to interstate movement (exception, immediate slaughter cattle) be marked permanently as to the state of origin. It is a known fact that animals frequently move through several states only to be sold as originating from another state than from what they originated. Disease spread is paramount with this type of animal movement.

2. Stricter enforcement of federal regulations regarding the movement of such animals. There is little, if any, curtailment on the movement of sick animals into public stockyards and/or to auction markets. If the animal appears to be clinically normal, they are allowed to move quite freely. Frequently sick animals are segregated from the apparently normal animals then the animals are moved to mingle with other cattle even though they may be in the incubation period of a disease. It is not uncommon at all to observe sick animals moving through public auction or public stockyards. Sick animals are classified as those that have abnormal temperatures and gross clinical signs of disease such as those observed with the various respiratory diseases.

3. A requirement that professional dealers have permanent, legible records of the animals sold and/or purchased by them for resale. These records should be made available to any prospective purchaser. Further, these animals should be individually identified and the purchaser should be knowledgeable of this at the time they are sold. Animals should not be sold for feeding purposes for a period of 21 days following vaccination for the various respiratory diseases that are viral in origin. If the animals are not vaccinated, they should be so identified.

4. All cattle dealers should be licensed and bonded.

5. Enforced regulations in the movement of cattle. Animals moved by rail should not move more than a 24 hour period and then rest in sanitary lots for a period of 12 hours. At that time adequate, clean water and feed should be given to the animals. If moved by truck do not move more than 24 hours and then rest for a period of 12 hours. No extension of this time period should be given. Periodic inspection should be made for overcrowding and down animals and all trucks moving across state lines should be so inspected.

6. All cattle coming in on a permit basis should be inspected by regulatory veterinarians and the permits released at the time the animals appear to be in a state of health so they can be considered equal to that of native cattle.

The cattle industry is a multi-billion dollar industry beset with problems pertaining to disease. A vast share of these problems fall in the category of interstate and intrastate movement. The industry is aware of these problems and are asking for help. Are we as regulatory and educational groups ready to lead or to be complacent? The challenge is past due.
MATERNAL ANTIBODY INTERFERENCE WITH IMMUNIZATION OF FEEDER CALVES

R. P. AZELTON, D.V.M.*
St. Joseph, Missouri

The ability of the cow to transfer her disease resistance to her offspring has been a known fact for generations.

Numerous studies in reference to this process demonstrates that the lacto-globulin fraction of the colostral milk carries those viral antibodies, iso-hemagglutinins, anti-toxins, and anti-bacterial antibodies that were present in the dam's body previous to calving.

It has also been demonstrated that the offspring has the ability to utilize these protective bodies in the same form, and at a level equal or higher to that which was present in the dam, provided that the colostrum was ingested within approximately the first twenty-four hours after birth.

This method of disease control has been very well utilized in species other than the bovine. The control of Canine Distemper starts with an immunization program directed to the bitch, resulting in maternal antibody protection up to sixteen (16) weeks.

The control of hog cholera starts with brood stock protection with the sow transferring its maternal anti-bodies to its offspring.

These two examples establish a foundation for control programs and protection in the young animal where the greatest death loss occurs in all species.

The ultimate objective in any venture is to obtain 100% results, but as most of you are aware, in the field of medicine, due to variations and unknowns, this is impossible.

Calf protection via maternal anti-bodies is not 100% either, due to many variables such as variation in maternal immunity or lack of, or insufficient colostrum milk intake during the first twenty-four hours of life.

The tremendous strides and increased knowledge in the field of bovine virology has produced many answers as well as problems.

The discovery of virus disease in cattle has presented a brand new and complicated picture to the cattle industry. Until recent years, killed vaccines were the only products available to assist in disease control. They were used and are presently being used with varying success. Killed products produced little or no problems, but the development of modified live viral vaccines present an entirely new concept completely different from their previous experiences and knowledge.

The M. L. V. vaccines produce a varying degree of stress due to the multiplication of virus and cellular destruction. A positive fact that they produce an immunity unheard of in comparison with killed products, magnified the use of these products faster than has ever been experienced before in history. A panacea-like attitude ensued. Problems of answerable and
unanswerable entities are presently facing us in reference to viral diseases incorporating M. L. V. vaccines.

It certainly scares me to think of the economic losses that have occurred in this area.

Antibody protection and antibody-antigen process has been known for generations. The use of a specific M. L. V. in face of existing specific antibodies produces this antibody-antigen reaction, and is purely a mathematical problem as to the M. L. V. Survival. Multiplication in extreme numbers are necessary to stimulate antibody production to produce immunity.

Any factor that interferes with the immunization process will deliver varying results as to the quality of immunity established, and in some instances, no immunity is developed.

It is a proven fact that maternal antibodies interfere with the effectiveness of M. L. V. vaccines. The percentage or the amount varies with the amount of anti-bodies present in the individual being vaccinated.

In recent years the widespread of I. B. R. (Infectious Bovine Rhinotracheitis) and B. V. D. (Bovine Virus Diarrhea) serum positive cattle has been demonstrated many times. In fact, it is extremely difficult to find a serum negative herd in the United States.

The alarming fact is that the serum anti-bodies vary from negligible to immune levels. With this in mind, transpose these levels via the colostral milk to the calf population. Not only is it alarming as to the variation in calf protection, but how would you constructively apply an immunization program incorporating a M. L. V. vaccine?

The variation in maternal protection as researchers have demonstrated is one important factor in the variation in age of reported cases of I. B. R. or B. V. D. infection.

With I. B. R. and B. V. D. being diagnosed with positive laboratory tests in animals from weeks to months of age, the utilization of MLV vaccines at an early age to gain protection has met with a head-on interference with varying levels of maternal anti-bodies. This varying interference has been the cause extended by investigators for so-called complete vaccine failures, partial or varying protection, and extended stressing viremic stages following vaccination.

Let's ask ourselves a few questions. When and how should I protect this pen of calves with an unknown history? What level of maternal antibodies does that calf have that is four months old for I. B. R. or B. V. D.? At what age should I vaccine my calves for I. B. R. and B. V. D. that are the offspring of vaccine immune cows?

These questions are quite thought provoking. The last question involving the length of full maternal protection is one area that should be explored in greater depths and magnitude. Researchers have delved into this matter, but the only well proven and documented maternal half life work is in Canine Distemper.

Researchers do have some reasonable idea with I. B. R. and B. V. D. Complete susceptibility in a calf is stated to occur from 4 to 6 months with
IMMUNIZATION OF FEEDER CALVES

I. B. R. and 6 to 9 months with B. V. D. Two to three months is quite a spread, especially in planning a prevention program. I think every effort should be extended to acquire all the knowledge possible in reference to the half life of I. B. R. and B. V. D. maternal antibodies. The true over-all facts in this entity will certainly be a major step in the planning of an immunization and preventive program.

In any planning, the positive known points must be used as the foundation and outline to arrive at a successful conclusion. With this in mind would not the adult I. B. R. - B. V. D. vaccination program be the first positive step? Secondly, the maternal antibody protection of calves would then take on a standard picture. With this known fact a calf or yearling immunization program could be formulated.

Although there will no doubt be exceptions to the rule, they will be in the percentage minority and a positive known approach has always succeeded.

The known fact that maternal anti-bodies interfere with the efficacy of MLV vaccines creates sometimes uncontrollable problems. The positive approach to a complete program controlling I. B. R. and B. V. D. should reduce the numbers of interference problems and be a giant step in the direction of controlling many of the infectious, contagious diseases in cattle.

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VIRUS ABORTION IN CALIFORNIA

GEORGE L. CRENSHAW, D.V.M.* and D. G. MCKERCHER, D.V.M., Ph.D.**

As laboratory diagnostic procedures have become more sophisticated, viruses have been increasingly incriminated as the causative factors of abortion in both beef and dairy cows in California.

The agents that have been most frequently involved are the infectious bovine rhinotracheitis (IBR) virus and the EBA agent. Occasionally the bovine viral diarrhea (BVD) virus has been incriminated on the basis of indirect evidence, but to our knowledge the parainfluenza-3 (PI3) virus has not been identified as a cause of bovine abortion in California.

Bovine Virus Diarrhea

Although the BVD virus may be involved in abortion more frequently than the evidence indicates, it has not been positively identified as a cause of abortion in California. Most of the evidence to this effect has been based on serological findings and, in view of the confusion regarding the interpretation of BVD serology, it is far from being conclusive. There have been cases of abortion, however, that have occurred subsequent to vaccination against BVD. In one particular herd the incidence was extremely low—six animals in a herd of 360 dairy cows. However, in this particular herd prior to vaccination approximately half of the calves dropped were weak; metritis occurred in approximately one-third of the cows following parturition; and the incidence of mastitis was high following parturition and above normal in cows that had freshened three months or less.

Subsequent to vaccination of the entire herd, which was done at the owner’s request, the aforementioned six abortions occurred. However, the incidence of metritis and mastitis dropped markedly, whereas the incidence of weak calves increased to the extent that during the next three months approximately two-thirds of the calves were born about two weeks prematurely. They displayed lesions suggestive of BVD, and frequently scoured between 6 and 24 hours of birth. The severity of this condition gradually subsided and five months after vaccination no problems which could be attributed to BVD or to BVD vaccination were observed.

The BVD virus was not isolated, but since that time improved techniques have resulted in recovery of the agent from calves manifesting similar clinical signs and pathologic lesions.

Calves with characteristic lesions dropped by vaccinated beef heifers have been under study, and the virus has been consistently recovered from the latter animals.

Infectious Bovine Rhinotracheitis

Evidence that abortion occurs as a result of either natural infection

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**Professor, Department of Veterinary Microbiology, University of California, Davis.
Presented at USLSA meetings, Phoenix, Arizona, October, 1967.
with the IBR virus or vaccination against this disease has been well documented. In addition, the occurrence of infectious pustular vulvo-vaginitis (IPV) and balanoposthitis, both of which are due to the IBR virus, is generally recognized. Abortion apparently does not result as a sequel to IPV infection. It appears that the only manner in which the virus can reach the uterus, and cause subsequent abortion, is via hematogenous transport. It is able to invade the blood stream from the respiratory tract but apparently not from the genital tract. Cattle can contract IBR from IPV affected cattle and abort as a consequence. IPV can, therefore, be considered as an indirect cause of abortion.

Abortions may occur in cows from several weeks to several months after the clinical signs of IBR first appear. In many cases, abortion may result with no other apparent sign of the disease. In vaccinated cattle abortions usually occur from 30 to 45 days after vaccination.

Abortions resulting from either the naturally occurring infection or from vaccination occur most frequently during the last trimester of pregnancy.

**Epizootic Bovine Abortion**

In California, EBA is unquestionably the most prevalent and important abortion syndrome. The causative agent is a member of the psittacosis-lymphogranuloma venereum (PLV) group of agents. The length of time this disease has existed in California is not known, but it has been widespread for the past 15 years. The causative agent was isolated in 1958 and since that time the disease has been studied extensively.

Epizootic bovine abortion is commonly called “foothill abortion” because it occurs in cattle that have been pastured on foothill or mountain ranges. Since more beef cattle are maintained under this type of regimen, the disease is seen more often in the beef breeds, although dairy cattle are equally susceptible to the disease. The areas in California where this disease is encountered are extensive, comprising practically all the foothill and mountain regions. No cases, however, have been reported in the extreme southern or northwestern counties.

Abortions usually occur during the last trimester of gestation. In some instances calves may be born alive but weak and febrile. It is possible also that some abortions occur earlier but are undetected.

In previously unaffected herds cattle of all ages abort, thereafter heifers primarily are involved. In heifers, from 20-80% may abort, whereas in previously unexposed aged cows the abortion rate is usually lower. Subsequently, most cows in an endemic herd are not affected by this disease, even though they may not have aborted.

Gross or histopathological changes in aborted fetuses are quite variable, and isolations of the virus have been obtained from stillborn calves with no characteristic lesions. In general, however, the causative agent is difficult to isolate.

In fetuses the main gross pathologic features are:

1. Subcutaneous edema with excessive pleural and peritoneal effusion.
2. Hemorrhage.
3. Liver damage.

The mode of transmission has as yet not been established. Although ingestion, inhalation, and venereal exposure have been attempted, the only way the disease has been reproduced experimentally has been by parenteral inoculation. It has been suggested that the agent may be transmitted by insect vectors, particularly ticks, but this has not been substantiated.

Cows or heifers that abort may manifest a transient febrile response. Retained placentas may be encountered in 25% of the animals that abort, and if this condition is not given prompt attention, a severe metritis and septicemia may result, but the percentage of animals so affected is usually low.

Diagnosis.

Diagnosis of EBA is difficult because of the inconstancy of lesions and possible confusion with leptospirosis, vibrionic abortion due to *Vibrio fetus intestinalis* and possibly IBR. Unlike the agent of enzootic abortion of ewes (EAE), the EBA agent is rarely present in placentas. Isolation of the agent from fetal tissues is extremely difficult and time-consuming, and it can be positively identified only by demonstrating that it is capable of reproducing abortion. Currently, the only available test is the complement fixation (CF) test and the correlation between CF antibody titers and abortion is poor.

In general, in making a diagnosis, a thorough investigation of the herd abortion problem must be conducted and this information, together with the laboratory examinations, used to obtain confirmation.

Prevention and Control.

Attempts to prevent EBA by the use of inactivated and live vaccines have been discouraging. Furthermore, natural infected cows that have aborted have been susceptible when challenged artificially. This finding indicates that the immunity may be weak, and, therefore, inadequate to protect against the amount of virus used in challenge.

Antibiotic Feeding Trial.

Since it has been recognized that the agent is sensitive to the tetracycline group of antibiotics, a trial was conducted to determine if Chlortetracycline (CTC)* fed to heifers could effectively control this disease.

Twenty-four (24) Hereford or Hereford-cross heifers from two to four months pregnant were separated into three groups consisting of one group of five animals and two groups of 10 and 9 animals each respectively.

Fig. 1: Group 1— 5 heifers—controls
Group 2—10 heifers— 5 gms CTC/head/day
Group 3— 9 heifers—2.5 gms CTC/head/day

*Furnished by American Cyanamide Company. Acknowledgment to Dr. Robert Schoeb for assistance in designing the feeding regimen.
Each animal was fed two pounds of unmedicated alfalfa pellets daily, plus alfalfa hay ad libitum. In order to permit the animals to adjust to the rather unpalatable medicated pellets, the unmedicated pellets were gradually replaced in Groups 2 and 3 by pellets containing 2.5 grams of CTC per pound. When these appeared to be adjusted to this ration, each animal in group 2 was placed on a daily regimen of two pounds of medicated pellets while those in Group 3 each received one pound of medicated and one of unmedicated pellets. Thus the daily intake of CTC was 5.0 grams for Group 2 and 2.5 grams for Group 3. When the heifers had been on the feeding regimen for ten days all 24 were injected intramuscularly with 50 aborting doses of the EBA agent.

<table>
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<tr>
<th>Group</th>
<th>No. Heifers</th>
<th>No. Abortions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(Controls)</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>(5 gm/head/day)</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>(2.5 gm/head/day)</td>
<td>9</td>
</tr>
</tbody>
</table>

Within ten days one control heifer aborted; and during the next 60 days three more of the controls aborted. During this same period of time, one heifer in each of the 2.5 gm and the 5 gm groups aborted. Both were heifers which had been inconsistent in the consumption of the pellets.

The other heifers were maintained on the respective CTC levels until calving. All gave birth to normal, vigorous calves with the exception of one in the 5 gm group whose calf died from complications of dystocia.

All heifers that were fed CTC have been retained and are being sent to a ranch of known EBA incidence for breeding and maintenance until June, 1968, and then they will be returned to the research farm. The purpose of this is to determine whether these animals are susceptible to the naturally occurring infection since the drug undoubtedly prevented multiplication of the agent.

Further trials, feeding reduced levels of CTC to be conducted at the University and in the field, are being planned.

**SUMMARY**

Viral infections have been incriminated as causes of abortion in California. The most significant entities are IBR and EBA viruses. Control of IBR by vaccination is well documented, however no satisfactory method of vaccination for EBA has been developed. Administration of Chlortetracycline on a continuous basis in feed successfully controlled this disease. Further trials are in progress to verify these results and determine the efficacy of lower levels of Chlortetracycline.

**REFERENCES**


REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES OF CATTLE—USLSA

John B. Herrick, Chairman

The committee gives top priority to a national cattle identification program to aid in epizootology. No long range disease control program will function efficiently and effectively without individual animal and herd identification. This committee has recommended that such action be taken by all segments of the industry in previous reports (1965 and 1966) to the Livestock Sanitary Association.

The concept on preconditioning of cattle prior to shipment and certification of such program was endorsed by the committee.

The movement of cattle, particularly feeder cattle, through the various market channels is one of the greatest source of disease spread. Inspections, identification and regulations need to be thoroughly studied and updated. Estimates indicate that stressed and diseased cattle moving through market channels produce a $10.00 to $20.00 loss for every animal so moved. All types of marketing and transportation need to be regulated more stringently to reduce the stress and predisposition to disease. Specifically, cattle need to be identified from the aspects of individuals and herd. Their origin as to farm or ranch and state needs to be known. The number of times and date of movement through public market must also be made available.

The two pertinent points pertaining to the above are:

1. Animal and herd identification: The Committee recommended that the officials of the United States Livestock Sanitary Association request the Secretary of Agriculture to immediately appoint a national task force to study and recommend a national animal identification program. Initially, this program should start with the cattle industry. This task force should include representative segments of the animal industry. Examples: animal producers, livestock organizations, marketing organizations, breed associations, livestock disease associations, and animal health related interests. This Committee shall be charged to investigate and implement a program of livestock identification at the earliest possible time.

2. Preconditioning: The Committee defined the term “preconditioning” to mean a program of preparing cattle to withstand the stress of movement and to prevent the spread of diseases peculiar to the movement of these animals. A preconditioning program may include what is appropriate from the following outline:

**PRECONDITIONING CERTIFICATE**

Certificate #_____________ Date_____________

The animals identified as to brand _________ and individual identification _____________. _______________ age,
sex, and total number of animals in this certification.

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<tr>
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<th>Date</th>
<th>Brand</th>
<th>Serial Number</th>
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<tr>
<td>WEANED PERIOD (Days ____ )</td>
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<td>MALIGNANT EDEMA</td>
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<td>SF4 (P13) VACCINE</td>
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<td>PASTEURELLA VACCINE</td>
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<td>BVD VACCINE</td>
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<td>LEPTO VACCINE</td>
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<td>GRUB TREATED</td>
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<td>WORM TREATMENT</td>
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<td>OTHER PATHOGENS</td>
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<td>SHIPPING DATES</td>
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Ration during preconditioning period: ____________________________________________________________

SELLER ____________________________________________

ADDRESS ____________________________________________

BUYER ____________________________________________

ADDRESS ____________________________________________

CERTIFIED BY ____________________________________________
The Committee endorses the immediate implementation of the program in that it will involve individual animal and herd identification as well as disease control measures essential to the animal industry. The Committee further urges continuous evaluation of the program. In some cases, aspects of this program may start with the breeding herd.

The Committee further approved adoption of the following items of previous committee recommendations:

a. State Livestock Sanitary Officials should be aware of the potential for disease transmission by the use of artificial insemination.

b. State Livestock Sanitary Officials should be aware of the existence of United States Livestock Sanitary Association's recommended regulation and uniform certificate for movement of bovine semen.


The Committee urged the United States Livestock Sanitary Association to recognize the efforts of the Study Committee on Leukosis in cattle and encourage full dissemination of their deliberations.

The Committee paid tribute to the sponsors of the First Symposium on Bovine Respiratory Diseases held in Athens, Georgia, June 1966. They encouraged full utilization of the information obtained from the proceedings of this Symposium.

The Committee approved of the resolutions on the items pertaining to the diseases of cattle presented by the Intermountain Veterinary Medical Association adopted June, 1967.

The Committee reminds United States Livestock Sanitary Association members that a supplement on neonatal diseases of cattle is available from the United States Department of Agriculture. This is to supplement the 1966 report.

The Committee recommends the institution of long range disease elimination and disease prevention procedures to be developed for breeding herds of cattle in the United States. This involves reevaluation of vaccines, testing procedures, and reporting of morbidity and mortality of the diseases affecting cattle.

The Committee recommends uniformity in testing by all diagnostic laboratories, particularly those diseases of cattle; namely viral disease, vibriosis and leptospirosis.
It is almost paradoxical that a disease costing the dairy industry between 225 and 500 million dollars a year should have received so little regulatory attention up to this time. Yet we all understand the reasons for postponing serious organized efforts to reduce the toll of this insidious disease. It is not of a single etiology. Several infectious agents plus a host of environmental factors are involved and control is largely a problem of management. In short there has been a very real problem in knowing just how or where to approach the problem from the standpoint of regulatory activity. Mastitis has been a "sleeping dog" which many have preferred to "let lie."

Sooner or later, however, most "sleeping dogs" awake and mastitis has been no exception. In this instance the over-riding concern has not been one of economics to the dairy industry, except indirectly, nor has there been concern that the disease would spread or in other ways bring harm to the dairyman or his animals. The "sleeping dog" was awakened by concern that the public should receive the most wholesome milk that could be produced. There was concern that the time had come to enforce laws on the statute books for many years that milk should be the "normal secretion of healthy udders from healthy cows." There was concern that freedom to market mastitic milk often increased the hazard of antibiotics being in the milk, and reduced the nutritive quality of milk. Finally there was the realization that mastitic milk could be detected and that effective methods of control were available.

The events of the past few years have demonstrated that detection is possible and means of control are now available. Control on the farm is largely a management matter, and, in my opinion, is one which must be left primarily in the hands of the dairyman, his veterinarian, and other local advisors. It does not now lend itself to centralized direction or to the usual regulatory practices of test and segregation or slaughter, although these procedures may be used by the owner on the individual dairy farm. In fact, these measures may be vital to control on the farm, and we may in the future wish to eradicate Streptococcus agalactiae altogether.

**BACTERIA COUNTS, CELL COUNTS AND MILK QUALITY**

Except for brucellosis and tuberculosis control, the greatest improvement in milk quality effective during the 20th century has been accomplished by attention to the milk after it has left the cow. Measures to keep dirt and debris out of the milk, refrigeration to reduce bacterial growth, mechanized milk handling and efficient equipment cleaning, together with almost universal pasteurization have virtually removed the threat of infectious disease being transmitted to humans by milk.

*Department of Clinical Pathology, University of California.*
DETECTION OF ABNORMAL MILK

Various tests and bacteria counts have been used for years to assure milk quality. High bacteria counts usually indicated poorly cleaned equipment or other contamination from outside sources, contaminated water, and/or poor refrigeration, and these possible causes must always be investigated and eliminated whenever excessive bacteria counts arise.

As milk quality improved, standards were gradually raised. When bacteria counts had to reach 100,000 or more to cause concern, they were almost invariably associated with problems of contamination and refrigeration. Now that we are often concerned about counts of 30,000, or even of 10,000 or less, udder infection is more often a major contributing cause. In such cases the tank milk will often, but not always, have a high cell count as well. On the other hand, milk often has an excessive cell count without an excessive bacteria count. The bacteria count is not necessarily above accepted standards in the presence of mastitis and cannot be depended upon to detect mastitic milk.

There is a variable relationship between cell numbers in an infected quarter and the number of bacteria in a quarter. When bacteria enter a quarter, they begin to multiply and exceed cell numbers. As bacteria and their by-products increase, substances are liberated which stimulate inflammation, and leukocytes which then pour in to combat the infection may soon far outnumber the bacteria, often destroying them altogether. At this point we may have many millions of cells and few, if any, bacteria (Fig. 1). Such milk is esthetically undesirable, often physically abnormal and is less nutritious than normal milk. This whole cycle can be completed within 24 hours in an acute situation. In chronic infections, cell and bacteria numbers can fluctuate in response to each other for months or years (Fig. 2).

This is why methods of measuring somatic (body) cells, chiefly leukocytes, have been introduced into quality control programs. Also for this reason one cannot predict cell counts from bacteria counts and vice versa. With large populations some average relationships can be shown, but for individual cows and herds, these relationships vary greatly. Thus to assure high quality milk, it is necessary to score milk on the basis of cell counts as well as on bacteria counts.

I have tried to emphasize that the relationship between bacteria counts and cell counts in milk is highly variable, that cell counts and mastitis levels may be high in milk with normal bacteria counts. Thus, to assure high quality milk, it is necessary to score milk on the basis of cell content as well as on bacteria counts and by means of other criteria.

At the same time I wish to emphasize that high bacteria counts may also be due to bacteria coming from the cow, either with or without high bulk milk cell counts. Streptococcus agalactiae may be responsible for very high bacteria counts (up to 300,000 or more). Staphylococci seldom cause counts that high but may also be responsible for high bacteria counts, often in the neighborhood of 30,000 bacteria or more. Therefore, identification of bacteria causing high counts is an important first step toward solving bacteria count problems. As milk handling procedures improve and bacteria...
count standards are lowered, infected cows may account for an increased percentage of high bacteria counts.

Whereas, there is a clear relationship between the cell content of the bulk tank milk and the mastitis status of the individual cows contributing milk to the tank,\textsuperscript{30} the relationship of the cell content or pathogenic bacteria count of the bulk milk to the number of infected cows is highly variable.

![Graph](image)

\textbf{Figure 1.} An example of early increases in bacteria numbers following infection in a quarter, after which the infection is destroyed as large numbers of leukocytes enter the gland. The whole process of infection, inflammation, and return to normal may occur within 24 hours, or may take somewhat longer.

This is due in part to the fact that milk from obviously mastitic cows is withheld from the tank and partly due to the variables illustrated in Figures 1 and 2. Evaluation of infection rate must still be made by culture of individual cows.
THE SIGNIFICANCE OF CELL NUMBERS IN MILK

Regardless of the kind or numbers of bacteria present, the number of cells in milk is a measure of the severity of mastitis. Cells in completely normal milk are low in number, probably less than 100,000 per ml and certainly less than 300,000 per ml in most cases although some have reported higher numbers. Lymphocytes and epithelial cells account for a substantial proportion of the cells from normal quarters. With irritation, however, neutrophils invade in large numbers so that the cell count may reach many millions. Because the cell type in the presence of inflammation is principally the neutrophil, the count is generally termed a "leukocyte count," although it is realized that a few epithelial and other kinds of leukocytes cells are included. Some, therefore, prefer the term "somatic cell count" which includes all types of body cells and is technically more correct. Or one may simply say "cell count." Whatever the semantics, all cell types are included in the counts and also contribute to the screening test results, but the neutrophils are responsible for the greatest variations in number.

Certain generalities are recognized which influence cell counts. Older cows tend to have higher counts, largely in relation to increased rates of infection. Counts do not always return entirely to normal after infection is removed, and some older cows have increased cell counts when infection cannot be proved. Others seem to reach a balance between moderately high

![Figure 2. A schematic example of the relationship between leukocyte and bacterial numbers in a chronic infection, in which bacteria are never completely destroyed. Numbers of leukocytes and bacteria fluctuate irregularly, as do the outward signs of inflammation. Number relationships may vary considerably from those selected for this example.](image)
levels of cells and low numbers of bacteria. Herds with excellent mastitis control programs can keep cows much longer and thus benefit from the tendency toward increased production with each lactation. Such herds may expect a somewhat higher incidence of low grade infections than herds made up principally of young cows. Counts of up to a million cells might be quite acceptable in herds with many old cows, whereas one might strive to keep counts in a young herd much lower. On the other hand, it was recently demonstrated that removal of infection in older cows resulted in the same average cell counts of 300,000 as existed in the heifers. The role of infection in causing high counts in cows of all ages is thereby emphasized, but excellent control of both infection and cell count should be possible regardless of age.

Cell counts also tend to vary with the lactation cycle. Cell counts or CMT scores tend to be higher in colostrum or just before drying off, probably partly as a result of volume changes. If milk volume drops as the cow is ending her lactation, and the total number of cells shed remains the same, the number per ml of milk will increase and the CMT score may increase. We believe these increased cell counts at the beginning and end of lactation are associated chiefly with cows that already have some inflammation present. Infection-free cows studied a few years ago in our laboratories did not show significant increases at any stage of lactation. Reduction in milk volume due to stresses, such as the stress of very hot weather is also accompanied by increase in cell count. Diurnal variations becomes important in individual cow studies or when herds are milked at other than standard intervals.

While it is probable that really normal cows at any stage of lactation and any age will not have a high cell count, we are not dealing with completely normal cows and herds. On a practical basis the number of old cows, or the number of heifers in a herd may distinctly influence the results of a screening test. Herds that freshen and dry on a seasonal basis may have an increase in screening test scores as a large number of cows approach the end of lactation.

The National Mastitis Council made allowance for these variables, including a low incidence of mastitis, when they stated that “presence of more than 500,000 leukocytes per ml. of mixed herd milk strongly suggests a significant incidence of mastitis in a given herd.” Further allowance for these and other variables is made by the establishment of a critical cell count level at about 1,000,000 cells in most milk quality programs and the requirement of several consecutive unsatisfactory tests before action is taken against a dairy. By these actions a workable compromise is achieved between the theoretically perfect product and that which is practically possible. The Abnormal Milk Program as approved April 6, 1967 by the National Conference of Interstate Milk Shipments allows a very generous tolerance of 1,500,000 cells.

At the moment I think a cut-off level of 1,000,000 cells is reasonable and generally possible of attainment. Perhaps two or three years should be allowed to enable all dairies to reduce cell levels below this level in areas
not previously having milk quality control programs. Substantial milk sheds have already surpassed this goal. Most dairies should now be able to stay comfortably below an allowable maximum of 1,000,000 cells, perhaps in the range of 500,000 to 750,000. At the moment it does not seem realistic to me to expect dairies to consistently market milk containing less than 500,000 cells although this may be possible in the future.

METHODS OF DETERMINING CELL CONTENT

Having established a permissible level of cells, our next problem is the selection of a practical testing procedure which will identify milks which are either above or below the specified level. Although this might seem on the surface to be relatively simple it becomes rather a complex matter to devise or select a method which at once is accurate, objective, repeatable, rapid, easily performed, inexpensive and not subject to change as the milk ages.

1. Direct Microscopic Somatic Cell Counts (DMSCC)

Of necessity the DMSCC has been the standard against which all other tests have been judged. Unfortunately, no standard methodology has been followed and the majority of workers have used methods not adequately documented and without satisfactory precision.

Prescott and Breed in reporting their method recommend that 100 fields be counted on duplicate films to assure an error below 15%. Very few investigators comparing cell count and screening tests have counted nearly that many fields. Strynadka and Thornton by counting 60 fields per sample obtained a variation of ± 50% from the mean, the highest count occasionally being three times that of the lowest. Paape et al. concluded it would be necessary to count 25 fields from approximately 200 smears to obtain a 95% confidence limit of approximately 500,000 cells per ml. Newbould and Phipps, after investigating sources of counting error, recommended counting procedures which resulted in a between-film coefficient of variation of less than 10 per cent in milk samples containing in excess of 350,000 cells. It is discouraging, however, to note that only two milks of high cell count can be counted per day by this method. Postle andBlob in a comparison of bulk milk screening procedures, concluded that the Breed smear method was the least reproducible of all.

It should be obvious, therefore, that some of the reported large cell ranges for a given screening test score are more revealing of variation from the counting procedures used than of variation in screening test performance. It should also be obvious that the DMSCC is too time-consuming and expensive for routine use in screening programs. Schneider and Jasper did, however, report on a simplified procedure whereby a DMSCC can be used simply to determine whether a given milk contains cells either above or below a specified number.

In recognition of the great confusion which exists with respect to
the conduct of the DMSCC, the National Mastitis Council has requested their Research Committee to recommend standardized procedures for the DMSCC. A sub-committee, of which I am a member, is working actively on this project. Procedures have been specified and are now subject to testing by the various laboratories involved. We hope soon to have a standardized procedure recommended by a widely representative group of experienced investigators. In the meantime, great care should be taken when interpreting work based on cell counts, especially when exact details of the procedure used are not given.

2. Electronic Counting of Cells in Milk

The success of electronic particle counters in such areas as hematology naturally encouraged investigation of their value for counting cells in milk. Serious problems arose initially because of the interference of fat droplets. Practical centrifugal methods have now been devised for eliminating the fat while retaining the cells and several methods have been published which appear to give good results. Further evaluation will probably reconcile some of the variations in methods to achieve maximal reliability and simplicity. Great accuracy and repeatability is achieved with electronic methods but labor cost is relatively high for use in routine screening and the equipment is too expensive for most control laboratories. Electronic counting of cells in milk may find its greatest usefulness in research laboratories in which accurate counts are necessary for a variety of reasons, including evaluation of simpler screening tests.

3. The Catalase Test

The catalase test is one of the older indirect tests used for estimating cell numbers in milk. It is a fairly satisfactory test but can be influenced by bacteria numbers as well as by cell numbers and by occasional extraneous influences. A major drawback is that of expense. It requires quite a lot of glassware and quite a lot of labor for performing the test and subsequent clean-up. The trend is away from use of the catalase test.

4. The Modified Whiteside Test

The Whiteside Test, as modified (MWT) by Murphy and Hanson, was the first of the widely used tests giving a gel-type reaction when the reagent (NaOH) was added to milk. The reaction involves leukocytes and apparently fat, calcium, and protein as well. Therefore, it is less specific than the detergent-gel tests. It requires more labor and equipment than the CMT. The MWT reaction apparently does not change as much with age as the milk grows older as do the other gel tests.

5. The California Mastitis Test (CMT)

The discovery of the California Mastitis Test (CMT) constituted an important breakthrough to the rapid, easy, and inexpensive evalua-
DETECTION OF ABNORMAL MILK

 tion of the approximate number of cells in milk. It is dependent upon a specific chemical reaction between the DNA in the nucleus of cells and the detergent in the reagent.\textsuperscript{2,19} We have had great success the past two years by using the CMT as our official screening test in the California Milk Quality Control Program. These are some of the reasons why it was selected:

a. The CMT has been subject to an extensive amount of critical evaluation both here and abroad. Although there are still some disagreements in interpretation, Schalm's original score ranges are still generally accepted.

b. By running a repeat test, cell ranges can be materially narrowed by using the refined range grouping of Schneider and Jasper.

c. The test is rapid and economical. Accuracy can be increased by repeated testing at much less expense than is incurred with a single run of most screening tests available.

d. Standardized reading is possible through suitable training. Concern over variations in reading was expressed early in our program but this was quickly reduced to a minimum. A movie has been produced by the State Department of Agriculture which has been very helpful in standardizing CMT scoring and in training testers.

e. Ease of the test makes repeated testing feasible. Frequent testing is practical, thus assuring against lapses in quality which might occur if testing intervals are prolonged. The producer does not know when to expect a test or, more correctly, he must expect a test at all times.

f. Careful microscopic cell counts are used to confirm the CMT whenever regulatory action is indicated. The dairymen is thus protected against possible error on the high side. Frequent testing protects the public and the dairymen against error in either direction.

g. Expensive glassware is unnecessary and reagent cost is low.

h. Very little time is consumed in testing. Public health and state laboratories have been able to absorb the Milk Quality Testing Program without budget augmentation.

i. The entire statewide program required the addition of a single technician to keep records, trouble-shoot and coordinate the testing and the reading of the tests. No other personnel were hired or shifted to this program. This is the kind of program all taxpayers, including dairymen, appreciate.

It is the hope in California to soon be able to keep all milk consistently scoring CMT 2 or higher off the market.\textsuperscript{5,9} Approximately 5 per cent of the dairies in the state are now in that category, a considerable improvement over the situation of 3 years ago.

6. The Wisconsin Mastitis Test (WMT)

The WMT is a variation of the CMT in which the volume of milk reagent mixture left in a tube is measured after a timed outflow through a small orifice.\textsuperscript{22,84} It is highly repeatable on a given milk sample
although an occasional erroneous result is obtained. It has the advantage of an objective numerical reading as contrasted to the subjective scoring of tests such as the CMT or MWT. It is more expensive in time and equipment than are the MWT and CMT. A similar test, called the Milk Gel Index is being successfully used in Canada.26

There are, however, in my opinion, some problems to be worked out prior to widespread use of the WMT as an official screening test. There is disagreement between the published reports12,22 correlating WMT test scores to cell counts. This may be related more to counting methods than to the WMT, but agreement is necessary on the appropriate correlation before screening significance can be attributed to a given score. Furthermore, some idea of the limits of confidence in terms of cell counts for a given score must be determined and scores established which could be used as cut-off points if cell counts are to be below selected levels. A third problem is that of deterioration of score with aging of the milk.11,12 This deterioration is more obvious with an objective test having more than 30 possible scores than with a subjective test such as the CMT or MWT having 5 possible scores.

7. The Brabant Mastitis Test (BMT)

This test, used in Holland, is a forerunner to the WMT except that photographic means are used to run large numbers of tests.7 Its accuracy is inadequate and equipment costs are high.

8. Chemical Measures of DNA

Direct correlations were obtained between chemical measures of DNA using the Feulgen reaction and cell counts in milk,18 although this technique has been questioned.35 At the moment chemical analysis is not a satisfactory procedure for routine milk quality testing.

Regardless of the test selected in an area program, care must be taken to standardize procedures so that all dairymen are treated equally. Every attempt should be made to secure an evaluation of the quality of milk as it leaves the cow. Excessive delays in pickup and further delays in testing may enable milk seriously deficient in quality to pass screening tests for cell content. Improper handling and refrigeration may add to the degenerative processes rendering milk unfit for testing.

Uniform results may depend more upon uniform procedures for collection, storage, testing and handling of the milk than upon selection of a uniform test from area to area. Without such uniformity in milk handling procedures, basic milk quality could vary greatly from area to area or from dairy to dairy even though the same test is used.

THE CURRENT DILEMMA

It is obvious that any of the procedures for estimating cell counts in milk are subject to objections on one ground or another. It is equally obvious that numerous milkshed areas have made great progress in improving
DETECTION OF ABNORMAL MILK

milk quality by using a variety of evaluation procedures (DMSCC, WMT, MWT, CMT and catalase). There is great interest in selection of a uniform test or tests but until a clear advantage can be shown for one or another, it may be preferable for different areas to demonstrate problems and progress associated with different approaches.

The National Mastitis Council has requested the Research Committee, which is working on standardized methods for the DMSCC, to recommend if possible, a standardized method for screening milk for cell count levels. When it is considered that it is taking a year to recommend standard methods for the DMSCC, it may be expected that evaluation and recommendations on screening tests may take even longer.

In the meantime, progress can be made on improved milk quality. We can, and are, advancing on the basis of knowledge at hand. It is not necessary to have a perfect test for progress. It is not even necessary to have a truly objective test as is evidenced by the great progress in disease control brought about by tests such as the tuberculin test or the tube or plate agglutination test. Our goals can be achieved by intelligent use of less than perfect tools and the experience gained thereby may bring us closer to optimum procedural methods. One thing is certain, inspite of whatever problems may exist with respect to testing procedures, the momentum already achieved will carry us inevitably to standards of mastitis-free milk considered impossible only a few years ago.

REFERENCES

Two meetings of the mastitis committee have been held during the year. One at the annual meeting of the National Mastitis Council held in Chicago February 23, 1967, and the other during the 1967 United States Livestock Sanitary Association meeting here in Phoenix. Six members and several interested guests were present at the Chicago meeting. Dr. Robert Schroeder, then president elect of the American Veterinary Medical Association and a member of this committee, presented a paper on mastitis control to the National Mastitis Council. His remarks stimulated a great deal of discussion. He urged that standardized methods, rules and procedures be drafted for the various states and the Federal Government. He stated, "We can no longer tolerate today's confusing situation with at least five screening tests in common use and with as many different approaches to regulatory control as there are states to promulgate them." He suggested that the U.S.L.S.A., because of its experience in disease control and eradication, take the initiative in this endeavor. As might be expected all members of the N.M.C. were not in full agreement with Dr. Schroeder's suggestions but concern regarding use of the various screening tests and need for uniformity was evident.

Recent action taken at the National Conference of Interstate Milk Shipments concerning marketing of abnormal milk (milk quality control) will undoubtedly result in demands by dairymen for more uniform and effective mastitis control programs. The first phase of the regulation approved by the Interstate Milk Shippers became effective July 1, 1967. The second phase which requires all interstate milk shippers listed in the quarterly publication "Sanitary Compliance and Enforcement Ratings of Interstate Milk Shippers" be certified as following a leukocyte indication test program becomes effective July 1, 1968. The third phase, effective July 1970 contains a penalty clause for non-compliance with leukocyte standards. Milk containing 1,500,000 or more leukocytes is considered abnormal and in excess of the standard by this organization.

The U.S.L.S.A. mastitis committee just completed a national survey on milk quality programs now in operation. A questionnaire was sent to each of the 50 state veterinarians. Of the 39 questionnaires returned, 35 indicated one or more milk quality programs now in operation. In 6 states the program is under direct supervision of the state veterinarian, in 1 (Los Angeles County), under the county veterinarian, in 12 states under the supervision of the State Department of Dairying or a similar department, 8 under the City Milk sanitarians supervision, 6 under the processors supervision, 12 under state or county health departments and 4 under the other supervision. As indicated by the above numbers, many states operate two or more programs. For example, the State Department of Dairying, City Sanitarian or health department, and processors may each test a portion
of the states milk supply and each may have its own regulations concerning quality milk control. Although only 6 programs were under direct supervision of the state veterinarian, he was often involved in an advisory capacity.

Tests used for milk leukocyte determinations varied considerably. A few programs used only the D.M.L.C. (direct microscopic leukocyte count). Most used one of the various screening tests followed by the D.M.L.C. on high count samples. Others used only screening tests. Nineteen states used the Wisconsin Mastitis Test (W.M.T.), 16 the California Mastitis Test (C.M.T.), 10 the Catalase Test, and 8 the Modified Whiteside Test (M.W.T.). Two or more of the above tests were used in a number of states. The D.M.L.C. was being used in 22 states either alone or in combination with one or more of the above screening tests.

Milk leukocyte number per ml. considered satisfactory by these regulatory agencies ranged from 500,000 to 2,000,000. Most programs used 1,000,000 leukocytes per ml. as the maximum number in normal milk.

Thirty-one state veterinarians indicated quality milk programs are helpful in stimulating mastitis control programs within their respective states, 6 failed to comment and 2 indicated no benefit was evident.

This survey clearly substantiates Dr. Schroeder's remarks to the N.M.C. concerning the need for uniformity not only within individual states but nationally.

To provide continuity of thought and effort for this and subsequent mastitis committees, this committee deemed it desirable to delineate specific long-range objectives. These objectives are as follows:

1. Create among dairymen, veterinarians, regulatory officials and extension workers a more general awareness of the importance of mastitis as an infectious disease of dairy cattle.

2. Encourage federal, state and other regulatory agencies to develop and improve mastitis control programs.

3. Encourage expanding activities in mastitis research.

4. Encourage the establishment of laboratory facilities for the diagnosis of mastitis.

5. Encourage and stimulate funding for diagnostic facilities, mastitis research and mastitis control programs.

6. Encourage veterinary practitioners to assume a more active role in mastitis control.

The following recommendations for U.S.L.S.A. action were approved:

*Streptococcus agalactiae* is a major udder pathogen in most areas of the United States. Mastitis due to this organism is an eradicable infectious disease causing serious economic loss to dairymen. In addition, there is a direct correlation between the prevalence of *Strepto-
coccus agalactiae infection and abnormally high leukocyte counts in milk. The mastitis committee therefore,

RECOMMENDS that the U.S.L.S.A. urge Federal and State regulatory officials responsible for animal disease control to institute appropriate measures to control and eradicate this disease. The committee further RECOMMENDS that:

1. The U.S.L.S.A. support, as an aid to mastitis control, the objectives of the milk quality control program recommended by the National Conference on Interstate Shipments.
What is swine tuberculosis? A current definition would state that swine can be infected with *Mycobacterium tuberculosis*, *M. bovis* and *M. avium*, that swine become infected with these organisms by contact with man, cattle and chickens and their waste products, and that the disease can be generalized, but is frequently limited to a very high percentage of the lesions in the head and mesentery lymph nodes.

The transmission of tuberculosis from man, cattle and chickens is well documented. The transmission from swine to man, cattle and chickens can occur when the disease is generalized or if the primary complex disease is in the intestines.

If tuberculosis in swine were limited to *M. tuberculosis*, *M. bovis* and *M. avium*, then the elimination of these organisms from the swine environment would lessen the disease from swine. If the organisms were established in swine, the transmission would be directly or indirectly from swine to swine. In that case the separation of swine and cattle, and the eradication of the disease in cattle would lessen the incidence of *M. bovis* infection in the swine but not necessarily eradicate the disease. This has occurred as demonstrated by the marked reduction of *M. bovis* infection in swine, however, the eradication of *M. bovis* infection in swine to be complete would necessitate the elimination of swine herds infected with *M. bovis*.

The *M. avium* infection in swine follows a similar pattern to that of *M. bovis* infection. Although there is no eradication program for chickens, comparable to that for cattle, a marked reduction in tuberculosis in chickens has occurred due primarily to animal husbandry practices of shipping birds to market before two years of age. Again, the elimination of an infected environment for swine and the destruction of infected swine herds should effect eradication of *M. avium* tuberculosis.

If tuberculosis in swine was as clear-cut as depicted, then all that is needed is a program of eradication based on the above statements and a successful program could be anticipated. Unfortunately, swine tuberculosis is not so simple.

Let me cite a few facts which we have found about cattle tuberculosis which is applicable to swine tuberculosis:

1. In addition to the disease produced by *M. bovis* in cattle, a tuberculosis-like (mycobacteriosis) disease or diseases is caused by a heterogeneous collection of mycobacteria that are designated Runyon Group III or slow growing non-pigmented organisms. These organisms run the gamut
of pathogenicity for highly pathogenic to nonpathogenic. Certainly in the light of all available information these organisms are not *M. bovis*.

2. An animal that is infected with one of these organisms develops some degree of sensitivity for some period of time to intradermal injections of mammalian and avian tuberculins. The degree of sensitivity depends largely on the extent of the disease in the animal and to its state as regard to progressity or regression rather than the specificity of the tuberculin. In generalized disease in a progressive state, large reactions to both mammalian and avian tuberculins will occur that are comparable to those produced in animals with progressive *M. bovis* infections. In general, the reaction to mammalian tuberculins will be greater than the response to avian tuberculin. In regressive generalized or primary complex disease, the reactions to both tuberculins will be smaller and in general, the reaction to avian tuberculin will be equal to or greater than to mammalian tuberculin. In other words, neither mammalian or avian tuberculin are specific. In general, the reactions are small to sensitivity of Group III mycobacteria in cattle to both mammalian and avian tuberculins. The disease in cattle caused by Group II mycobacteria is, generally, a primary complex type although there are exceptions.

3. The Group III mycobacteria probably enter the body through breaks in the skin, as evidenced by skin lesions and granulomas in the body nodes, and by ingestion as evidenced by the finding of the organisms in the ileocecal valve and walls of the intestines. We have infected calves with Group III mycobacteria by aerosol and by intruterine and intradermal routes. Most of the organisms produce a primary complex disease. The only evidence of the disease is microscopic lesions in the lymph nodes, particularly body nodes.

4. The source of the Group III organisms is unknown. Assuming that the mode of invasion is as above, then we can conjecture that the organisms are present in the soil. If these organisms are of soil origin, and most soils carry these organisms as normal inhabitants, then eradication of these diseases would be difficult if not impossible.

Presently, a very high percentage of the cattle branded as reactors are reported at kill as NGL animals. Very likely the cause of the hypersensitivity is due to infections of Group III mycobacteria. There may be other causes of sensitivity but Group III mycobacteria have a high priority. In addition, the animals classified as “suspects” have sensitivities likely due to Group III mycobacteria. Reversions in this group are high as would be expected.

What do we know about swine tuberculosis? The following is a citation of accepted information:

1. Swine may become infected by contact with *M. bovis* infected cattle, and by contact with *M. avium* infected chickens or their waste products.

2. The gross and microscopic lesions are very similar to those in cattle. Differences have been reported but there is no agreement by pathologists.
3. The lesions produced by *M. avium* and *M. bovis* do not differ significantly.

4. The sensitivity induced by *M. bovis* and *M. avium* can be detected by both mammalian and avian tuberculins. In general, a greater response will occur to the homologous tuberculin but this is not always true.

5. It is likely that either *M. bovis* and *M. avium* can spread in the herd. In many cases, the disease is limited to a primary complex type and there may be little or no spreading of these cases.

6. Where generalized disease occurs, the disease spreads to other swine in the herd and to other susceptible species in contact with the swine or their waste products.

If tuberculosis in swine were limited to *M. bovis* and *M. avium*, an eradication program based on the tuberculin test and/or trace-back would be effective. Unfortunately, the atypical mycobacteria produce a disease in swine indistinguishable from that induced by *M. bovis* and *M. avium*.

In 1960, we became interested in such a disease because tuberculosis was discovered in the university herd. At that time, our interest was primarily one of service.

The results of this outbreak are extremely interesting. At the time of the tuberculin testing, 32 swine that had been used on a copper deficiency experiment were slaughtered prior to tuberculin testing. Tissues were collected for bacteriologic and histopathologic examination. The results are presented in Table 1. Sixty-five percent yielded Group III mycobacteria.

### TABLE 1

**Bacteriologic and Histopathological Data on Swine Used on a Copper Deficiency Experiment**

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Tested</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Group 3 Isolated</td>
<td>21</td>
<td>65.0</td>
</tr>
<tr>
<td>No Pathologic Changes</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Group 3 Isolated</td>
<td>3</td>
<td>37.0</td>
</tr>
<tr>
<td>Granulomas Present</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Group 3 Present</td>
<td>18</td>
<td>90.0</td>
</tr>
<tr>
<td>No Pathologic Test</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Group 3 Isolated</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Ninety percent of the animals with tissues showing granulomas yielded Group III mycobacteria. Thirty-seven percent of the animals showing no significant changes histopathologically yielded Group II mycobacteria.

Thirty-four swine from the pole barn were slaughtered. The results are presented in Table 2. Thirty-five percent of the swine yielded Group III mycobacteria. One hundred percent of the animals with microscopic lesions were positive for Group III. Twenty-six percent of the animals with no significant pathologic changes yielded Group III mycobacteria.

Seventy-four swine from the farrowing barn, concrete slabs, and
pasture were slaughtered. The results are presented in Table 3. Fifty-seven percent yielded Group III mycobacteria. Forty-four animals had granulomas and 68 percent yielded Group III organisms. Thirty animals failed to show microscopic lesions. Four percent yielded Group III mycobacteria.

An examination of these data show that a relatively high percentage of the swine sent to slaughter were infected with Group III mycobacteria. The organisms were practically in pure culture. They appeared to be identical from cultural appearance. There were no signs of dissociation.

The tuberculin testing of the herd using mammalian and avian tuberculins also indicated a high rate of infections. A total of 1574 tuberculin tests were made on animals in the swine herd. There were 227 reactors (14.4%) and 220 suspects (13.97%) for a total of 447 reacting animals (28.37%). These are presented in Table 4.

In another experiment, an endocrine swine study, 115 tuberculin tests were made over a period of a year as presented in Table 5. Fifty-four (46.9%) were listed as reactors. Some of these swine were slaughtered and Group III mycobacteria were isolated.

TABLE II
BACTERIOLOGIC AND HISTOPATHOLOGIC DATA ON SWINE KEPT IN A POLE BARN

<table>
<thead>
<tr>
<th>Table II</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Tested</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Group 3 Isolated</td>
<td>12</td>
<td>35.0</td>
</tr>
<tr>
<td>No Pathologic Changes</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Group 3 Isolated</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Granulomas Present</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Group 3 Isolated</td>
<td>6</td>
<td>100.0</td>
</tr>
</tbody>
</table>

TABLE III
BACTERIOLOGIC AND HISTOPATHOLOGIC DATA ON SWINE FROM THE MSU SWINE HERD

<table>
<thead>
<tr>
<th>Table III</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Tested</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>No Pathologic Changes</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Group 3 Isolated</td>
<td>12</td>
<td>40.0</td>
</tr>
<tr>
<td>Granulomas Present</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Group 3 Present</td>
<td>30</td>
<td>68.0</td>
</tr>
<tr>
<td>Total—Group 3 Isolated</td>
<td>42</td>
<td>57.0</td>
</tr>
</tbody>
</table>
These observations on tuberculin testing and isolations of Group III mycobacteria from the university swine present an excellent demonstration of an extensive outbreak of swine tuberculosis due to acid-fast organisms other than *M. bovis* and *M. avium*.

### TABLE IV

**The Results of Tuberculin Testing of the MSU Swine Herd**

<table>
<thead>
<tr>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests</td>
<td>1,574</td>
</tr>
<tr>
<td>Reactors</td>
<td>227</td>
</tr>
<tr>
<td>Suspects</td>
<td>220</td>
</tr>
<tr>
<td>Total Reactions</td>
<td>447</td>
</tr>
<tr>
<td>Negative</td>
<td>1,127</td>
</tr>
</tbody>
</table>

### TABLE V

**The Results of Tuberculin Testing of Swine Used in the Endocrine Experiment**

<table>
<thead>
<tr>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests</td>
<td>115</td>
</tr>
<tr>
<td>Reactors</td>
<td>54</td>
</tr>
<tr>
<td>Suspects</td>
<td>14</td>
</tr>
<tr>
<td>Total Reactions</td>
<td>68</td>
</tr>
<tr>
<td>Negative</td>
<td>47</td>
</tr>
</tbody>
</table>

**DATE**

<table>
<thead>
<tr>
<th>Number</th>
<th>Reactors</th>
<th>Suspects</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>9–8–61</td>
<td>41</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>12–7–61</td>
<td>30</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>1–5–62</td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>1–11–62</td>
<td>30</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>8–29–62</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

We have no information on this herd that would show the disease was transmissible within the herd. The fact that in some groups, the number of infected pigs was exceedingly high, would indicate that the disease was readily transmissible or that the pigs were exposed to a common contaminant from feed, water or the environment. Thus we have no knowledge of the source of the organisms. During the period of infection in the swine herd, no continued attempt was made to send reactor animals to slaughter. Many were slaughtered but some were left in the herd. A good
sanitation program was instituted and continued. The disease disappeared as evidenced by a lack of tuberculin reactions in the herd in 1965 and 1966. Many explanations could be offered for the disappearance of the disease but none could be documented.

There is, however, one interesting fact in regard to the Group III mycobacteria infecting swine. They did not cause infection in cattle. Organisms were injected intradermally into calves and no infection was detected.

During the outbreak in the university herd, animals in a swine evaluation program, were tuberculin tested. Swine breeders submit three swine for ratings. These swine are never in contact with the university herd. A total of 159 tests were made over a period of 12 months at 13 different times. Eight reactors (5%) and 12 suspects (7.5%) for a total of reacting animals of 12.5% were found (Table 6).

These data on tuberculin testing indicate that the herds from which these swine were obtained were infected. These data do not necessarily mean that the infections were due to Group III mycobacteria.

<table>
<thead>
<tr>
<th>Table VI</th>
<th>The Results of Tuberculin Testing of the Swine in the Swine Evaluation Program</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests....</td>
<td>159</td>
</tr>
<tr>
<td>Reactors.</td>
<td>8 5.0</td>
</tr>
<tr>
<td>Suspects.</td>
<td>12 7.5</td>
</tr>
<tr>
<td>Total Reactions...</td>
<td>20 12.5</td>
</tr>
<tr>
<td>Negative...</td>
<td>138 86.0</td>
</tr>
</tbody>
</table>

In another completely isolated area a small herd of swine consisting mostly of SPF stock were under study. Four sets of tests were made over a period of 8 months. The total number of tests were 58 with 7 reactors (12.06%) and 4 suspects (6.89%) (Fig. 7). Again the causative organism was not determined.

Reports of the university outbreak were reported by Beck, et al.¹ and Mallmann.²

A comparative infectivity study in swine was made of M. bovis, M. avium and the Group III mycobacteria isolated from the university herd.³

In brief, each animal received 2 mg (wet weight) or organisms either orally or intradermally. Thirty-nine crossbred pigs, 8 to 12 weeks old were used. Twenty-one litter mates were housed in contact with the treated pigs. Fifteen litter mates were also housed separately as a control. The pigs were killed at various times and their tissues were examined bacteriologically and histopathologically.
These studies can be summarized as follows:

1. Lesions were observed in tissues from which acid-fast organisms were isolated following administration of Group III mycobacteria of swine origin.

2. Group III mycobacteria of swine origin produced more extensive disease than M. avium and less extensive than M. bovis.

3. Lesions caused by M. bovis, M. avium and Group III of swine origin could not be differentiated pathologically.

4. No disease was produced following the intradermal injection of Group III mycobacteria isolated from floor scrapings of swine pens.

5. There were no acid-fast bacilli isolated from the uninoculated pigs.

6. Transmission occurred after Group III mycobacteria of swine origin were administered per orum.

7. Transmission occurred after M. avium or M. bovis was administered per orum and after M. avium was administered intradermally.

8. In comparative tuberculin tests, avian tuberculin induced greater

**TABLE VII**

**BACTERIOLOGIC AND HISTOPATHOLOGIC DATA OF LESIONS OBTAINED FROM SWINE AT A PACKING PLANT**

<table>
<thead>
<tr>
<th>Number</th>
<th>Number Isolated</th>
<th>Group III</th>
<th>M. avium or III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michigan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Gran.</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>None.</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Ohio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Gran.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Illinois</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Gran.</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Missouri</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Gran.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>None.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gran.</td>
<td>3</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>None.</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>15</td>
<td>4</td>
</tr>
</tbody>
</table>
reactions than mammalian tuberculin in swine injected with *M. avium* and Group III of swine origin. There were a number of exceptions in the first tests made two months after inoculation.

9. Lesions were more marked in the swine killed in the early stages of the experiment. Few lesions were detected in swine killed at the end of the experiment.

To determine whether or not the Group III infection that occurred in the university swine herd was an unusual experience, lesions from swine collected at a Detroit packing plant were examined. These results are presented in Table 7. Thirty-six swine were tested from Michigan, Ohio, Illinois and Missouri. Group III mycobacteria were isolated from 17 animals, *M. avium* from four animals and 15 were reported as either *M. avium* or Group III because they were not tested by chicken inoculation.

These data represent a small number of swine but over half of the swine were infected with Group III and of the cultures tested by chicken inoculation 17 out of 21 were infected with Group III mycobacteria.

In Table 8 are presented recent data on isolations of acid-fast mycobacteria from lesions of swine obtained from a Detroit packing plant.

*TABLE VIII*

**Isolation of Mycobacteria from Lesions of Swine from a Packing Plant**

<table>
<thead>
<tr>
<th>Number</th>
<th>Origin</th>
<th>Type of Granuloma</th>
<th>Culture Isolated</th>
<th>Non-Progressive</th>
<th>Progressive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unknown</td>
<td>M. avium</td>
<td>Not tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Michigan</td>
<td>III</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Ohio</td>
<td>III</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Michigan</td>
<td>M. avium</td>
<td>Few</td>
<td>Many</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Unknown</td>
<td>Pseudochrome</td>
<td>Not tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Unknown</td>
<td>III</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Michigan</td>
<td>M. avium</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Unknown</td>
<td>III</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Unknown</td>
<td>III</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Ohio</td>
<td>Pseudochrome</td>
<td>Not tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Ohio</td>
<td>III</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Missouri</td>
<td>III</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Unknown</td>
<td>III</td>
<td>+</td>
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<td></td>
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<td>14</td>
<td>Unknown</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Indiana</td>
<td>III</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Illinois</td>
<td>III</td>
<td>Not tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Ohio</td>
<td>III</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of swine with Group III infection—11.
Number of swine with *M. avium* infection—3.
Group III mycobacteria were isolated from 11 swine and *M. avium* from three swine. Group III mycobacteria when injected into chickens did not produce gross or microscopic lesions and generally no acid-fast bacilli were isolated. A relatively large number, equal to the total of isolations reported in Table 8 produce microscopic lesions in chickens but no gross lesions. These are presently classified as atypical *M. avium*.

These data together with those of the university swine herd and the experimentally induced disease in swine indicate that Group III infections are not uncommon and likely are more frequent than *M. avium* infections.

There may be three types of mycobacterial infections in swine namely: *M. avium*, Group III mycobacteria and a possible third group that are intermediate between *M. avium* and Group III mycobacteria.

As previously stated, in general, the reaction to avian tuberculin was greater than that to mammalian tuberculin. Reactions of mammalian and avian tuberculins to Group III infections in swine are comparable to those obtained from infections due to *M. bovis* and *M. avium*.

The diagnostic value of the mammalian and avian tuberculins may not be limited to the diagnosis of *M. bovis* and *M. avium* infections and any evaluations must take into consideration the Group III mycobacteria. It must also be remembered that the size of the reactions and the relationship of size of reaction induced by mammalian and avian tuberculins are largely dependent upon whether or not the disease in each animal is in progressive or non-progressive stage.

Unfortunately, little is known about the source of Group III mycobacteria infections in swine as well as in cattle and man. In 1957, Baumann and associates isolated an organism from swine that differed from *M. avium*. They named this organism *M. suis*. It is quite possible that they were correct. The Group III infection may be a distinct disease of swine, not transmissible to cattle and chickens. Whether it is or not, we know nothing about the epidemiology of the disease, the source of the organisms, and other species of animals and birds that may carry the disease. Further we know nothing about these organisms that produce microscopic lesions, but no gross lesions in chickens but gross lesions in swine. Are these *M. avium* conveyed to swine from chickens that have lost their virulence for chickens by passage through swine or are they Group III mycobacteria with some degree of virulence for chickens? Are we dealing with three types of infection, namely: *M. avium*, Group III mycobacteria and an atypical *M. avium* infection?

We can speculate and theorize but to know we must have facts. Swine tuberculosis at present is an enigma.

**ACKNOWLEDGMENT**

The authors wish to acknowledge the help of Dr. Ronald Scott, Animal Health Division, USDA and Dr. James A. Ray, Department of Microbiology, Tuberculosis Project.

These studies were made possible through a co-operative agreement with Animal Health Division, Agricultural Research Service, USDA.
REFERENCES


TUBERCULOSIS IN A BULL STUD

A. R. McLAUGHLIN, D.V.M.*

A report on the discovery of bovine-type tuberculosis in a large bull stud. Although the source of the infection has not been determined, details of the outbreak and factors contributing to the spread of the infection are discussed. Protective measures which have been instituted to prevent the reintroduction and spread of the infection are cited.

Somewhere in these United States is a tuberculosis infected herd! We don't know where it is. We think it is probably a purebred dairy herd! We also think that a bull was exposed to bovine tuberculosis in this herd where it became infected, and eventually was the source of the disease in a large bull stud. The stud had established what it thought was a rigid animal health program, but Mycobacterium bovis bridged the barriers! The result was near disaster!

The bull stud purchases approximately 25 mature and 100 young bulls each year. A negative tuberculin test at the herd of origin is required, not only for the bulls, but also for all mount animals. On arrival at the stud, the cattle are held under quarantine for approximately 30 days. At the end of the quarantine period, another tuberculin test is conducted. Animals then enter one of the 4 units on the premises, which are the dairy unit, the beef unit, the rearing unit (dairy and beef bulls, 1½ to 5 years of age), and the adolescent unit (bulls 6 to 18 months old). Although mount animals are assigned to either the dairy or beef unit, there is also a barn to keep them in after they have lost their appeal to a particular group of bulls. A hospital barn and two other barns which could be used as isolation areas, completed the facilities.

Twice a year all cattle on the premises are tuberculin tested by veterinarians employed by the stud. It is their responsibility to maintain the rigid animal health standards of the organization.

In November 1965, the bull stud tested 299 cattle. The test revealed one suspect, a 12 year old Holstein bull in the dairy unit, with a response of less than a twofold increase in the caudal fold. In the past 5 years there had been 5 other suspects. They had been shipped to slaughter or retested by state-federal veterinarians and in all of these cases either post mortem examination or retest results were negative. This case was different! This 12 year old Holstein bull had bovine tuberculosis!

A comparative cervical test was immediately conducted and the results were suspicious. There was a response to mammalian tuberculin within 24 hours, but by 72 hours it had practically disappeared. The bull was moved to one of the isolation barns and held for a retest. Two months later, another comparative cervical test disclosed no reaction to avian tuberculin.

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TUBERCULOSIS IN A BULL STUD

Tuberculin and approximately 70x130 mm. induration to mammalian tuberculin. The animal was shipped to slaughter. Extensive granulomatous lesions were found in the suprathyroid and bronchial lymph nodes. Laboratory findings were compatible with a diagnosis of tuberculosis. Arrangements were made to test the balance of the herd, starting with the dairy bull barn.

This building is an ultramodern, air-conditioned structure, 280 feet long and 72 feet wide. Approximately one-third of the floor space is a semen collecting area with the remainder housing 63 bulls and 5 mount animals. There are 13 box stalls along both sides of the building. Two opposing rows of 19 tie stalls each, separated by a raised feed alley, are down the middle. Each bull has an individual watering cup and manger. Manure disposal is into a tank located beneath the floor. Heating, cooling, and ventilation are accomplished by forced air, with 75 percent recirculation when the outside temperature is above 50° F. Air outlets are in two rows along the length of the ceiling. There are only two air returns located in the wall which divided the building. Mount animals are kept away from the bulls in a separate area.

Cattle were tested on the left side of the neck with 0.1 ml. each of mammalian and avian tuberculins. Disposable 1.0 ml. plastic tuberculin syringes equipped with 26 gauge x 3/8" needles, were used. Injection sites were in the middle third of the neck where the hair was removed with an electric clipper. Avian tuberculin was injected 5 inches below the nuchal crest and the mammalian tuberculin was injected approximately 5 inches below the avian injection site. Prior to injection, the normal skin thickness was measured with a modified Hauptner Dermal Thickness Gauge. Tests were read at 24, 48, and 72 hours. Both increased skin thickness and induration were determined—the former with the Hauptner Gauge; the latter with a millimeter ruler. All animals in which the response to mammalian tuberculin exceeded that of the avian, were removed. These were 12 Holstein bulls; 2 Jersey bulls, and 3 mount animals. Prior to slaughter, semen was collected from 9 of the animals that showed the greatest reaction and forwarded to the National Animal Disease Laboratory for examination including guinea pig inoculation. The results were negative.

The animals were slaughtered at an establishment operating under supervision of the Livestock Slaughter Inspection Division, C&MS, USDA. Lesions of tuberculosis were found in four of the Holstein bulls and both of the Jersey bulls. The mount animals had no visible lesions of tuberculosis. The carcass of one of the Jersey bulls was condemned because of generalized disease. Selected lymph nodes and lesion material from 4 of the animals were forwarded to the National Animal Disease Laboratory for examination and culture. \textit{M. bovis} was isolated in all cases.

The complete urino-genital tracts of the 9 animals from which semen had been collected, were removed and examined at the Central Animal Health Laboratory, Wisconsin Department of Agriculture. No significant findings were reported.

The following week, animals in the beef unit, the hospital, and the mount barn were tested. There were 3 reactors at the beef barn: 2 bulls and
mount; none at the hospital, and 8 at the mount barn. On post mortem examination, lesions of tuberculosis were found in 3 of the mounts—the one which had been at the beef unit, and 2 which were in the mount barn. All had recently been used at the dairy unit.

The adolescent and rearing units were tested, using 0.2 ml. mammalian tuberculin. There were 4 more reactors, 2 in each barn. None had visible lesions.

The dairy unit was apparently the main focus of infection! All units were thoroughly cleaned and disinfected after removal of reactor animals with an officially permitted disinfectant.

A quarantine had already been issued which restricted the movement of animals from the premises. The question arose as to the handling of the semen collected from the bulls. The organization ships frozen semen interstate and internationally. Three states and several foreign countries have requirements governing the health status of bulls whose semen is offered for import. Animal Health officials in the states involved were notified directly. The foreign countries were notified through the Animal Health Division, USDA, Hyattsville, Maryland. All semen in field refrigerators and in storage on the premises collected from the bulls found with lesions of tuberculosis was thawed and discarded. Semen collected and processed after January 1, 1966, from other reactor bulls had not left the premises and was also discarded. Approximately 150,000 ampules of semen from 16 bulls were taken to a municipal dump where they were destroyed under the supervision of veterinarians from the Animal Health Division, USDA.

A retest of the dairy bulls was conducted 45 days after the initial test. The dose was 0.2 ml. mammalian tuberculin in the right cervical area. Skin thickness was measured before injection and 48, 72, 96, and 144 hours after injection. Induration was measured at 24, 48, 72, 96, and 144 hours. Those animals with at least 2.0 mm. increased skin thickness at 48 or 72 hours and persistent induration at the 96th hour were removed for slaughter. At the 24th hour, Arthus responses to tuberculo-protein were observed in 17 of the 51 animals tested. However, these responses diminished and only 4 Holstein bulls were classified as reactors. No visible lesions were reported on post mortem examination. Of the 4 reactors, 3 had close contact with 2 of the bulls that disclosed lung lesions after the first test. Pooled lymph node samples from each of these 4 animals were submitted to NADL. M. bovis was isolated from one.

There was a total of 6 isolations of M. bovis from the herd: the original suspect, the 4 dairy bulls with lesions, and the no-lesion reactor. The remainder of the stud was retested and found negative on April 1, 1966. A single cervical injection was used.

Because it is possible that an animal with open lung lesions could have been present and not have reacted to the tuberculin test, it was decided to secure bronchial swabs from the remaining dairy bulls. An instrument was devised by one of the veterinarians at the stud, consisting of speedometer cable with a crank on one end and a cotton swab on the other, protected by a 4½ foot length of 3/8" neoprene tubing. The swab end was sealed with a piece of paper toweling secured with masking tape to prevent
contamination during passage into the trachea. The devices were autoclaved and transported in polyethylene bags.

Animals were restrained in steel stocks. A lead rope was snapped into the nose ring and passed through another ring mounted on the wall directly in front of the stocks. Tension on the rope positioned the bull's head for passing the tube into the nostril. With the aid of a Frick speculum, the operator manipulated the device through the larynx and down the lumen of the trachea. The swab was then pushed through its covering of protective paper, the crank was turned several times, and the swab was returned into the tube. The apparatus was withdrawn, the swab protected with a sterile plastic bag, and taken to a bacteriologist. Artificial media were inoculated on the premises and transported to the National Animal Disease Laboratory for incubation and animal inoculations. Unfortunately, results were inconclusive because of heavy overgrowth of *Proteus spp.* and other contaminants. The source of contamination was thought to be mucus from the upper respiratory tract which was pushed in front of the tube during its passage down the trachea. When the protective seal was broken, the swab became contaminated. Further refinement of the technique to eliminate contamination would offer a means of locating animals with "open" tuberculosis which may have been missed by the tuberculin test.

In late May, the entire herd was retested, using 0.1 ml. tuberculin in the caudal fold revealing one reactor, a mount animal in the dairy unit. No lesions of tuberculosis were found on post mortem examination and no acid-fast bacteria were isolated from tissues submitted for laboratory examination. After this test, the quarantine was released from all units except the dairy bull barn.

After negative retests in July and November 1966, the quarantine of the dairy bull barn was also released. During the outbreak, each unit in the stud was tuberculin tested at least three times. Five tests were conducted of the dairy bulls. In all, 1382 tuberculin tests were made. There were 33 reactors: 22 at the dairy unit; 3 at the beef barn; 8 at the mount barn, and 2 each at the adolescent and rearing units. In addition, 25 mount animals were slaughtered because of exposure to tuberculosis. In accordance with established policy, the entire stud will be tested at least once each year, for 5 successive years by a full-time regulatory veterinarian.

During the entire period, no new bulls were added to the exposed dairy bulls. In February 1967, the 30 remaining animals were transferred to another premise 10 miles distant, which was also owned by the organization. This group of animals that was exposed to bovine tuberculosis will be maintained as a separate unit in semi-isolation until the last individual is shipped for slaughter. As of today, only 24 remain. All 23 animals, slaughtered since the reactors were removed, have received a thorough post mortem examination. No further tuberculosis has been found!

The dairy unit has been thoroughly cleaned, disinfected, and restocked. As soon as the infection was discovered, investigations were initiated to determine its source. Information was sent to animal health officials in the states of origin of all reactors. In most cases, investigations were conducted immediately and all were reported negative.
Because of possible mechanical introduction, all herds owned by personnel working on the premises were tested. These investigations revealed nothing.

Considering a possible human source, 32 employees who could have had even casual contact with the bulls were tested. One individual with a known arrested case of human-type tuberculosis reacted to PPD-S. He had never worked with the dairy bulls!

In past years, cull animals had frequently been slaughtered at a plant which had only minimal post mortem inspection or had been sold without identification through a public stockyard. On the theory that tuberculosis may have been missed on postmortem examination or found and not traced to the stud, the origin of all animals culled in the past 4 years was investigated. Results were again negative. The source of infection has still not been identified!

We are reasonably certain, however, of the means of transmission within the stud. Inhalation may have played a significant role. The design of the air-conditioning system was such that there was almost continuous recirculation of air. Furthermore, there were only 2 air returns in the entire bull barn. They were located in the wall which divided the building. Although there was air filtration, it was not designed to remove particles in the size range of tubercle bacilli.

Most important in the spread of the disease, however, were certain practices used in semen collecting and handling of the bulls. Barn employees wore cotton work gloves while handling the bulls by their rings. The gloves which became saturated with nasal discharges and saliva were not changed between bulls and provided an excellent means of bull to bull transmission. Cotton lead ropes which snapped into the bulls' rings were used interchangeably with no disinfection between animals. Although mounts were disinfected in the perineal area to prevent dissemination of venereal infections, no consideration was given to the withers, which were frequently wet with oronasal discharges. Had the disease gone undetected for any greater period of time, the interchange of mounts between units would certainly have played an important role in spread of the disease to other units from the dairy barn.

The ventilating system has been modified. Exhaust fans have been installed in the roof of the building. During the past summer, there was recirculation of air for only 8 hours. The cotton gloves have been replaced by heavy rubber gloves, which are disinfected between animals. An individual nylon lead rope, which is disinfected after each use, has been provided for each animal. Mount animals are washed with disinfectant from the neck to the rump after each service. There is no longer any interchange of mounts between units.

To further reduce possible human factors in mechanical transmission of disease, protective clothing has been provided for employees to wear only while on duty. Outer garments must be changed when the employees move between units. Rubber or disposable plastic footwear is also provided for employees and visitors at each unit.

To prevent reintroduction of tuberculosis, more rigid animal health
requirements have been imposed. A negative tuberculin test is now required, not only for the individual animal, but also the entire herd of origin. Animal Health officials in states of origin are contacted to determine herd histories. Other herds with which the bulls may have had contact, are also investigated. At the admittance area, all animals are now quarantined for 60 days. The comparative cervical tuberculin test is conducted on arrival and again before release into the stud. Some animals have been rejected because of significant response to mammalian tuberculin, in spite of a negative caudal test at the herd of origin. The stud has established these new animal health standards to protect its investment and its reputation.

The excellent cooperation of the following is gratefully acknowledged: Dr. A. A. Erdmann and Dr. A. I. Moyle, State-Federal Cooperative Program, Madison, Wisconsin; Diagnostic Services (NADL), Animal Health Division, ARS, USDA, Ames, Iowa.
COMMENTS ON THE EPISODE OF BOVINE TUBERCULOSIS IN A POPULATION OF BULLS USED FOR ARTIFICIAL INSEMINATION

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The technical details of a significant episode of bovine tuberculosis have been presented by another observer. How could this have happened? Where did it come from? Why did it spread so rapidly? What did it cost? . . . are all reasonable questions and quite in order. Concomitantly . . . What implications do I see in this experience? . . . is a reasonable question for me to answer.

How Could This Episode of Tuberculosis Have Occurred?

Herd health management was in strict compliance with all regulations and exceeded usual recommendations. Veterinarians held full responsibility and all necessary authorities. But—after 25 years under a rigid system of routine tuberculin testing, the barrier system failed to function and protect the herd the first time *Mycobacterium bovis* is known to have challenged the system.

It is quite reasonable to question if all test results were valid. Did the veterinarians conducting the tests perform precisely and accurately at the time of injection and were the diagnostic readings made in a thoroughly conscientious manner? Were any chances taken? Was there any cover up?

I submit that there has been no evidence of such nor was there ever any reason for such. Especially, the organization's veterinarians who live with all problems of the herd and the consequences thereof, would have had, personally, everything to lose and nothing to gain by dereliction of their duties.

Where Did the Infection Come From?

This question is as yet unanswered.

There were no notable exceptions to the entry procedures established for the cattle.

All employees were examined by appropriate medical procedures and found free from tuberculosis.

The possibility of an infectious visitor being responsible has been considered. The possibility of sabotage has been considered. These routes of infection seem quite improbable. No supporting evidence exists. Further, transmission of tuberculosis at a single exposure seems quite improbable.

Tracebacks into herds of origin of involved cattle were initiated promptly. Traceback data were quickly at hand from all such herd, with a single exception—, the herd of origin of the principal “suspect.” This bull
at slaughter had been found to have the most advanced tuberculous lesions. The carcass was condemned and tanked because of extensive bronchial and mediastinal node lesions and advanced involvement of the lungs. This bull had been tuberculin tested and found negative at herd of origin and six times thereafter by three different veterinarians.

The bureaucratic obfuscation and frustration of the effort to trace back the only existing logical lead to source was seriously harmful to effective conduct of the investigation and to the rational and decisive handling of this very serious problem. Local animal health officials and veterinarians responsible for the herd were left “cliff hanging” for 15 months before learning that the herd of origin of the animal most important to the investigation was negative.

Why Did Tuberculosis Spread so Rapidly?

The unusual design of the Dairy Unit has been suggested as contributory to the rapid spread of the disease or even as the “cause” of the disease. The Dairy Unit is fully enclosed and air conditioned. Its air circulating and air conditioning systems were poorly and inadequately designed for their intended purposes. Too little air was being moved and much of this was being recirculated in an undesirable pattern. Humidity was high.

Nevertheless, it is believed far more probable that the very nature of routine handling of bulls under AI conditions provided overwhelmingly the mechanism for transmission. Bulls were continuously handled by their nose rings with bare hands or cloth gloves and with ropes and snaps. “Teasing” at collection time commonly involved licking and nosing of mounts by successive bulls.

The environment and practices of a typical AI collection room are little different than if one designed a method to assure transmission of bovine tuberculosis.

What Did the Experience Cost?

Total of the purchase prices of the 20 bulls lost exceeded $200,000. Much of their value, of course, had already been depreciated.

Ampules of frozen semen destroyed totalled 146,816. Value at sale of these ampules through usual channels would have approached $300,000.

Loss of 35 mount animals, effects upon the routine of operations, effects upon employees involved, and extraordinary costs of handling a problem of such magnitude simply can’t be calculated.

The staff of veterinarians and the executives of the affected organization have the highest of praise for the extraordinary and effective manner in which Animal Health personnel functioned in this trying emergency. Thanks are due many—but especially Drs. A. R. McLaughlin, A. I. Moyle, and A. A. Erdmann. State-Federal Cooperative Animal Disease Eradication Programs, Madison, Wisconsin, Dr. A. F. Ranney, Chief Staff Veterinarian, Tuberculosis Eradication, Animal Health Division, and Drs. E. P. Pope, and C. D. Van Houweling, National Animal Disease Laboratory.
What Implications Do I See in This Experience?

A. I am of the opinion that the scheme of moving individual cattle on a standard interstate health certificate which requires only a negative tuberculin test result for the individual is a great tradition and a fine scheme. It is highly efficacious—if not perfect—but, only in the absence of tuberculosis.

When occult tuberculosis is present in the herd of origin, a negative tuberculin test of an individual bovine as reported on a health certificate has decidedly limited technical value. Confidence is misplaced. The recipient herd is in dire jeopardy!

Recently, as possible, it has been the stated policy of USDA to slaughter the entire herd when presence of bovine tuberculosis in the herd is established. It has been reported to this Association by USDA veterinarians that in their detailed examinations at slaughter of individual cattle comprising tuberculosis infected herds, that usual caudal tuberculin tests had not always designated those individuals in which lesions of tuberculosis were found post mortem. For example, in one herd submitted to slaughter, of five animals found with lesions, two were non-reactors.¹

Three other herds in which tuberculosis was known to exist revealed similar findings when the entire herds were slaughtered. Eighteen cattle were found to have tuberculous lesions post mortem. They had been recent non-reactors ante mortem.²

B. Encountering bovine tuberculosis three more times since the principal experience related herein is convincing evidence of the present importance of bovine tuberculosis.

1) In the fall of 1966 a Canadian importation of more than 100 Charolais cattle from France was found on completion of elaborate diagnostic and quarantine procedures to include some tuberculous individuals. Two of the unaffected group were owned in part by the organization and destined for its premises.

2) In the spring of 1967, 25 Angus cattle were purchased at an auction market in Nebraska for use at that market for training of ranchers in AI techniques. The next day, advice was received from an animal health official that the cattle had originated from a known tuberculosis infected herd and were part of a group in which tuberculosis lesions had been demonstrated. They were not used for their intended purpose and were shipped to slaughter. Loss was small. Inconvenience was considerable.

3) In the spring of 1967, a tuberculosis reactor was disclosed on routine stud-entry procedures. On investigation, a “cloudy” background relating to two grossly infested herds located in Kansas and Minnesota was established.*

* Since presentation of this paper, there has been a fourth encounter. While a new Holstein bull was undergoing routine quarantine procedures in spring 1968, it was learned via unofficial sources that this Holstein had previously been in the same barn as a Charolais which had just responded to a tuberculin test. This Charolais was another of the group referred to in 1) above.
C. I must express concern over the plans for deemphasizing regular, routine tuberculin testing, especially in valuable herds. That the destruction of the entire herd upon diagnosis of tuberculosis is good epizootically is unquestioned. But, such may prove to be disastrously economically to owners of affected herds. The degree to which tuberculosis progresses into a herd is often a function of frequency of tuberculin testing. Herd owners should be warned that they must now take more initiative in protecting their property from tuberculosis.

The cattle industry of the USA, especially the dairy industry, seems committed in the future to much larger and more valuable herds. Dairy herds of 500–1000–2500 are already extant and there will surely be many more large herds in the future.

What of these herds when they become bovine tuberculosis infected? Is it reasonable to give bovine tuberculosis opportunity to flourish for as long as a year in a stud of bulls used for AI?

D. It is obvious that new and improved methods are essential for the more precise diagnosis of bovine tuberculosis. These must be used as indicated for the maintenance of a high level of security in individual herds by the veterinarians responsible for health of these herds.

Artificial Insemination and Bovine Tuberculosis

During 1966 in the USA artificial insemination with semen originating from the established commercial AI organizations was used as a method of breeding a USDA reported total of 7,933,723 heifers and cows. Of these, 7,286,580 were dairy cattle and 647,143 were beef cattle.

USDA reported that in 1966 47.9% of the nation's 15,201,000 dairy heifers and cows and 1.9% of the nation's beef heifers and cows located in an estimated 540,265 herds were inseminated with semen supplied by commercial AI organizations. There were 2,367 bulls in service in 35 studs. In 1966 some of these bulls produced semen which was used in more than 50,000 cows, a few bulls produced semen used in a hundred thousand cows or more. Additionally, many thousands of cattle were inseminated with semen collected and processed by itinerant "custom freezers" from on-the-farm and on-the-ranch herd bulls.

Bovine semen is a live animal tissue preserved by certain chemical additives and by lowering of temperature—, usually to very low temperatures such as the −320° F. of liquid nitrogen. Semen may be kept in the frozen state for decades. Most biological entities—bacteria, protozoa, viruses—are almost as well preserved in the frozen state by the methods employed as are bovine sperm cells.

It has long been known—though not commonly remembered—that lesions of bovine tuberculosis occasionally are found to affect the genital organs of cattle. Williams in his Diseases of the Genital Organs of Animals (2nd Ed. 1939) discussed the subject at considerable length. He stated;
“Writers have accorded genital tuberculosis only a minor place. Its occurrence was admitted, but directly or inferentially it was regarded as rare.”

The route of Mycobacterium bovis to the affected site may be either hematogenous, producing secondary genital lesions, or from contact with the opposite sex at coitus, producing primary genital lesions.

Williams cited several instances of bulls with primary tuberculous lesions involving the penis and prepuce, and of bulls with tuberculosis orchitis. He cited the report of Richter (1932) of transmission of tuberculosis by a bull with orchitis to numerous cows which developed tuberculosis metritis and extensive peritoneal adhesions.

Williams pointed out an unusual frequency of failure of the tuberculin test to disclose cattle with genital lesions.

Venereal transmission of bovine tuberculosis to about 50 cows within a herd from service of a bull with tuberculous orchitis was reported from Denmark in 1935 by Plum. Transmission of tuberculosis to females via contaminated instruments has long been recognized.

For the past 30 years the AI industry and animal health officials have assumed postures toward the relationship of bovine tuberculosis to artificial insemination which might be described as cavalier in the first instance and one of tacit acknowledgment in the second instance.

A Code of Minimum Health Standards for Bulls Used in AI was recommended to the National Association of Animal Breeders in 1955 by an AVMA Subcommittee on Animal Reproduction and Artificial Insemination. This Code included a recommendation that bulls destined for use in AI be tested for tuberculosis before entrance into AI and annually thereafter. In 1962 a Subcommittee on Artificial Insemination of the USLSA Committee on Diseases of Cattle developed and recommended a model regulation for the States relative to AI which included testing of bulls for tuberculosis prior to their entering use in AI and annually thereafter. Three of fifty states, Virginia, Montana, and Washington, in order of their actions, regulate movement and use of bovine semen. These state regulations were all instituted prior to the creation of the model USLSA regulation in 1962.

Recommendations have probably had the effect of encouraging tuberculin testing of many—if not most bulls used as sources of semen in AI—especially within the recognized AI industry. The same can not be said of bulls whose semen is collected and processed on farms and ranchers by itinerant “custom freezers.”

In 1966, as already discussed, it was evinced that bovine tuberculosis can appear and spread rapidly in a well managed stud of bulls used for artificial insemination. Only the fact that these bulls were under a program of routine, twice yearly testing disclosed the existence and spread of the infection in an early phase, permitting prompt termination of progress of the disease in the affected herd. In consideration of the rapid spread underway, the number of affected bulls and the fact that the disease could have had six more months of uncontrolled and unrecognized progress had the herd not been on an unusual (and unpopular) twice yearly schedule of tuberculin testing, it would have been completely possible for genital lesions
to have developed in these bulls and for widespread semen borne transmis-

sion of tuberculosis to have occurred.

1966 was a remarkable year for two additional reasons.

Goyings (1966) in Michigan demonstrated the transmissability of atypical Mycobacterium to heifers by the route of intrauterine inoculation.

From France, Roumy (1966) has reported in considerable detail an enzootic of bovine tuberculosis transmitted by artificial insemination. A bull in service from 1957 was found on slaughter in 1963 to have generalized tuberculosis, including a pelvic lesion involving the rectum and internal urethra. Soon thereafter, cows were found infected with tuberculosis in previously uninfected herds. Semen from this bull had been used to inseminate more than a thousand cows on several hundred farms. More than a hundred of the cows inseminated with semen from this bull were found on slaughter to have tuberculous lesions of the uterus, peritoneum, and/or regional nodes.

During 1963 this bull had a 60-90 day non-return rate of 57% on 262 inseminations as compared with a stud average of 71%. Of 66 females inseminated in February 1963, 35 were later identified as having genital tuberculosis. On one farm there was extension of the disease to a herd of pigs.

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IMPROVED HISTOPATHOLOGIC TECHNIQUES FOR EXAMINATION OF GRANULOMATOUS LESIONS FROM CATTLE


Introduction

Specimens from cattle with granulomatous lesions suspected of being tuberculous are frequently submitted in formalin for histopathologic examination and in chilled chloramine T for bacteriologic culturing. Histopathologic findings when new fuchsin (NF), auramine O (AO), and hematoxylin-eosin (HE) staining were used, were correlated with the results of mycobacterial culturing on 711 specimens received between July 1, 1966, and December 31, 1966.

Experimental Design

Histopathology case reports were divided into four groups with regard to tuberculosis. I. Mycobacteria and lesions characteristically produced by these organisms were found using HE and NF staining. II. Lesions indicative of tuberculosis were found in the HE stained tissue sections, mycobacteria were not found in the section stained with NF, but they were disclosed in the tissue sections stained with AO. III. Lesions indicative of tuberculosis were found with HE staining, but mycobacteria were not disclosed using NF and AO staining. IV. Tuberculosis lesions were not found in tissue sections stained with HE and mycobacteria were not found in NF stained sections.

All cases in each group were compiled and if mycobacteria were isolated, the type was listed as *M. bovis, M. avium*, and unclassified mycobacteria. The results of mycobacterial culturing were compared with histopathologic findings in each group.

Materials and Methods

Formalin fixed specimens were processed by conventional methods for histopathologic examination. From each case, sections were stained with HE for cellular details, and NF for staining mycobacteria. Additional sections were stained with auramine O when tuberculous lesions were noted, but mycobacteria were not found in the NF stained sections.

Fresh specimens were cultured for mycobacteria and isolates were typed according to procedures established in the Bacteriology Section, Diagnostic Services.*

*From the Diagnostic Services, National Animal Disease Laboratory, Ames, Iowa. Dr. McDaniel is Chief of Diagnostic Pathology; Dr. Muhm is Leader of Granulomatous and Neoplastic Pathology; Dr. Howell is a Veterinary Pathologist in Granulomatous and Neoplastic Pathology; Dr. Ellis is Chief of Diagnostic Bacteriology.
Results

The results are summarized in Table I.

Group I

When tissue sections stained with HE and NF were examined, both mycobacteria and lesions compatible with tuberculosis were found in 159 cases. Mycobacteria were isolated from 111 or 70 per cent of the cases; 79 were *M. bovis*, 29 were *M. avium*, and 3 were unclassified mycobacteria.

Group II

Tissue sections from 44 cases in this group contained lesions indicative of tuberculosis, but mycobacteria were not found in NF stained sections. However, mycobacteria were found in additional tissue sections stained with

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<td><strong>CATTLE CASES SUBMITTED FOR TUBERCULOSIS EXAMINATION</strong></td>
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*Hematoxylin-eosin.
†New fuchsin.
‡Auramine O.

Auramine O staining increased the number of cases in which mycobacteria were found from 159 to 203 (28 per cent).

Group III

Tissue sections from 87 cases contained lesions suggestive of tuberculosis, but no mycobacteria were found in NF or auramine O stained sections. Mycobacteria were isolated from 25 or 29 percent of the cases; 17 were *M. bovis*, 6 were *M. avium*, and 2 were unclassified mycobacteria.

Group IV

Neither tuberculous lesions nor mycobacteria were found in 421 cases.
However, mycobacteria were isolated from 38 or 9 per cent of the cases; 14 were *M. bovis*, 14 were *M. avium*, and 10 were unclassified mycobacteria.

**Discussion**

Auramine O staining was found to be a valuable and reliable addition to conventional tissue stains used for histopathologic examination of cattle tissues suspected of being tuberculous. Use of this stain increased the rate of detecting mycobacteria in granulomatous lesions 28 per cent. The probability of isolating mycobacteria from cases in which mycobacteria were seen with auramine O staining was the same as when the organisms were seen with NF staining. Mycobacteria were isolated from 70 per cent of the cases in both groups.

Some reasons for discrepancies between histopathologic and bacteriologic results are as follows: (1) Specimens may be so small they cannot be divided so both specimens always contain lesions and culturable mycobacteria. (2) Mycobacteria may not be culturable when they reach the laboratory. (3) Mycobacteria can be isolated from tissue in which no gross lesions are seen. Ten of the 14 animals in group IV from which *M. bovis* was isolated were reactors, suspects, or slaughtered during herd depopulations due to *M. bovis* infection.

**Summary**

Auramine O staining is reliable for detecting mycobacteria in tuberculous lesions from cattle. Use of this stain increased the rate of detecting mycobacteria 28 per cent.

Mycobacteria were isolated from 70 per cent of tuberculosis suspicious cases in which the organisms and lesions were detected histopathologically, 25 per cent of those cases in which only granulomatous lesions were detected, and 9 per cent of those in which neither granulomatous lesions nor mycobacteria were detected.

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A. F. Ranney* D.V.M., M.S.

Presented at the Seventy-first Annual Meeting
United States Livestock Sanitary Association
Phoenix, Arizona
October 19, 1967

Tuberculosis was responsible for the loss of hundreds of thousands of dollars to one bull stud. How long can we continue to procrastinate with this disease? As soon as this outbreak occurred, the breeding organization took immediate action and went the extra mile to protect the livestock industry of which it is a part. Unfortunately, this organization has encountered the disease four distinctly different times in the past two years. These may be summarized as follows:

1. In the first encounter mentioned, the source of the infection is still undetermined. The loss included 20 valuable breeding bulls and 35 mount animals which were difficult to replace. Almost 150,000 ampules of frozen semen were destroyed.

2. The organization was negotiating for the purchase of a breeding bull that proved to be a reactor when tested. Loss to the owners was $20,000. Two states were involved in this investigation. Bovine tuberculosis was recently reported in the herd where this breeding bull was born.

3. In another state, 25 beef-type cattle were purchased at an auction market to be used at an artificial insemination training school. The day after the purchase it was learned that these 25 animals originated in a herd from which several cattle with lesions of tuberculosis at time of slaughter had also originated. The organization immediately sent the group of animals to slaughter.

4. Two bulls were purchased in a foreign country for use in the bull stud. These bulls were part of an international shipment of animals and were jeopardized by the presence of tuberculosis in the shipment.

Today, few people become concerned about bovine tuberculosis unless it strikes home—in their herd or in the herd of an acquaintance.

As a basis for future action, we should consider the past is prologue. We find that many of the points which are currently being discussed about tuberculosis have been repeated many times. Ten years ago, a committee of three top veterinary scientists, Drs. William H. Feldman, William A. Hagan, and Jacob Traum, who contributed many years of research and special studies on the subject of tuberculosis and the progress of the eradication program, made the following pertinent comments:

"Pernicious in effect was the complacency on the part of many who,

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*Chief Staff Veterinarian, Tuberculosis Eradication, Animal Health Division, Agricultural Research Service, United States Department of Agriculture.
wrongly believing that tuberculosis of cattle was a vanishing disease, thought that there was no reason to spend additional time and money in suppressing a disease that had all but been suppressed. Irrepressible optimism regarding the disappearance of bovine tuberculosis in America still prevails. This optimism probably was engendered, in part at least, by the celebration of so-called ‘Achievement Days’ in the various states when they were first designated as modified-accredited areas. These celebrations apparently instilled in the minds of many the belief that the goal had been achieved and the objectives reached when, of course, only a first-stage objective and not the final one had been reached.

* * * *

“We direct attention to the importance of satisfactory identification of all animals included in the tuberculosis eradication program. It is obvious that for the satisfactory planning, execution, and evaluation of the results of the program it is important to have clear and permanent identification of the animals. In problem herds, it is particularly important that the animals be positively identifiable and that accurate records be kept and reported of all tuberculin reactions as interpreted by the veterinarian, irrespective of the subsequent disposal of these animals.

* * * *

“We believe that the Department should carefully consider whether the continuation of modified-accredited areas is useful or wise. Unfortunately it appears that some officials have been more concerned with retention of the accredited status of their states than with final eradication of the disease.

* * * *

“We commend the progress which has been made in recent years in finding bovine tuberculosis infected herds by tracing slaughtered animals. If a practical plan of identifying all bovine animals could be evolved, this procedure would be much more effective; however, it has been yielding very useful results and every effort should be made to utilize it as fully as possible.”

There are some very important facts to report about the tuberculosis eradication program for the fiscal year 1967.

Among the cattle population of approximately 108.5 million, there were 6,012,215 cattle tested with tuberculin. This is the lowest number of cattle tested since 1924 when 5.3 million were tested. The infection rate in 1924 exceeded 3 percent and the total cattle population was just under 66 million. In 1924 the number of tests applied was equal to 8 percent of the total cattle population as compared to only 5.5 percent in 1967.

Reactors, among the cattle tested in fiscal year 1967, totalled only .06 percent. This is the lowest rate on record. At this point, we might ask: What is the future course that will lead to the eradication of bovine tuberculosis?
An analysis of the reduction in carcass condemnation rates on regular kill meat inspection, because of tuberculosis, indicates a disturbing trend in the last ten years.

Reduction in these rates for the past 30 years, from 1938 to 1967, indicates a ten-fold decrease every twelve years. When extrapolated, this suggests an eradication date of 1995. However, the reduction rate for the last ten years indicates that 20 years will be required to achieve the same ten-fold decrease. When this is extrapolated, it suggests that eradication will be achieved not in 1995, but about 19 years later or in the year 2014. These projections are determined by calculating the regression of number of condemned animals per 10 million slaughtered on years and projecting the regression line to the point of eradication.

This is a clear indication that we need to review our program procedures as they are actually being carried out in the field. We need to determine what should be done and what can be done with available resources in order to continue the reduction rate decrease experienced over the past 30 year period, or what can be done to proceed more expeditiously.

Two facts stand out from an analysis of program data. The first is the apparent long-standing infection in a high percentage of the infected herds when they are first known to be infected. With modern husbandry and marketing practices this has two implications: (1) Animals are capable of disseminating a tremendous amount of infection to other herds before being detected, and (2) the tuberculous animals that are shipped to slaughter are not being used to full advantage as a means of locating infected herds. The second fact supports the latter implication: Data strongly suggests that about 60 percent of our tuberculous animals that are reported on regular kill meat inspection are not being traced to the source of infection.

The reason for this is quite clear from the data available. Few animals that are reported with lesions of tuberculosis on regular kill have adequate identification. Again, there are two reasons for this. First, many cattle are not identified adequately through the marketing process. Secondly, some slaughtering processes are such that it is impossible to maintain the identity of an animal until the meat inspection examination is completed.

It is mandatory to the efficient operation of the tuberculosis eradication program that these deficiencies be corrected.

Based on program data and varying degrees of intensity of program procedures, the four projected lines on the inverted semilog chart, figure 1, illustrate what may be expected in eradicating bovine tuberculosis from our domestic cattle.

This chart depicts data from 1946, which is six years after the country was recognized as a modified-accredited area. From 1938 until 1967, the average decline in carcasses condemned because of tuberculosis as reported by Federal meat inspection was at the rate of 16.75 percent yearly. However, since 1958 the rate has declined to approximately 10.74 percent annually.

Suppose that we continue at the 10.74 percent rate of decline experienced in the past ten years from 1958 to 1967. The graph shows (line 1)
that we could expect to reach our objective of eradication about the year 2014. This might be compared to the movements of an early model airplane.

If we proceed at the rate of decline experienced over the past 30 year period we could anticipate crossing the eradication line in 1995 (line 2). This step-up could be compared to the speed of a modern propeller airplane.

Suppose we step-up our program activities and intensify our procedures comparable to the speed of a jet plane as contrasted to the propeller plane (line 3). With this increased speed we could eliminate bovine tuberculosis in cattle by the end of 1982. However, this is still far from the ideal.

We are in the rocket age and should advance our program to keep up with the times. If we move at top speed and give immediate and detailed attention to all aspects of the program we could anticipate eradicating bovine-type tuberculosis from cattle nearer to the year 1970 (line 4). This will take immediate concentration on (1) liquidating by slaughter *M. bovis* herds as soon as discovered, (2) identifying at least 90 percent of the known tuberculous animals with the source of the disease to uncover infection that may have been spread from these animals, and (3) full compliance with the intent of the Uniform Methods and Rules.

When the rocket goes into orbit, the major cost of the program of tuberculosis eradication has been expended. Likewise, a surveillance pro-
gram to find and eradicate bovine tuberculosis from cattle in the next three years should put us in a position of maintaining from then on a surveillance program at relatively little cost. This surveillance program must be such as to detect promptly any new infection that may creep in.

The number of herds found with infection indicative of bovine-type tuberculosis continues to decline. This is the sixth successive year that detailed records have been maintained on a herd basis. It will be noted in figure 2 that 86 herds were found with infection indicative of bovine tuberculosis last year. This is a 22.5 percent decrease from the prior fiscal year of 1966. This may denote encouraging progress; however, we must ask ourselves whether the figures reflect actual field conditions.

The map, figure 3, shows the location of the 86 herds diagnosed in 1967. They were located in 23 states and Puerto Rico. The figure in each state indicates the number of M. bovis herds reported. It will be observed that these herds are scattered country-wide. This illustrates a very important point: It is impossible to predict where the next infected herd will be found. Thus it is essential that we develop and maintain a highly efficient national surveillance program.

For several years the percentage of infected herds found as a result of traceback as compared to routine testing has shown a marked increase: From 32 percent in 1963 to 56 percent in 1966.
**Tuberculosis Eradication**

**LOCATION OF INFECTED HERDS**
(Indicative of M. bovis)

23 States and P.R.: Total Herds-86

**FISCAL YEAR 1967**

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

Figure 3

**Tuberculosis Eradication**

PERCENT OF HERDS WITH LESIONS INDICATIVE OF M. bovis Found Through Routine/Traceback Testing

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Routine Testing</th>
<th>Traceback Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1963</td>
<td>68% (145 Herds)</td>
<td>32% (68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(213 Herds)</td>
</tr>
<tr>
<td>1964</td>
<td>64 (117)</td>
<td>36 (67)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(184)</td>
</tr>
<tr>
<td>1965</td>
<td>62 (87)</td>
<td>38 (54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(141)</td>
</tr>
<tr>
<td>1966</td>
<td>44 (49)</td>
<td>56 (62)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(111)</td>
</tr>
<tr>
<td>1967</td>
<td>60 (52)</td>
<td>40 (34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(86)</td>
</tr>
</tbody>
</table>

Routine Testing | Traceback Testing

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Figure 4
Figure 4 illustrates a reverse change in this ratio for 1967. It should be pointed out, however, that eleven owners with infected herds maintained their cattle in community pastures. Nine of these were recorded with the data for routine testing as individual herds. The other two were recorded as regular kill traceback cases. It is difficult to present all data in a strictly unbiased manner. Possibly it would have been more appropriate to include these eleven herds as one unit for each community pasture.

Historically, considerably more area testing has been carried on in dairy herds than in beef-type herds. It would be expected that the ratio of infected herds found as a result of routine testing as compared to traceback would be higher in dairy cattle than in beef-type cattle. This is borne out by the data in figure 5. Here, again, we should explain that

![Figure 5: Tuberculosis Eradication]

the nine infected herds recorded as being associated in community pastures are included in the 13 beef-type herds recorded as found by routine testing procedures.

It must be realized that the degree of emphasis that is placed upon a respective procedure for finding infection will have a bearing on the resulting data.

From time to time, data has been shown to compare the cost of routine testing to traceback in detecting infected herds. The speed of detection and the swiftness with which the infection is eliminated have also been considered.
The chart, figure 6, illustrates by "reasons for tests" the percentage of cattle tested in 1967 to locate herds with infection indicative of bovine tuberculosis. The circles on the left show the number of cattle tested by reason and the percentage of the total tested. The circles on the right show how many infected herds were found opposite the reason for applying the test. It is easy to note that traceback is an effective method to find infected herds. Only one percent of the total tests were made following traceback procedures. This one percent resulted in finding 39 percent or 34 of the 86 infected herds. While this data is impressive from one point of view, it is of considerable concern from another. We do not find infected herds early enough. Of the 86 \textit{M. bovis} herds discovered during the past fiscal year, 26 or 30 percent had over 20 percent reactors. The number of animals classified as reactors in these 26 herds was 38 percent of the cattle tested. Of the reactors in these 26 herds, 40 percent disclosed tuberculous lesions on meat inspection examination.

The stimulation that the use of the "Red Flag Herd" category has had in concentrating attention on herds with repeated infection has been phenomenal. The number has dropped from 239 in 1960 to 18 at the end of the 1967 fiscal year. The number of herds with this designation from 1961 to the present, their location, and the number of years they

\begin{table}[h]
\begin{tabular}{|l|c|c|}
\hline
\textbf{REASON FOR TESTS} & \textbf{CATTLE TESTED} & \textbf{INFECTED HERDS} \\
\hline
\textbf{ROUTINE} & & \\
\textbf{AREA TESTING} & 2,440,949 & 24 \\
\textbf{HERD ACCREDITATION} & 1,083,138 & 6 \\
\textbf{MILK ORDINANCE} & 1,272,384 & 15 \\
\hline
\textbf{TESTING FOR SALE OR SHOW, IMPORTS, AND OTHERS} & 1,028,055 & 7 \\
\textbf{TRACEBACK} & 54,546 & 34 \\
\hline
\textbf{REGULAR KILL (ANH635's) REACTORS, EXPOSED} & 5,879,072 & 86 \\
\hline
\end{tabular}
\end{table}

Figure 6
have been infected, are shown in figure 7. Members of this Association need not be reminded that concentration on "Red Flag" and other infected herds is first on the list of priority items for tuberculosis eradication as recommended by the Committee on Tuberculosis and Paratuberculosis.

The graph in figure 8 shows the number of herds that have been known to be infected for a long period of time as compared to those in which the infection has been known to exist for a short time. The drop in percentage of herds with infection in a prior year between 1965 and 1966, from 44 percent to 25 percent, was explained in part in last years' report as being a result of liquidating herds with long-standing infection. It appears that we have not made comparable progress during 1967 since there are more herds reported today with a history of prior infection than there were in 1966. The number of herds liquidated because of tuberculosis is recorded by fiscal years as follows: 1964—8; 1965—22; 1966—11, and 1967—16. While the number of herds known to be infected has been decreasing annually, the data again points out the need for intensifying our efforts.

The 32 herds with recurrent infection are further illustrated in figure 9 by depicting the year or years that infection was reported in each of these herds. It should be noted that 16 or 50 percent of the herds with
Tuberculosis Eradication

**Herds Reported with infection**
*(Indicative of M. bovis)*

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>1965</th>
<th>1966</th>
<th>1967</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>141</td>
<td>111</td>
<td>86</td>
</tr>
</tbody>
</table>

- **Fiscal Year 1965**: 44.68%
- **Fiscal Year 1966**: 25.23%
- **Fiscal Year 1967**: 37.21%

- ☐ No Record Prior Infection
- □ Infection Reported in a Prior F. Y.

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**Figure 8**

**Tuberculosis Eradication**

**RECURRENT INFECTION REPORTED IN 32 HERDS**

<table>
<thead>
<tr>
<th>No. Infected Herds</th>
<th>Current Herd Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fiscal Year of Recurrent Infection</td>
</tr>
<tr>
<td></td>
<td>IN Quarantine</td>
</tr>
<tr>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

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**Figure 9**
infection reported in 1967 and a prior fiscal year were also reported to have had infection in each of three or more years. We should also be cognizant of the fact that only 8 of these herds were sent to slaughter in their entirety as shown in the last column. An equal number, or 8 herds, have already been released from quarantine. The possibility that infection still exists in these herds to raise its ugly head at a later date or be a source of infection for other herds is tremendous. This is borne out by the data in this chart.

The interest and attention directed toward liquidating infected herds when first discovered is encouraging. However, this phase of the program must be expanded if we are to reach our objective in the near future. The provision for Federal indemnity payments for exposed animals in an infected herd has been available since 1963. This payment is predicated upon an equal or greater amount from local sources. In 1965 the maximum Federal payment for an exposed individual animal when liquidating an infected herd was raised from $25 to $100 for a grade animal and from $50 to $200 for a purebred animal. We see in figure 10 the 38 states that presently have authority for indemnity payments to liquidate infected herds and thus hasten eradication.

There have been several instances where the liquidation of an infected herd has been seriously delayed because provision for state indemnity...
was lacking at the time that the infection was found. Fourteen herds were liquidated in six states and Puerto Rico during fiscal year 1967. The location of these herds is shown in figure 11.

The need for intensifying our eradication procedures regardless of the status of the area is borne out by the fact that 27 or 4 percent of the 608 counties listed as accredited-free were found to be associated with infection indicative of *M. bovis* during the past year. These 27 counties are included among the 49 counties in 11 States in this category since being listed in the free status. Figure 12 illustrates the wide distribution of these cases. In many instances, precise information even in accredited-free areas is not available to determine the specific movements of infected animals. Thus a measure of the spread of the disease is frequently lacking.

We might comment here about one case in New Hampshire, the first State to be accredited-free. This case was particularly well documented as to the source of the animal, the date of entry into the State, its location while in the State, and its route to slaughter where the tuberculosis was found. The fact that the movement involved a state line undoubtedly contributed to this comprehensive information which is often unavailable when movements involve local political subdivisions. This supports the philosophy of accrediting areas as free on a statewide basis rather than on
ACCREDITED BOVINE TUBERCULOSIS – FREE AREAS
608 COUNTIES IN 23 STATES, P. R. & V. I.

Prepared July 1, 1967

Figure 12

TUBERCULOSIS ACCREDITATION
(STATEWIDE BASIS)

September 1967

ACCREDITED TB-FREE
(M. bovis in Cattle)

2 States and V.I.

Figure 13
an individual county basis where animal movements are more informal than when crossing state lines.

As the incidence of a disease declines, administrative changes are frequently required in keeping with efficiency of operation. In recent years this Association has supported a general shift from accredited status on an individual county basis to accreditation, either modified or free, on a statewide basis. There are now 27 states designated as modified-accredited on a statewide basis and two additional, New Hampshire and Maine, plus the Virgin Islands, that are listed as Accredited Tuberculosis-Free (\textit{M. bovis} in cattle). These are depicted in figure 13. Savings from an administrative and clerical standpoint because of this change are considerable.

The volume of area testing has declined 43.5 percent in the past five years from 4.3 million in 1963 to 2.4 million in 1967. The development

\textbf{Tuberculosis Eradication}

\textbf{LESION CASES-REGULAR KILL (6-35)}

\textbf{Indicative of \textit{M. bovis}}

\textbf{1965-1967 Inclusive (615 Cases)}

\begin{tabular}{|c|c|}
\hline
\textit{M. bovis} Confirmed & \textit{M. bovis} Not Confirmed \\
\hline
26\% & 74\% \\
(158 CASES) & (457 CASES) \\
\hline
\end{tabular}

\begin{tabular}{|c|c|}
\hline
Traced To Infected Herds & Not Traced To Infected Herds \\
\hline
40\% & 60\% \\
(64 CASES) & (88 CASES) \\
\hline
\end{tabular}

\begin{tabular}{|c|c|}
\hline
Traced & Not Traced To Infected Herds \\
\hline
\textbf{58\%} & \textbf{92\%} \\
\textbf{(164 CASES)} & \textbf{(193 CASES)} \\
\hline
\end{tabular}

\begin{tabular}{|c|c|}
\hline
\textbf{With Bacteriological Examination} & \textbf{No Bacteriological Examination} \\
\hline
\textbf{58\%} & \textbf{42\%} \\
\textbf{(164 CASES)} & \textbf{(193 CASES)} \\
\hline
\end{tabular}

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\textbf{Figure 14}
classified as being indicative of *M. bovis* infection. It may be observed that for the three-year period, from 1965 to 1967, there was a total of 615 cases of which 158 were confirmed bacteriologically as *M. bovis*. This leaves 457 cases not confirmed. Of the 158 confirmed cases, only 40 percent were successfully identified with an infected herd. The 60 percent not identified with an infected herd strongly suggests that this is a phase of the program that should have more concentrated attention than it has had to date. It might be expected, as is shown, that the traceback of confirmed *M. bovis* cases would result in finding a much higher percentage of infected herds than would be found as a result of traceback of cases unconfirmed or those not examined bacteriologically.

It may be observed, from the chart, that 37 percent of the total infected herds found were discovered as a result of tracing cases that were not proven *M. bovis* bacteriologically. This supports the statement frequently made that "positive laboratory results are significant, but negative results are inconclusive."

This same chart, bars 3 and 4, shows the findings as a result of traceback on 264 cases which were examined bacteriologically and not confirmed as *M. bovis* as compared to 193 cases that were not similarly exam-
ined. This data substantiates the belief that greater emphasis and study should be given to each individual case than may at first seem necessary to assure ourselves that infected herds are not overlooked.

Last year, your Committee on Tuberculosis and Paratuberculosis supported an extension of the program to include greater attention to avian-type tuberculosis through a four-phase project—Education, Identification, Epidemiology, and Eradication. This program has been outlined in considerable detail during the year. Unfortunately, educational and promotional material in connection with the renewed attack on avian-type infection has been slow in developing. However, 16 states are currently enrolled in Phase I, Education, and 17 more plus Puerto Rico expect to be enrolled by the end of the fiscal year. This is illustrated by the map, figure 15.

The need for attention to avian-type infection is supported by the fact that approximately 16 percent of the bacteriological isolations from bovine specimens made for the tuberculosis eradication program at the National Animal Disease Laboratory are classified as *M. avium*.

The data presented in this report indicates that our best ingenuity is demanded to develop adequate identification and traceback procedures to effectively and economically locate tuberculosis herds.

The high proportion of infected herds that have experienced repeated infection justifies the liquidation of *M. bovis* herds. The release of exposed non-reacting animals from quarantine perpetuates the disease.

While statistics suggest that there is a record low prevalence of bovine tuberculosis in cattle, the challenge has never been greater for the eradication of this age-old enemy of man and animal.

THE TIME FOR ACTION IS NOW!
REPORT OF THE COMMITTEE ON TUBERCULOSIS AND PARATUBERCULOSIS


1. As in the past, a midyear meeting of the committee was held in April. A meeting has been scheduled for April, 1968. The committee feels that these mid-year meetings are advantageous in organizing work for the annual meeting.

2. "Standards for the Histopathologic and Bacteriologic Diagnosis of Tuberculosis" which were adopted by the Conference of Veterinary Laboratory Diagnosticians on October 16, 1967 were reviewed by the committee. It was agreed that these should be accepted as minimum standards for laboratories examining specimens in connection with the cooperative tuberculosis eradication program.

3. Accredited herds: It has been suggested that the establishment and maintenance of individual accredited herds be discontinued. This was reviewed by the committee at a previous meeting and was again considered at this meeting. The Committee recommends that accredited herds be continued as a part of the official program. It is further recommended that attention be given to the strict adherence to the standards for accredited herds as outlined in the Uniform Methods and Rules.

4. The committee has been requested to consider the matter of using the double injection (caudal and vulva) in routine tuberculin testing. This matter has been covered in detail in a paper by Dr. R. M. Scott, "Reasons for Not Using Double Tuberculin Injection." The subject was also discussed at a conference of tuberculosis epidemiologists at Lansing, Michigan in August, 1967. Since it has been definitely established that the use of the double injection causes interference, the committee recommends that only the single injection be used in routine testing.

5. Papers entitled "Tuberculosis in a Bull Stud" were given by Dr. A. R. McLaughlin and Dr. D. E. Bartlett. The committee wishes to commend the authors for their excellent presentations and recommends that these papers be called to the attention of the membership. These papers were not presented at the general session due to time limitations but will be printed in the proceedings. The committee recommends that
all animals going into A.I. studs or other valuable herds originate from
herds that are tested and negative to tuberculosis, and that the seller
make available to the purchaser all official records pertaining to the
herd and the individual animals.

6. A paper entitled "Improved Histopathologic Techniques for Examina-
tion of Granulomatous Lesions From Cattle" was given by Dr. H. A.
McDaniels. The committee recommends that this paper be called to the
attention of the membership. This paper was not presented at the gen-
eral session due to time limitations but will be printed in the Procede-
nings.

7. Dr. A. F. Ranney presented statistical data pertaining to the Market
Cattle Identification Program for fiscal year 1967. After a careful
review of this data, the committee recommends that diligent efforts
be continued to get as close as possible to 100% of the slaughter
cattle backtagged and completely identified through accredited slaugh-
ter, and that accredited establishments be reviewed periodically to be
sure that inspection and identification standards are being met.

8. It has been called to the committee's attention that in some instances
the existing policy of not retesting for tuberculosis in less than 60 days
has interfered with interstate shipments to states requiring a 30 day
test. The committee recommends that states consider accepting tests
for 60 days which will coincide with the existing policy.

9. It has been called to the committee's attention that there are instances
in which it has been established beyond reasonable doubt that bovine
tuberculosis exists in herds that have not been adequately tested and
are not under official quarantine. It is the unanimous opinion of the
committee that the accredited status of the county should be revoked
when these two conditions exist.

10. The committee recommends that the following changes be made in the
Uniform Methods and Rules for Tuberculosis Eradication:

(a) Change reference to the Animal Disease Eradication Division to
the Animal Health Division wherever it appears.

Part I: Definitions

(b) Delete paragraph two relative to advanced tuberculosis. Replace
with the following: "A case will be considered *M. bovis* infection
when a pathologic (granulomatous) lesion in cattle suspected of
being tuberculosis is found unless a satisfactory examination at an
accredited laboratory justifies a diagnosis other than bovine-type
tuberculosis. Exception—lesions that occur only in the mesenteric
lymph nodes."

Part II: Individual Accredited Herd Plan

(c) Paragraph 2, line 4: change the word "advanced" to "bovine."

(d) Paragraph 8, line 8: after "additions to" insert the words "and
removals from."

Part III: Modified Accredited Area Plan
(e) Paragraph 12 (f), line 3: delete the word "tested" and replace with "over 24 months of age in the area."

Part IV: Accredited Tuberculosis Free Areas

(f) Delete from the introductory paragraph the following words: "a county, or a block of several counties, which is part of a progressive plan for complete state coverage."

(g) Paragraph 13(a): delete the words "During the three year period from effective date of this part, an area—." Replace with "A state—."

(h) Paragraph 14: delete entire paragraph and replace with the following: "The listing of states as accredited tuberculosis-free will be preceded by a thorough review. The history of tuberculosis in the state, the efforts to find any tuberculosis that may exist, and the compliance with the Uniform Methods and Rules for Tuberculosis Eradication will be taken into consideration in recognizing a state for Accredited Tuberculosis-Free (M. bovis in cattle) status."

(i) Paragraph 16, lines 2 and 3; delete the words "and cattle within the area that have associated with those found to be infected"— and replace with the following: "and all animals that have associated with the infected animal for any period of time whatever"—.

(j) Paragraph 21(d): delete entire paragraph. Replace with the following: "Feeder cattle from a modified accredited area without individual identification may be moved directly to a premise and maintained in quarantine under control of the state livestock sanitary officials, provided that they are identified on the premises and are moved to slaughter to accredited establishments under permit at the end of the feeding period."

(k) Paragraph 25: delete entire paragraph. Replace with the following: "Unless an area is disqualified, it may maintain its status of Accredited Tuberculosis-Free (M. bovis in cattle) by continuing to meet the requirements as outlined in 13(b) or (d)."

11. The Animal Health Committee of NASDA has requested that the Tuberculosis Committee of the USLSA evaluate the Johne’s disease problem in the United States and that the USLSA’s views on the matter be reported to NASDA’s Animal Health Committee at its 1968 meeting by the president of the USLSA.

No statistically sound survey of the extent of infection throughout the United States has ever been conducted. Most states contain infected herds. Many of these herds contain purebred animals and are a source of breeding stock for other herds.

Diagnostic tests presently in use are not sufficiently accurate to eradicate the disease from infected herds; however, research work is under way that shows promise in two areas: (1) An experiment is in progress to determine the feasibility of using a vaccine in herds of cattle infected with the disease. (2) A technique is being developed for isolating
the causative agent from fecal specimens of infected cattle for diagnostic purposes. It is our understanding that the results of these experiments will be ready for publication within a year.

With the development of an effective diagnostic procedure and an effective vaccine, it is hoped that it will become possible to eliminate this disease from infected herds. A vaccine has been developed that is quite effective in controlling the disease in sheep but has not been used in cattle, in this country, except on a special study basis, as it causes a marked sensitivity to tuberculin.

The committee recommends:

1. that research be continued and expanded in an effort to develop effective diagnostic and preventative measures,
2. that epidemiological and special field studies be instituted and developed, and
3. that the U. S. Department of Agriculture instigate a statistical survey to determine the incidence of the disease.
EPIDEMIOLOGIC STUDIES OF *LEPTOSPIRA GRIPPO-TYPHOSA* and *LEPTOSPIRA HARDJO* INFECTIONS IN IOWA CATTLE


Iowa City and Ames, Iowa

INTRODUCTION

*Leptospira pomona* has been reported as the major causative serotype of leptospirosis in cattle in the United States (1)(2). Increasing evidence of infection caused by serotypes other than *Leptospira pomona*, primarily *Leptospira grippotyphosa* and *Leptospira hardjo* have been reported in domestic animals (3) (4) (5) (6) (7) (8) (9) (10) (11) (12) (13) (14) (15). These infected animals are potential human health hazards (16) (17).

In 1952, serologic evidence of the hebdomadis sero-group (closely related to the Mallersdorf strain of *L. sejroe*) was reported in Iowa cattle (18).

In Iowa in 1962, serologic evidence of infections with predominant *L. grippotyphosa* and *L. hardjo* titers was observed in a dairy herd following abortions. (19) (20).

From the Comparative Medicine Section, Institute of Agricultural Medicine, College of Medicine, University of Iowa, Iowa City, Iowa (Diesch, McCulloch, Crawford and Braun) and the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa (Bennett). Dr. Diesch's present address is College of Veterinary Medicine, University of Minnesota, St. Paul and Dr. Braun, Wisconsin State University, Whitewater, Wisconsin.

This investigation was supported in part by Public Health Service Grant CC-00071, National Communicable Disease Center, Atlanta, Georgia.

The purpose of this paper is to present evidence of the complex problems associated with the diagnosis of *L. grippotyphosa* and *L. hardjo* infections in Iowa cattle.

MATERIALS AND METHODS

Epidemiologic Procedures

This report is part of a project entitled "The Epidemiology of Leptospirosis in Iowa." These studies were conducted in a 10-county area located in Eastern Iowa.

Phase A: In the summer and fall of 1964, following a human outbreak of leptospirosis, 744 blood specimens from beef and dairy cattle were collected from 52 herds in Bertram township where the human outbreak occurred (21). The blood samples were collected during a phase of the State, Federal Brucellosis Eradication Program.
Phase B: Within the 10-county area, selected studies were made on cattle herds if serologic evidence of *L. grippotyphosa* or *L. hardjo* was found. The veterinary practitioner and herd owner were initially interviewed to determine if clinical signs of leptospirosis had been observed. Epidemiologic studies were subsequently conducted.

Serologic Procedures

The initial blood specimens collected were in most cases submitted to the Veterinary Diagnostic Laboratory, Iowa State University, where they were screened for leptospiral agglutinins by a macroscopic plate procedure using commercial *L. pomona* antigen (22). Selected samples were further screened by the macroscopic slide agglutination test using 12 leptospiral serotype antigens (Difco)* divided into four pools (23).

Pool I, *L. ballum*, *L. canicola*, *L. icterohaemorrhagiae*
Pool II, *bataviae*, *L. grippotyphosa*, *L. pyrogenes*
Pool III, *L. autumnalis*, *L. pomona*, *L. sejroe*
Pool IV, *L. australis*, *L. hyos*, *L. mini georgia*

Sera reactive to any of the Difco pools were screened by the microscopic agglutination test at a final 1:100 serum dilution against the above leptospiral serotypes plus *L. hardjo*. Sera positive by the microscopic agglutination test to any of the 13 serotypes were titered for leptospiral antibodies. Leptospires grown in Stuart's liquid medium without phenol red** (Difco) were used as antigens in the microscopic agglutination test. Density was adjusted by nephelometry (Coleman Jr. Model 9) to 400 Coleman units***.

Isolation Procedures

Voided urine from cattle was filtered through a Swinney adapter with a 0.45µ Millipore filter**** and directly inoculated into artificial media. Kidney tissue was ground with mortar and pestle and diluted with Stuart's liquid medium (Difco) from 10⁻¹ to 10⁻⁵. Two to three drops from each kidney tissue dilution and urine specimen were inoculated into 2 tubes containing 7-9 ml of bovine albumin polysorbate 80 (24) and 2 tubes of Fletcher's (Difco) semisolid media. In some instances, 5-fluorouracil***** (25) was added (100 mcg/ml) to media to retard growth of microbial contaminants. Inoculated tubes of media were incubated at 29°C and examined at regular intervals for leptospires by darkfield microscopy for a minimum of 70 days.

Weanling guinea pigs or hamsters were inoculated intraperitoneally with 1cc and ½cc respectively of 10% kidney suspension or undiluted

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* Difco and Company.
** Difco and Company (Thiamine and KH₂P0₄ added, pH adjusted to 7.4).
*** Coleman Standard (80 units) was made to read 5 on scale and antigens adjusted to read 25 on scale.
**** Millipore Filter Corporation, Bedford, Massachusetts.
***** Hoffman-LaRoche, Nutley, New Jersey.
urine for isolation attempts. Blood specimens were collected from the animals and cultured two times between four and eight days post inoculation. These animals were killed at 30 days, the serum specimens screened for antibodies by the macroscopic slide agglutination test and kidneys cultured for leptospires. Isolates were definitely typed by the Veterinary Public Health Laboratory, National Communicable Disease Center, Atlanta, Georgia.

Results

Phase A: (See Table I)

Of the 744 bovine serums collected in Bertram township, the predominant microscopic agglutination serotype titers of 1:100 or higher were as follows: *L. pomona* 13.2%; *L. hardjo*, *L. sejroe* 9.9%, *L. grippotyphosa* 4.2%; other (*L. autumnalis, australis, canicola*) 1.0%; no predominant titer, 2.2%; total of 30.5% positive.

<table>
<thead>
<tr>
<th>Predominant Serotype</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. pomona</em></td>
<td>13.2%</td>
</tr>
<tr>
<td><em>L. sejroe/hardjo</em></td>
<td>9.9%</td>
</tr>
<tr>
<td><em>L. grippotyphosa</em></td>
<td>4.2%</td>
</tr>
<tr>
<td>Others</td>
<td>1.0%</td>
</tr>
<tr>
<td>No predominant titer</td>
<td>2.2%</td>
</tr>
<tr>
<td>Total</td>
<td>30.5%</td>
</tr>
</tbody>
</table>

*1:100 or higher titer (MA Test, live antigens).

Phase B: The following are data collected from several case studies made during 1964 and 1965.

Herd Case I.

During March, 1964, five heifers aborted. Blood specimens collected on March 16, and 24 were negative when screened for *L. pomona*, but three serums had microscopic agglutination titers against *L. hardjo* and *L. sejroe*. Two were from a 100-Hereford-stock-cow herd on pasture and three from a 140 cow herd located in another pasture. All five had aborted eight-month-old fetuses. The cattle had not been vaccinated against leptospirosis.

On April 21, blood specimens were collected from 21 cattle selected
from the two herds. Attempts were made to select cows that had recently aborted or were repeat breeders. On May 15, repeat blood specimens were collected from 11 cattle (10 repeat) from both herds. On May 20th, one cow, a repeat breeder, was slaughtered and found to be 3 months pregnant. Blood was collected for serology. Urine, kidneys and placenta were collected for isolation attempts. Leptospires were not isolated from specimens obtained.

The prevalence of leptospiral antibodies (microscopic agglutination test) was based on the predominant titer observed on blood specimens collected from each animal of 27 tested during a two-month study period: L. hardjo and/or L. sejroe—18 cattle, 1:100-1:1600 (67%); L. pomona—2 cattle, 1:400-1:1600 (8%); L. pomona, L. sejroe (4%)—1 cow, 1:400 (4%). Six or 22% were negative.

Of 25 cows tested, 18 (19 had serologic evidence of infection) were found pregnant or delivered live, normal calves, six aborted and one cow was a repeat breeder. The herd bull had a 1:400 titer against L. hardjo, L. sejroe.

Herd Case II. (See Table II)

On January 14, 1965, blood specimens from 25 Hereford beef cattle were tested for brucellosis; 10 reactors were branded and sold for slaughter. On March 10, the 15 remaining cattle were retested for brucellosis; four reactors were subsequently branded and sold. Serologic evidence of L. hardjo, L. sejroe and L. pomona was found. Between January 14 and March 20, five cows had aborted. On March 29, the farmer marketed the 11 remaining cattle. Blood and kidney specimens were obtained at the abattoir. Pregnancy information was not available.

The prevalence of leptospiral antibody titers (microscopic agglutination
test) was based on the predominant titer observed on blood specimens collected from each cow during a 19-day period: *L. hardjo* and/or *L. sejroe* 9 cows, 1:100-1:1600 (60%); *L. pomona*—2 cows 1:100-1:1600 (13.5%). Two exhibited no predominant serotype (*L. pomona, L. hardjo*)—1:100-1:1600 (13.5%). Two cows (13.5%) were negative. Leptospires were isolated from kidney tissue of one cow in bovine albumin polysorbate-80 medium containing 5-fluorouracil. The isolate was identified as *L. hardjo*. In the serum collected from the cow from which the isolation was made, titers of 1:100 to 1:400 were found against both *L. hardjo* and *L. sejroe*. This serum was serologically negative for brucellosis.

**Herd Case III.** (See Table III)

On July 22, 1965, a Holstein cow aborted a five-month-old fetus; 12 days later (August 3) a veterinarian removed the retained placenta. Blood specimens from the cow that previously aborted and two others were submitted and found *L. pomona* negative on the macroscopic plate test. The

<table>
<thead>
<tr>
<th>Table III</th>
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<tbody>
<tr>
<td><strong>Herd Case Study No. 3—1965—Iowa</strong></td>
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<tr>
<td>Prevalence of leptospiral antibodies (M. A. Test) based on predominant titer observed on blood specimens collected from each animal.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Predominant Serotype</th>
<th>Titer Range Observed</th>
<th>Number of Cattle</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. hardjo</em> and/or <em>L. sejroe</em></td>
<td>1:100-1:400</td>
<td>3</td>
<td>15%</td>
</tr>
<tr>
<td><em>L. grippotyphosa</em></td>
<td>1:100-1:1600</td>
<td>8</td>
<td>40%</td>
</tr>
<tr>
<td><em>L. pomona</em></td>
<td>1:8400</td>
<td>1</td>
<td>5%</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td>8</td>
<td>40%</td>
</tr>
<tr>
<td>Total Tested</td>
<td></td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

*Isolation: *L. grippotyphosa*—bovine albumin—polysorbate 80 medium—containing 5 F U.

specimen from the cow that aborted was Pool II positive on the macroscopic slide agglutination test. The microscopic agglutination titer was 1:1600 against *L. grippotyphosa*. The other two were negative by all tests.

On August 24, follow-up studies were initiated. The herd of 20 mixed breed cattle had not been vaccinated against leptospirosis. Blood and urine specimens were collected from the cow that aborted and the two others; all three had been purchased at a salesbarn in March, 1965. Repeat specimens were obtained 3 weeks later.

On October 1, another cow (one of the two) aborted a 7½ month-old fetus. The cow had a retained placenta. Blood and urine specimens were collected. On October 13 and Nov. 17, 117 days later, blood specimens
were obtained from the entire dairy herd. The serologic titer of the first cow that had aborted ranged from 1:1600 (August 3)—1:100 (October 13) against *L. grippotyphosa*. Serologic titers from the second cow that aborted on October 1 went from negative (Aug. 3) to 1:6400 (Oct. 13) against *L. grippotyphosa*.

On October 20 the two cows that aborted were sold for slaughter and their kidneys obtained for isolation attempts. On December 14 the 15 remaining cows were examined for pregnancy (five had been sold for slaughter).

The prevalence of leptospiral antibody titers (microscopic agglutination tests) was based on the predominant titer observed on blood specimens collected from each cow during a three-month period: *L. grippotyphosa*, 8 cows, 1:100 to 1:1600 (40%); *L. hardjo* and/or *L. sejroe*, 3 cows, 1:100 to 1:400 (15%). *L. pomona*, one cow, 1:6400 (5%). No evidence of leptospiral antibodies was found in the sera of 8 cows (40%).

Of 12 cows with titers (1:100 or higher), two aborted, four delivered live, normal calves, four carried live fetuses, and two were not examined for pregnancy. Of seven cows which did not exhibit leptospiral antibodies, four delivered live, normal calves, and two were pregnant, and one was not examined. Although evidence of leptospiral infection was found in the herd, only two of 16 known pregnant animals aborted.

Leptospires were isolated in bovine albumin polysorbate—80 medium containing 5-fluorouracil from the kidney tissue of the second cow that aborted. The tissue was collected 20 days post abortion. The isolate was identified as *Leptospira grippotyphosa*.

**Herd Case IV**

On July 31, 1965, an Ayrshire cow aborted a seven-month-old fetus. The following day the cow was treated for milk fever. A blood specimen submitted for *L. pomona* testing was negative on the macroscopic plate test. The serum screened by the macroscopic slide agglutination test was positive in Pools II, III and IV. The microscopic agglutination titer was 1:400 against *L. grippotyphosa*. On August 2, another cow aborted and the herd of 11 Ayrshire cows was vaccinated with *L. pomona* bacterin. On August 20, signs of pyometra were observed in the cows that had previously aborted. Another cow had a temperature of 103.6, pulse 82, anorexia, ataxia, and decreased milk production; a blood specimen was collected for isolation attempts. Blood and urine specimens were collected from the remainder of the herd. On September 10 the cow that initially aborted was sold for slaughter and the kidneys collected.

The serologic titer of the first cow that aborted increased from 1:400 (August 2) to 1:1600 (August 20) against *L. grippotyphosa*; from negative to 1:6400 against *L. hardjo*; negative to 1:400 against *L. sejroe* and 1:400 against *L. pomona*. The second cow that aborted had titers of 1:1600 against *L. grippotyphosa*; 1:400 against *L. hardjo* and *L. pomona*. The cow that exhibited clinical signs went from a negative serologic test (Aug. 20) to 1:1600 (Sept. 30) against *L. grippotyphosa*, but did not abort. Another
cow artificially bred six times, was sold to slaughter and had a 1:400 titer against *L. grippotyphosa* (Aug. 20). Leptospires were not isolated from specimens collected.

The prevalence of leptospiral antibody titers (microscopic agglutination test) was based on the predominant titer observed on blood specimens collected from each cow during a two-month period: *L. grippotyphosa*—4 cows, 1:100-1:1600 (36%), *L. hardjo* and/or *L. sejroe* one cow, 1:100-1:6400 (10%), 1 cow (10%) 1:1600 (no predominant serotype, *L. pomona*, *L. hardjo*). Five or 45% were negative.

Of the eight cows that were pregnant or delivered live, normal calves during the study period, three had leptospiral antibodies. One was not examined during this period.

Additionally, four other farms where cattle had aborted were investigated. The initial serologic screening procedure on the macroscopic slide test was *L. pomona* negative on the sera of eight cows that had aborted. The prevalence of leptospiral antibody titers (microscopic agglutination test) was based on the predominant titer observed on blood specimens collected from each cow during the study period: all eight cows exhibited titers ranging from 1:100 to 1:1600 against *L. hardjo* and/or *L. sejroe*.

**Discussion**

In Iowa, the isolation of *L. grippotyphosa* and *L. hardjo*, together with serologic evidence of infected cattle with and without clinical signs of disease, supports previous findings that bovine leptospirosis is associated with serotypes other than *L. pomona*.

**The Livestock Industry**

In Iowa and the United States leptospirosis continues to cause severe losses to the livestock industry. In the United States the average annual loss from 1951-1960 in dairy cattle and milk production was estimated at $12,189,000 per annum—(26). Estimates for beef cattle loss are not available. The reported bovine cases of leptospirosis declined from 23,417 cases in 1962 to 10,276 in 1966 (27) (28). Despite this reported evidence of decreasing incidence, we are of the opinion that leptospirosis is a major livestock disease. From research data accumulated by us and others in Iowa during the past decade, it is apparent that many cases have not been diagnosed and subsequently not reported. The complexity of leptospiral infections and subsequent losses continues to be an enigma to the livestock industry.

In case reports I, III and IV, 26 of the cows with serologic titers of 1:100 or greater were found with live fetuses, or delivered live, normal calves during the study period in which ten cows in the herds aborted. Wide-spread serologic evidence of infection was found in other cattle in the herds, most of which appeared clinically normal.

Evidence of wide-spread *L. pomona*, *L. hardjo* and *L. grippotyphosa* infections was found in the Bertram township prevalence study. The majority of herd owners interviewed by questionnaire reported no signs of
leptospirosis in their herds. However, a herd owner rarely observes leptospirosis when the disease is sub-clinical or when it occurs in nonpregnant or early pregnant cows; and bull or steers. Occasionally acute leptospirosis; is observed in feedlot cattle resulting in severe illness and death.

Serotypes other than *L. pomona* are being reported in an increasing number of new hosts. Therefore, new approaches and methods of prevention and control must be developed and provided by the veterinary profession.

The Practicing Veterinarian and Regulatory Programs

The establishment of clinical diagnosis of leptospirosis by the practicing veterinarian is often difficult and complex. In the reported cases associated with *L. grippotyphosa* and *L. hardjo* infections, the practitioner, depending on laboratory support in establishing a diagnosis did in all cases initially received an *L. pomona* negative report from the diagnostic laboratory. This evidence and findings reported by others (13) (14) (15) further substantiates the need for multiple serotype testing of blood specimens submitted.

The practitioner must, however, rule out other infections and noninfectious causes of abortion. A diagnosis of leptospirosis must be based on herd history and clinical signs with laboratory support. Repeat blood specimens are necessary to demonstrate a significant rise or fall in antibody titer (29), but this procedure may take weeks to accomplish. Cultural methods and subsequent identification of organisms (if isolated) are impractical when immediate results are needed. The practitioner must take immediate action and is therefore unable to delay diagnosis and treatment of the herd.

Treatment of bovine leptospirosis with antibiotics and supportive methods are used, but are generally unsatisfactory. Despite treatment, the animal may remain a carrier. Subclinically infected animals may be shedding leptospires; these are often not detected or treated and are a major problem in prevention and control programs.

Vaccination against *L. pomona* is widely used in Iowa and in the U. S. From 1953-1964, 24 million doses of vaccine were manufactured (30).

State or Federal regulatory programs for control or eradication of leptospirosis are practically nonexistent. In 1966 the USLSA Leptospirosis Committee reported, "This disease is not ammendable to eradication because of various serotypes encountered, the wide range of hosts and the inability to detect the carrier animal," and the committee urged expanded research (31). For control and prevention it appears that vaccination with approved bacterins is justified (14) (31) (32). However, evidence exists that the vaccinated animal that becomes infected may remain a carrier—vaccinated animal (33). Research is needed to determine cross protection immunity against other leptospiral serotypes by use of single or multiple serotype bacterins. A multiple serotype vaccine must protect against the serotypes prevalent in a specific geographic area. In Iowa, on the basis of preliminary clinical and laboratory evidence, these serotypes in addition to *L. pomona* are *L. grippotyphosa* and *L. hardjo*. 
**Diagnostic Laboratories**

The Iowa Veterinary Diagnostic Laboratory screens all blood specimens (leptospirosis requests) for *L. pomona*. Special requests are tested for other serotypes. *L. pomona* is considered to be the major infecting serotype in Iowa cattle, but other serotypes are causing infections. From 1963 to 1966, the Veterinary Diagnostic Laboratory of Ames, Iowa, averaged 33,794 bovine leptospirosis test requests per year with 6.6% of the specimens serologically positive against *L. pomona*. Scr should be screened for other serotypes and also titered, but due to the large number of requests and for economic reasons, it has not been possible. To determine the infecting serotype(s), more reliable and rapid diagnostic methods are needed. The microscopic agglutination test is difficult and costly to conduct on a large volume of samples. In the cattle studied in this report, laboratory evidence of leptospiral infections was initially determined by the pooled antigen screening (Difco), but additional testing was necessary to identify predominant serotype reactions. Serologic results are often difficult and confusing to interpret due to cross agglutination titers, mixed infections or possibly recent response to vaccination. Rapid screening methods and the ability to determine *L. pomona*, *L. grippotyphosa* and *L. hardjo* antibodies are needed. Surveillance by geographic locations is necessary to determine the prevalent serotypes that are causing infections. The veterinary profession must be constantly alert to merging or changing patterns of prevalent serotypes. Consideration of new and improved diagnostic methods are needed for identifying specific leptospiral organisms and serum antibodies. The fluorescent antibody method has been found useful in detecting leptospiral organisms in aborted fetuses and tissues that are grossly contaminated by other bacteria—(34).

**General Comments**

Leptospirosis, in addition to causing major losses to the livestock industry, remains a human health hazard. Since 1947, Iowa physicians have reported 150 human cases of leptospirosis. Not only veterinarians but also physicians have difficulty in establishing a definitive diagnosis because of protean clinical manifestations (16).

Fifteen years ago, in December, 1952, at a Symposium on Leptospirosis, one of the speakers on bovine leptospirosis stated that:

“Although a great deal of information has been accumulated relative to bovine leptospirosis, we do not possess a good base knowledge of all the mechanisms involved in the disease and its dissemination. . . . A rapid, reliable, uncomplicated serologic test has not yet been devised. . . . We need to know of a drug that will eliminate the carrier state.” And finally, he stated that: “It is hoped that a surge of activity in research and field studies will resolve the problem within a few years” (35).

Although much knowledge has been accumulated during the past fifteen years, the complex problems associated with leptospirosal infections
in cattle and other animals in Iowa persist. We must accept the challenge and greatly expand research and increase all efforts to prevent, control and finally eradicate this major livestock disease.

Acknowledgments

The authors wish to thank the following practitioners for valuable contributions during the investigation of these cases: Drs. Ramsey and Krob, Tipton, Iowa; Drs. Barrington, Longtin and Fick, Muscatine, Iowa; Dr. Hanna, Springville, Iowa and Drs. Stamy and Reinhart, Norway, Iowa. Appreciation is also due Dr. Vaughn Seaton, College of Veterinary Medicine, Iowa State University and Drs. Grant Blake and M. E. Pomeroy, Federal and State Veterinarians for Iowa, respectively.

From the Comparative Medicine Section, Institute of Agricultural Medicine, College of Medicine, University of Iowa, Iowa City, Iowa (Diesch, McCulloch, Crawford and Braun) and the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, Iowa (Bennett). Dr. Diesch's present address is College of Veterinary Medicine, University of Minnesota, St. Paul and Dr. Braun, Wisconsin State University, Whitewater, Wisconsin.

This investigation was supported in part by Public Health Service Grant CC-00071, National Communicable Disease Center, Atlanta, Georgia.

REFERENCES

13. Hanson, L. E., Schnurrenberger, P. R., Marshall, R. B., and Scherrick, G. W.:


22. Fort Dodge Laboratories, Inc.


The presence of Leptospirosis in the Wisconsin animal population has been suspected for many years. Serological diagnostic service by the Wisconsin Animal Health Laboratories has been offered to Wisconsin herd owners since the middle 1950's when the Stoenner Plate Test was first employed. As research workers uncovered more evidence of the existence of a number of species of Leptospirae in addition to \textit{L. pomona}, selected serum samples from cattle and swine were submitted to the National Animal Disease Laboratory at Ames, Iowa in the early 1960's for serological testing. Tests were conducted against twelve different serotypes of Leptospirae by the National Animal Disease Laboratory. Results from these tests gave serological evidence to the presence of 7 or 8 species of Leptospirae in Wisconsin farm animals. Early in 1964, it was decided to offer to veterinary practitioners serological tests for these more commonly occurring serotypes of Leptospirae on sera from animals suspected of being affected, utilizing an agglutination-lysis procedure. Stock cultures were obtained from the National Animal Disease Laboratory, Ames, Iowa and from Walter Reed Hospital. These stock cultures are maintained on Fletcher's semi-solid media, transfers being made each 4 to 6 months. Antigen is produced by use of Stuarts Media, transfers being made each 3 to 5 days. Only antigen 7 days old or older is utilized for conducting the agglutination-lysis test. Known positive and negative sera to each of the serotypes being employed is run with each day's tests as controls. Dilutions are made to represent dilutions of 1:10, 1:100, 1:1,000, 1:10,000 and 1:100,000 after antigen addition. The 1:10 dilution is discarded and not tested due to lack of significance of positive reactions at this dilution, such as cross reactions between serotypes, vaccination titers, etc. Each dilution is read as positive if both the number of free Leptospirae is less than 50% of the negative control, and the amount of agglutination is more than 50% of the positive control in the control dilutions, where no Leptospirae are seen and no lysis occurs. Readings are adjusted for the occurrence of lysis. Gross lysis may occur in the lower dilutions with the succeeding higher dilution showing over 50% agglutination.

The following tables list the results for cattle and swine for the three years from July 1, 1964 through June 30, 1967. For the purposes of this report, reactions at the 1:100 dilution and above are considered positive.

**Leptospira pomona—Cattle**

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Negative</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY 1965</td>
<td>147</td>
<td>1,919</td>
<td>7.1</td>
</tr>
<tr>
<td>FY 1966</td>
<td>236</td>
<td>2,818</td>
<td>7.7</td>
</tr>
<tr>
<td>FY 1967</td>
<td>176</td>
<td>2,518</td>
<td>6.5</td>
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</tbody>
</table>
Leptospirosis in Wisconsin

### Leptospira icterohemorrhagiae—Cattle

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<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Negative</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY 1965</td>
<td>438</td>
<td>1,567</td>
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<tr>
<td>FY 1966</td>
<td>307</td>
<td>2,706</td>
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<tr>
<td>FY 1967</td>
<td>60</td>
<td>2,634</td>
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### Leptospira hardjo—Cattle

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<tr>
<td>FY 1965</td>
<td>481</td>
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<td>550</td>
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<tr>
<td>FY 1967</td>
<td>139</td>
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### Leptospira autumnalis—Cattle

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<tr>
<td>FY 1966</td>
<td>330</td>
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<tr>
<td>FY 1967</td>
<td>184</td>
<td>2,422</td>
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### Leptospira hebdomadis—Cattle

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<th>Positive</th>
<th>Negative</th>
<th>% Positive</th>
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</thead>
<tbody>
<tr>
<td>FY 1965</td>
<td>125</td>
<td>1,941</td>
<td>6.1</td>
</tr>
<tr>
<td>FY 1966</td>
<td>105</td>
<td>2,911</td>
<td>3.5</td>
</tr>
<tr>
<td>FY 1967</td>
<td>53</td>
<td>2,641</td>
<td>2.0</td>
</tr>
</tbody>
</table>

### Leptospira grippotyphosa—Cattle

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Negative</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY 1965</td>
<td>66</td>
<td>2,000</td>
<td>3.1</td>
</tr>
<tr>
<td>FY 1966</td>
<td>142</td>
<td>2,652</td>
<td>5.1</td>
</tr>
<tr>
<td>FY 1967</td>
<td>29</td>
<td>2,665</td>
<td>1.1</td>
</tr>
</tbody>
</table>

### Leptospira ballum—Cattle

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Negative</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY 1967</td>
<td>100</td>
<td>2,504</td>
<td>3.8</td>
</tr>
</tbody>
</table>

### Leptospira pomona—Swine

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Negative</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY 1965</td>
<td>4</td>
<td>63</td>
<td>6.0</td>
</tr>
<tr>
<td>FY 1966</td>
<td>14</td>
<td>70</td>
<td>16.7</td>
</tr>
<tr>
<td>FY 1967</td>
<td>28</td>
<td>134</td>
<td>17.3</td>
</tr>
</tbody>
</table>

### Leptospira icterohemorrhagiae—Swine

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Negative</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY 1965</td>
<td>23</td>
<td>37</td>
<td>38.3</td>
</tr>
<tr>
<td>FY 1966</td>
<td>22</td>
<td>62</td>
<td>26.2</td>
</tr>
<tr>
<td>FY 1967</td>
<td>28</td>
<td>133</td>
<td>17.4</td>
</tr>
</tbody>
</table>
Leptospira hardjo—Swine

<table>
<thead>
<tr>
<th></th>
<th>FY 1965</th>
<th>FY 1966</th>
<th>FY 1967</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>28</td>
<td>48</td>
<td>22</td>
</tr>
<tr>
<td>Negative</td>
<td>39</td>
<td>36</td>
<td>139</td>
</tr>
<tr>
<td>% Positive</td>
<td>41.8</td>
<td>57.1</td>
<td>13.7</td>
</tr>
</tbody>
</table>

Leptospira autumnalis—Swine

<table>
<thead>
<tr>
<th></th>
<th>FY 1965</th>
<th>FY 1966</th>
<th>FY 1967</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>4</td>
<td>25</td>
<td>41</td>
</tr>
<tr>
<td>Negative</td>
<td>43</td>
<td>59</td>
<td>112</td>
</tr>
<tr>
<td>% Positive</td>
<td>8.5</td>
<td>29.8</td>
<td>26.8</td>
</tr>
</tbody>
</table>

Leptospira hebdomadis—Swine

<table>
<thead>
<tr>
<th></th>
<th>FY 1965</th>
<th>FY 1966</th>
<th>FY 1967</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>62</td>
<td>84</td>
<td>161</td>
</tr>
<tr>
<td>% Positive</td>
<td>7.5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Leptospira grippotyphosa—Swine

<table>
<thead>
<tr>
<th></th>
<th>FY 1965</th>
<th>FY 1966</th>
<th>FY 1967</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Negative</td>
<td>61</td>
<td>62</td>
<td>151</td>
</tr>
<tr>
<td>% Positive</td>
<td>9.0</td>
<td>26.2</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Sera from other species of domesticated animals have been similarly tested during this period with some positive results but the numbers are too small to lend much significance to them. Periodic surveys have been conducted on wildlife in cooperation with the Conservation Department. Considerable serologic evidence was found as to the existence of several serotypes of Leptospira in deer and fox in Wisconsin.

Serum samples submitted to the laboratory for routine testing for intrastate or interstate movement and for private purposes are subjected to the Stoener Plate Test as a screening test and all positives are tested by the agglutination-lysis method for *L. pomona* only. These three year results are:

Leptospira pomona serology—routine samples

<table>
<thead>
<tr>
<th></th>
<th>FY 1965</th>
<th>FY 1966</th>
<th>FY 1967</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive &amp; AL tube positive</td>
<td>1,123</td>
<td>893</td>
<td>227</td>
</tr>
<tr>
<td>Stoener Plate Negative</td>
<td>8,027</td>
<td>7,807</td>
<td>5,908</td>
</tr>
<tr>
<td>% Positive</td>
<td>12.3</td>
<td>10.3</td>
<td>3.7</td>
</tr>
</tbody>
</table>
Leptospira pomona serology—routine samples

Swine

<table>
<thead>
<tr>
<th></th>
<th>Stoenner plate</th>
<th>AL tube positive</th>
<th>Negative</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY 1965</td>
<td>32</td>
<td>3,193</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>FY 1966</td>
<td>63</td>
<td>2,703</td>
<td></td>
<td>2.3</td>
</tr>
<tr>
<td>FY 1967</td>
<td>35</td>
<td>2,631</td>
<td></td>
<td>1.3</td>
</tr>
</tbody>
</table>

A number of canine and feline sera are tested each year for the previously discussed serotypes plus L. canicola. Of interest is the fact that only one positive canine and no positive feline samples have been recorded during this three-year period for L. canicola.

Isolation attempts by use of Ellinghausen Media are now being carried out in collaboration with serological procedures on cases exhibiting clinical symptoms, but results have not been completed in sufficient quantities to be of significance.

Attempts at explanation of the marked decrease in percent of positives during the past year are unsatisfactory. It is known, however, that a considerable increase in vaccination with available vaccines has been practiced during the past two or three years. I believe evidence gained from this summary as well as evidence being accumulated in many other parts of the country should give credence to the desire of many veterinary practitioners for the licensing of vaccines prepared from species of Leptospirae other than those now permitted.
LEPTOSPIRA HARDJO INFECTIONS IN CATTLE

L. E. Hanson, D.V.M., Ph.D. and B. O. Brodie, D.V.M., M.S.
Urbana, Illinois

Leptospirosis has been considered a major disease of cattle in the United States for approximately 20 years. During this period, *L. pomona*, since its isolation in the U. S. in 1948, has been accepted as the primary leptospiral serotype affecting cattle and swine in the United States. However, a number of studies have indicated that *L. hardjo* is also widely distributed in the cattle population in the U. S. and in Canada. Roth reported the first isolation of *L. hardjo* in the United States in 1960 and suggested that it was apparently responsible for the positive *L. sejroe* reactions previously obtained with many cattle sera. Alexander and Evans (1962) confirmed that *L. sejroe* titers were the result of *L. hardjo* infections. However, Roth, *et al.* (1960) were not able to demonstrate a definite relationship between *L. hardjo* infections and clinical signs in cattle. Later Sulzer, *et al.* (1964) in Nebraska isolated *L. hardjo* from the urine of a cow following an abortion. Robertson, *et al.* (1964) in Canada also isolated *L. hardjo* from a cow following an abortion. In Illinois (Hanson, *et al.*, 1965) *L. hardjo* has been isolated from the urine of a cow following an abortion and from kidney tissues of 2 feeder cattle (Martin, *et al.*, 1967) not exhibiting related signs.

The purpose of this paper is to provide additional information concerning the pathogenesis of *L. hardjo* infections.

**Material and Methods**

Sera collected from cattle located on 2 state institutional farms and sera submitted by practicing veterinarians were tested with the microscopic agglutination (MA) test. The microscopic agglutination test was conducted according to the procedure described by Roth, *et al.* (1960) utilizing 5 leptospiral serotypes: *L. hardjo*, *L. grippotyphosa*, *L. canicola*, *L. icterohaemorrhagiae* and *L. pomona*. In addition *L. hyos*, *L. ballum* or *L. autumnalis* were also used for varying intervals.

Liquid and semisolid albumin polysorbate medium (Ellinghausen, *et al.*, 1965) was used for cultivation of leptospires from urine, blood and tissues of field and experimental animals. New isolants were first identified with known antisera and confirmation was obtained from Dr. A. M. Alexander at the Walter Reed Army Institute of Research WHO/FAO Leptospiral Reference Laboratory.

The animal exposure studies were conducted with newly purchased calves and pregnant cows demonstrated to be serologically negative to *L. hardjo*, *L. pomona*, *L. grippotyphosa* and *L. canicola*. The animals were maintained in isolation units throughout the observation periods. The 4 calves were inoculated with isolant 2966-421, originally isolated from the
urine of a cow a few days following an abortion (Hanson, et al., 1965). The culture had been maintained in liquid and semisolid medium for approximately 2 years prior to inoculation of the calves. Two calves (7932 and 7933) were inoculated intraperitoneally with 1 ml. of the liquid culture of isolate 2966-421. The third calf (240) received 1½ ml. intraperitoneally and a few drops on a conjunctival membrane. The fourth calf (241) was exposed by placing 2 drops of liquid leptospiral culture on a scarified conjunctival membrane.

Two pregnant cows were utilized in exposure studies. The first cow was a Jersey in approximately 9 months of its gestation period and the second cow was a Holstein in approximately 80 days of its gestation period. The culture (SC 1769) was isolated from a steer (Martin, et al., 1967) and had been cultivated in media for only a few serial passages. Both animals were inoculated intracervically with cultures of leptospires administered with a syringe attached to a plastic inseminating tube.

Tissues collected at autopsy were fixed in 10 percent formalin. The tissue sections were stained with both the hematoxylin and eosin stain and with the Levaditi's method.

Results

Serologic studies—

Serologic tests conducted on sera submitted to the State Diagnostic Laboratory from 1959 to 1966 indicate *L. hardjo* is a common serotype in Illinois cattle (Table I). The reactor rates varied from a low of 6.1 per-

![Graph showing reactor rates of L. hardjo in Illinois cattle sera](image)
cent in 1962 to a high of 22.1 percent in 1966 with an average reactor rate of 13.4 for the 8 year period. A comparison study of *L. pomona* and *L. hardjo* reactor rates in one large state herd at the Dixon Springs Agricultural Research Center shows no correlation between the reactor rates of the 2 leptospiral serotypes (Table II). All animals in the herd were vac-

![Graph showing reactor rates](image)

**TABLE II** Annual Reactor Rates of *L. pomona* and *L. hardjo* in the Dixon Springs Agricultural Center Cattle Herd

inated with *L. pomona* bacterin since 1964 with an apparent reduction in *L. pomona* activity as shown by a low reactor rate since 1964. In contrast *L. hardjo* remains very active as shown by high reactor rates and isolation of organisms from the kidney of 2 steers this past year.

In an attempt to collect clinical information involving cattle with *L. hardjo* antibodies, a herd history was requested of each veterinarian submitting positive *L. hardjo* serum samples. A summary of the information from the history reports is shown in Table III. One thousand and forty-four sera were submitted from 58 herds and represented 29 percent of the total herd populations. Abortions were reported in 82.7 percent of the herds and 89.7 percent of the herds had abortions and/or breeding problems. Abnormal milk was reported from only one herd.

Eight *L. hardjo* isolations have been made from cattle from 4 herds in Illinois. One of the 8 isolations was made from the urine of a cow following an abortion (Hanson, et al., 1965). The remaining isolations were made from kidney homogenates of animals collected at time of slaughter. The sera of all 8 infected animals had *L. hardjo* antibodies present.
Experimental Disease in Calves and Cows

Calves 7932 and 7933 which were inoculated intraperitoneally with 1 ml. of a liquid culture of isolant 2966-421 had no clinical signs. Leptospires (L. hardjo) were isolated after 2 months of incubation from blood collected from both calves on the second and fourth day following inoculation.

| TABLE III |
| HISTORIES ASSOCIATED WITH CATTLE HERDS WITH POSITIVE L. hardjo MICROSCOPIC AGGLUTINATION REACTIONS |

<table>
<thead>
<tr>
<th>Number Herds</th>
<th>Sera Tested</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>0/0 of Herd</td>
</tr>
<tr>
<td></td>
<td>Sera Sample</td>
<td>Sample Rate</td>
</tr>
<tr>
<td>58...........</td>
<td>1,044</td>
<td>29.0%</td>
</tr>
</tbody>
</table>

No isolations were made from urine samples. The first detectable titers (1:100) were demonstrated in sera collected 11 days post inoculation. The kidneys of both calves had white foci present at necropsy examination. An interstitial nephritis was present in kidney tissues. No leptospires were isolated from the kidneys or observed in tissue sections stained with the Levaditi's method.

No clinical signs were observed in either calf 240 which was inoculated both intraperitoneally and on the conjunctiva and calf 241 inoculated on scarified conjunctival tissue. No leptospires were isolated from calf 240 and the calf failed to develop a MA titer at the time of the necropsy examination 19 days post inoculation. Leptospires were isolated after 4 months of incubation from a blood sample collected from calf 241 8 days post exposure. The serum collected on day 12 had a MA titer of 1:10 and a MA titer of 1:1000 4 days later. The calf when necropsied 21 days following exposure had numerous white foci present in both kidneys. A moderate interstitial nephritis was observed in the histologic sections. No isolation of leptospires was made from the tissues taken at necropsy.

Experimental Infections Conducted in Pregnant Cattle

In the Jersey cow inoculated via the cervix with 1 ml. of a semisolid culture L. hardjo (SC 1769) at 9 months of the gestation period, no clinical signs were observed during the first 10 days following inoculation. On the eleventh day the cow's body temperature increased to 104.6, the urine was blood tinged and a premature calf was delivered. The calf died 15
minutes after birth and was necropsied. The serum sample taken on the same day was negative but a sample taken 10 days later had a microscopic agglutination titer of 1:10,000 for *L. hardjo*.

Leptospires were observed in the medium inoculated with the blood tinged urine after 5 months of incubation. No leptospires were isolated from blood or milk samples of the cow or from tissues from the calf. A moderate interstitial nephritis was present in the histologic sections of the kidneys of the calf.

The Holstein cow inoculated via the cervix with *L. hardjo* isolant SC 1769 at approximately 80 days of the gestation period, had no observable clinical signs for 20 days post inoculation. On the 21st day a dead fetus was aborted. Serum samples taken on day 13 had *L. hardjo* MA titer of 1:10 on day 19 and a titer of 1:1000 on day 21. No leptospires were isolated from the fetal tissues or from blood and urine samples of the cow.

**Discussion and Summary**

The serologic evidence reported in this paper indicates *L. hardjo* is a common leptospiral serotype in Illinois cattle as the yearly reactor rates varied from a low of 6 percent to as high as 22.1 percent. The findings are consistent with results reported by other workers (Galton, *et al.*, 1956; Hoag, 1957; Byrne and Chamber, 1959).

Information obtained from veterinarians concerning the clinical signs associated with herds containing cattle with *L. hardjo* antibodies indicated abortions and stillbirths occurred in 82.7 percent of the herds. However, the associated abortion rate within the herds was much less than anticipated with *L. pomona*. Repeat breeding was also associated with *L. hardjo* infections. Although abnormal milk was reported as occurring in only 1 herd, Mitchell, *et al.* (1960) had previously observed mastitis in 4 of 12 cows from 1 herd.

The experimental studies conducted in young calves and pregnant cows indicated *L. hardjo* was less pathogenic than *L. pomona*. *L. hardjo* organisms were isolated from the blood of 3 of the 4 calves but no associated clinical signs were observed. However, both gross and microscopic lesions were present in the kidneys of the infected calves.

The pregnancy of both cows was interrupted following inoculation of leptospires in the cervix. The Jersey cow inoculated late in the pregnancy period delivered a live weak calf that died shortly after birth. The second cow which was inoculated in the first quarter aborted a dead calf and no organisms were isolated from the cow or calf. Although inoculation, via the cervix, provided a severe stress, the isolation of leptospires from the cow's urine and the presence of interstitial nephritis in the kidneys of the premature calf indicated a systematic infection with *L. hardjo* occurred in the Jersey cow.

The extended periods of incubation required to grow some of the *L. hardjo* cultures is similar to results obtained by Roth, *et al.* (1960) and Robertson, *et al.* (1964).
LEPTOSPIRA HARDJO

College of Veterinary Medicine, University of Illinois.

This investigation was supported in part by Federal Hatch Grant 70-302 and Communicable Disease Center Grant COO190. The authors would like to thank the many veterinary practitioners who provided the sera and herd history information and the technical assistance of Rachel Marlowe, W. R. Manuel and M. K. Coad.

REFERENCES

REPORT OF THE COMMITTEE ON LEPTOSPIROSIS


The committee reviewed the previous years report and information that has become available during the current year. Infections with *Leptospira pomona* remain the predominant cause of leptospirosis in livestock. Additional bacteriologic and serologic evidence has accumulated indicating that important economic losses are caused by *L. grippotyphosa* and *L. hardjo* infections of cattle and *L. grippotyphosa* infections of swine. Therefore it is recommended that: (a) all diagnostic laboratories routinely examine sera for the presence of *L. grippotyphosa* and *L. hardjo* agglutinins, (b) prophylactic agents be made available for immunizing cattle and swine against infections with these serotypes, and (c) diagnostic laboratories maintain and/or continue programs of surveillance for other leptospiral serotypes known to be present in the United States.

Leptospirosis is enzootic in nature, occurring cyclically in geographic areas. The broad spectrum of hosts, both domestic and wild animals, contributes to the complexity of evaluating the incidence of infections. Current testing and reporting procedures do not adequately reflect the actual incidence of leptospirosis.

The accepted serologic procedure is the microscopic agglutination (agglutination-lysis) test, and diagnostic laboratories are urged to adopt it as this test provides valid information on the status of individual animals and herds. In the establishment of herd diagnosis selective sampling is imperative to minimize the cost but still give meaningful serologic information. It is recognized that the microscopic agglutination test is expensive. However, the 1:100 serum dilution is satisfactory for screening large numbers of samples against multiple antigens. Caution should be exercised in the interpretation of serologic results obtained with macroscopic agglutination tests. The problem of obtaining valid information is more acute when polyvalent plate antigens are employed due to the occurrence of false reactions.

It is recognized that leptospiral agglutinins may persist for months or years in most species of livestock and that a specific diagnosis must be based on the relationship of clinical signs of leptospirosis with serologic evidence. The definitive diagnosis of leptospiral infections requires isolation of the specific serotype. Although serologic results may suggest that new serotypes are present in livestock, isolation and identification are essential to determine their actual relationship to disease. The United States Department of Agriculture, Animal Health Division, National Animal Disease
Laboratory, Ames, Iowa has established a leptospiral typing laboratory to assist in characterizing isolates.

Due to the increasing clinical, serologic and bacteriologic evidence of *L. grippotyphosa* and *L. hardjo* infections, the committee recommends that temporary licenses be issued to interested biological companies to produce a polyvalent bacterin (*L. pomona*, *L. grippotyphosa* and *L. hardjo*) for use in cattle. It is further recommended that the biologic activity for each of the serotypes included in the polyvalent bacterin be at least equivalent to that in currently available monovalent *L. pomona* bacterin. Efficacy testing of currently available bacterins in the specific species for which they are recommended should be continued and extended to include the polyvalent bacterin.

Financial support for research on the epizootic, prophylactic and chemotherapeutic aspects of the disease is necessary to maintain an effective research effort.

The committee wishes to reiterate the opinion expressed in previous reports that leptospirosis is not amenable to eradication because of the numerous serotypes encountered, the difficulty of detecting carrier animals and the wide range of domestic and wild animals hosts.

We respectfully submit this report to the Executive Committee for approval and suggest that the work of this Committee be continued.
NEW ZEALAND’S PRE-PLANNING TO COMBAT FOREIGN ANIMAL DISEASES
S. JAMIESON, M.R.C.V.S., D.V.S.M., Ph.D.
Director, Animal Health Division,
Department of Agriculture, New Zealand

Before the discovery of New Zealand by Europeans the only mammals present were two species of dog, one species of rat and two of bats. Captain Cook is credited with the first introduction of domestic stock in 1769 when he presented two pigs to a Maori chief. From then until 1814 desultory gifts of domestic stock were made, mostly pigs and goats and a few sheep. In 1814 the Reverend Samuel Marsden brought with him from New South Wales, Australia, cattle, sheep, poultry, cats and dogs, turkeys and geese, and in January 1815 the first calf was born in New Zealand. Mr. Marsden said at the time, “Milk, butter, beef and labour these cattle will soon produce to the inhabitants; and if the number of settlers should be increased, they will greatly promote their support and comfort.” When Darwin visited New Zealand in 1835 he recorded seeing many cattle, sheep and pigs. Today New Zealand supports 60 million sheep, six and three-quarter million cattle, 5 million poultry, three-quarter million pigs and two and three-quarter million humans. Mr. Marsden proved to be a prophet.

It was characteristic of the early settlers that the quarantine of imported stock was established before even the establishment of a state veterinary service or a department of agriculture.

Thus New Zealand, because of its geographical isolation, the establishment of early quarantine controls, and the early introduction of animal disease control legislation in 1849, today enjoys an excellent record of freedom from the major diseases which beset domestic stock. Sheep Scab was introduced in 1840 and eliminated in 1885. The first outbreak of Swine Fever was recorded in 1933 around the port of Wellington and was eliminated in a comparatively short time by the total slaughter of 1,920 infected or in-contact pigs. Since then one isolated case occurred in 1953 around the port of Auckland. Since 1953 New Zealand has been free of Swine Fever and a recent serological survey (gel test) indicates that the position has been maintained. Pleuro-pneumonia was introduced in 1861 in cattle from Australia but was rapidly eradicated by slaughter by 1864.

In 1952 scrapie was diagnosed in Suffolk sheep imported from the U.K. two years previously. The disease was eradicated by 1954 and all infected or in-contact farms were quarantined for three years.

New Zealand has never experienced foot-and-mouth disease, rinderpest, Newcastle disease, rabies, sheep pox or tick fevers of any species, equine anaemia or bluetongue, and is free of scrapie, swine fever, and pleuro-pneumonia. The prime purpose of the Animal Health Division in New Zealand is to maintain this happy situation.

Historically, New Zealand’s animal disease legislation has always placed...
considerable emphasis upon measures designed to prevent foreign animal diseases entry into the country, and because of this and our isolation the popular belief arose that we had done all that needed to be done to preserve our now accepted traditional freedom from foreign animal diseases. About 15 years ago, however, the then Assistant Director of the Division, Mr. A. D. G. M. Laing, promoted the need to consider our ability to handle and control foreign animal disease. In this endeavour he had some measure of success, but it was not until the last five years that the implications of this problem came to be fully realised.

Foot-and-Mouth Disease Control. Veterinarians’ Duties at Infected Place.

It is recognised today, as a result of world experiences, that no matter how efficient animal import control and quarantine is designed legislatively and efficiently applied, the end-result is never absolute and the probability of the entrance of foreign animal diseases exists. This probability is enhanced in recent years by the increasing global rapidity of travel and the increasing movement of animals and people. Distance is no longer a favourable factor in the formulation of animal quarantine principles.

PRE-PLANNING AND DIAGNOSTIC AND CONTROL METHODS

Some few years ago a review of the aspects of foreign animal diseases control was initiated in the Animal Health Division. This started by the examination of the following:

(a) The existing legislation.
(b) The strategic distribution of Animal Health staff.
(c) The ability of Animal Health staff to recognise foreign animal diseases and to implement immediately control measures.
(d) The integration of other Government departments, which have specialist knowledge and personnel into any scheme of national foreign animal disease control.

(a) The Existing Legislation

In the event of an outbreak of serious foreign animal disease the principle of eradication by total slaughter of affected and in-contact animals has always been accepted in New Zealand, and the legislation designed accordingly. Powers exist also for the proclamation of an animal disease emergency to exist either in any part or whole of New Zealand as the gravity of the situation demands. Powers also exist to conscript any registered veterinarian residing in New Zealand or any fit male over the age of 18 years residing within 5 miles of an Infected Place to render assistance to prevent the spread or limit the disease.

Critical Paths of Foot-and-Mouth Disease Control in Freezing Works and Abattoirs.

In reviewing the legislation the only major defect was the absence of Regulations detailing the specific actions to be taken in an Infected Place and Infected Area, particularly in relation to foot-and-mouth disease.

Consequently, foot-and-mouth disease control regulations came into force in 1966 and detail the actions on Infected Places and Infected Areas as described in Appendix I.

The basic principles in our plan of control of highly infectious diseases are:

1. Slaughter of infected and in-contact animals at fair market value compensation.
2. Total prohibition of movement of animals, people, and things on any Infected Place.
NEW ZEALAND'S PRE-PLANNING

(3) The immediate imposition of an Infected Area of 30 miles radius around any Infected Place, in which the movement of livestock is totally prohibited except under licence issued by a veterinary Inspector with powers to extend this area to meet the exigencies of the disease situation.

(4) The cleansing and disinfection of all Infected Places after slaughter and disposal of carcasses.

(b) The Strategic Distribution of Animal Health Staff

The combined national coverage of Animal Health veterinary staff and Meat Division veterinarians gives a well distributed availability in all regions of New Zealand except in some of the more isolated hill farming areas. Those areas, however, are serviced by experienced lay inspectors, trained to think of the possibility of foreign animal diseases. To supplement those areas in particular a scheme of part-time employment of veterinarians in practice was instituted by the Department some three years ago so that in an emergency the services of any veterinarian can be called upon.

The logical culmination in considering the distribution of staff to meet foreign disease emergencies was to review their availability at all times. The United Kingdom system of having staff compulsorily available at week-ends and holiday periods has undoubtedly contributed materially to its ability to control and eradicate foot-and-mouth disease with rapidity.

Today New Zealand has a "Stand By On Call Service" on all week-ends and holiday periods. This service is integrated with the local district
police services so that any farmer, in any part of New Zealand, who suspects the existence of a foreign animal disease at any time, has now a rapid contact with the State veterinary service.

(c) The Capabilities of Animal Health Staff

Some important problems were evident in the development of plans for the control and eradication of foreign animal diseases. Because of our freedom from major animal diseases, veterinarians in New Zealand have had no practical experience in the field diagnosis of these diseases, nor in the application of control measures. Recognising the necessity for rapid recognition of foreign animal diseases, the New Zealand Government instituted a scheme of overseas training for departmental veterinary staff and for veterinary practitioners, with particular emphasis on the field diagnosis of foot and mouth disease. For these facilities we have to thank our veterinary colleagues in Thailand, and for specialised training at Gros Ilc, Canada, Plum Island, the U.S.A. and Pirbright in the United Kingdom.

The production of manuals of instructions and the pre-planning of procedures were relatively simple, but the haunting question remained as to whether a paper-army, however enthusiastic, could cope when suddenly faced with the fact that foreign animal disease had entered New Zealand.

My own experience in the United Kingdom convinced me that without continuing training in the practice of control methods then our efforts would fail. Consequently, a permanent training school has been set up in New Zealand and to date 60 veterinarians from general practice and all
departmental veterinarians have undertaken a course of instruction in foreign animal diseases control.

The course is concerned primarily with the field procedures of disease control and this is ably assisted by the farmers in the area. The location is on a Government farm which becomes the Infected Place and all farmers within a radius of 10 miles co-operate to the fullest extent by allowing their farms to comply with the restrictions and examinations which would factually take place in a genuine Infected Area. At this course veterinarians are trained in the practical application of the Foot-and-Mouth Disease Regulations of 1966 and the operation of administrative manual instructions. It is our aim to train, by an intake of about 60 veterinarians per year, all veterinarians in New Zealand, whether Government employed or not, and to make the course a continuing one.

The operation of a field training school with its involvement of some 20 farms highlighted the need to study such matters as the normal movement of animals, the rapidity with which traceback procedures could be expected to produce results, the relationships of domestic stock with wildlife, the rapidity of laboratory diagnosis and above all the education of the farming community without creating a national state of alarm.

THE MOVEMENT OF ANIMALS AND TRACEBACK PROCEDURES

At certain times of the year there are massive movements of stock to export freezing works for slaughter. This movement presented little problems to our purposes because these animals are all well documented as to their origin and are subject to ante- and post-mortem examination.

A review of the movement of animals at saleyards showed that the majority were moved within a radius of 50 miles of the saleyard, and that although there are 218 stock saleyards in use the origin and destination of these animals were reasonably well documented so that it does appear that traceback procedures should produce a high percentage of success. In addition, the Department of Agriculture has promoted the need for farmers to maintain a register recording their individual purchases and sales of stock.

The question of wildlife control in relation to an outbreak of foreign animal disease was the subject of joint study by the Department of Forest Services and the Animal Health Division. Members of the Forest Services have attended our courses of instruction and a manual of procedure has been produced so that in the event of an outbreak of foreign animal disease Forest Service personnel know exactly what their duties are, and are trained to carry them out.

The final planning was done with representatives of the Police and the Transport Department, the members of which will be responsible for the setting up of road blocks and disinfecting units in infected areas.

Since few serious diseases exist in New Zealand it has always been Government policy to depend on overseas diagnosis for foreign animal disease like foot-and-mouth disease, rinderpest, and pleuro-pneumonia. It is estab-
lished Divisional policy that where there is good clinical evidence to suspect an outbreak of serious animal disease the diagnosis would be made clinically and measures taken to eradicate the disease without necessarily waiting on laboratory confirmation. For instance, any vesicular disease in ruminants would be regarded as foot-and-mouth disease and treated as such in the first instance. Since vesicular stomatitis does not exist in New Zealand it would be as important to us to eradicate the disease by slaughter as it would be to eradicate foot-and-mouth disease.

We have demonstrated that if samples are sent from New Zealand at midday, New Zealand time, to Pirbright in the United Kingdom, the preliminary results can be obtained within 60 hours New Zealand time.

**SUMMARY**

The New Zealand Department of Agriculture is fully aware of its responsibility to prevent foreign animal diseases entering New Zealand, but realistically considers that it is necessary to have a highly organised programme of control and eradication in the event of these diseases entering the country. To this end it has aimed at the overseas training of staff in the recognition of foreign animal diseases and has concentrated its programme on the training of personnel in the procedures to be adopted and used in the field in the eradication of foreign animal diseases. The outbreak of a serious animal disease like foot-and-mouth disease is regarded in New Zealand as a national emergency and all Government departments which have a part to play have been brought into the pre-planning of procedures of duties.

New Zealand has shown its ability to eradicate disease in the past and it is certain that in the event of a serious outbreak of disease all those concerned will know exactly what their duties are, and should be able at a moment's notice to implement efficiently the procedures necessary to control and eradicate serious disease. The final stages in planning have been the complete documentation on all veterinarians in New Zealand as to their experience and ability in the recognition of serious animal diseases and to the setting up of a single sheet critical path procedure which is available to all veterinarians in New Zealand.

The main policy of the New Zealand Animal Health Division is to keep New Zealand free of serious animal diseases, preferably by preventing the entrance of those diseases, but if they do enter to be as highly trained and skilled as possible to ensure rapid and complete eradication.
THE INCIDENCE OF NEUTRALIZING ANTIBODIES TO DUCK PLAGUE* VIRUS IN SERUMS FROM DOMESTIC DUCKS AND WILD WATERFOWL IN THE UNITED STATES OF AMERICA

A. H. DADBIRI and W. R. HESS

Duck plague is a disease which has not been previously detected in the United States. Outbreaks have been reported in Holland from 1923 to 1963, in China in 1958,1 in India in 1963,2 and Belgium in 1964.3

In January, 1967, the Duck Research Laboratory at Eastport, Long Island, New York, reported observing lesions suggestive of duck plague in ducks from commercial duck farms in Suffolk County, Long Island, New York.4 The disease was subsequently confirmed by exhaustive cross-protection and serologic tests conducted at the Plum Island Animal Disease Laboratory (PIADL)5. At that time, it appeared that the disease outbreak was confined to one commercial duck farm of 4 flocks.

Shortly thereafter, the virus was isolated from a dead mute swan found close to a stream to which the diseased ducks had access.

To determine the incidence of the disease among ducks raised commercially in Suffolk County, where approximately 66% of all the ducks in the United States of America are produced, personnel at the Animal Health Division, Agricultural Research Service, U.S. Department of Agriculture, collected blood samples from ducks on 12 major commercial duck farms. In addition, serum samples were obtained from ducks raised commercially in California, Illinois, Indiana, Michigan, and Ohio. Also, to establish the possible exposure of migrating birds to the disease and their role in its dissemination, blood samples were taken from wild waterfowl that were nesting on the Atlantic coast.

The disease occurred on 4 other Suffolk County commercial duck farms in March, April, May, and June, and also was detected on 2 premises where a variety of waterfowl species were kept. One of the latter premises is located outside Suffolk County.

In this report, the results of testing a total of 2936 serums from waterfowl are reported. The information gathered is prerequisite for establishing the relative number of ducks having neutralizing antibodies to duck plague virus. Thereby, the question of whether this disease is alien or endemic in this country may be resolved.

MATERIALS AND METHODS

Virus. Chicken embryo-adapted duck plague virus was utilized in all

*The Long Island duck disease was designated duck virus enteritis by New York State and ARS regulatory officials (9-Code of Federal Regulations—Part 83—duck virus enteritis (duck plague)).


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It was obtained as chorio-allantoic membranes (CAM) from infected chicken embryos (CE) through the courtesy of Dr. Jac. Jansen of the Institute of Infectious Diseases, Utrecht, The Netherlands. Stock virus was prepared by inoculation of 8- to 9-day-old CE into the chorio-allantoic sac (CAS) and collection of the CAM and chorio-allantoic fluid (CAF) from dead embryos. A 20% suspension was prepared from the CAM with CAF and Hanks’ balanced salt solution (BSS). After centrifugation at 800 g for 20 minutes at 6°C, the supernatant fluid was divided into small aliquots, placed in glass containers and stored at -40°C until used.

**Serums.** A total of 2936 serum samples were tested and their sources and geographical locations are presented in Tables I, II, and VII.

### TABLE I

**Source of Serums from Ducks on Commercial Farms That Were Tested for Duck Plague Antibodies**

<table>
<thead>
<tr>
<th>State</th>
<th>County</th>
<th>Number of Farms</th>
<th>Number of Serums</th>
</tr>
</thead>
<tbody>
<tr>
<td>New York</td>
<td>Suffolk, L. I.</td>
<td>13</td>
<td>1,726</td>
</tr>
<tr>
<td>Ohio</td>
<td>Wyandot</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>Indiana</td>
<td>La Porte</td>
<td>1</td>
<td>52</td>
</tr>
<tr>
<td>Indiana</td>
<td>Kosciusko</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>Illinois</td>
<td>Piatt</td>
<td>1</td>
<td>104</td>
</tr>
<tr>
<td>California</td>
<td>San Bernardino</td>
<td>1</td>
<td>69</td>
</tr>
<tr>
<td>California</td>
<td>Riverside</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>California</td>
<td>Sonoma</td>
<td>1</td>
<td>131</td>
</tr>
<tr>
<td>Michigan</td>
<td>Ottowa</td>
<td>12</td>
<td>336</td>
</tr>
</tbody>
</table>

| Total      |               | 2,501           |

**Serums from waterfowl in the field:** Blood specimens were collected from waterfowl by personnel of the Animal Health Division, ARS, USDA. They were placed in sterile, rubber-stoppered, glass, vacuum tubes. Whole blood was obtained from waterfowl in New York State and delivered to PIADL within 24 hours. Upon arrival, the serums were separated by centrifugation, placed in sterile containers, identified, and stored at -20°C until tested. Samples from areas outside New York State were submitted as serums.

**Serums from experimentally-infected domestic ducks:** The serums were taken from 34 ducks of a larger group of breeder ducks that had been exposed experimentally to the disease. The 34 ducks had disease signs and had recovered, whereas the majority of the group died. Blood samples were obtained by cardiac puncture at 0, 21, and 42 days postinoculation (DPI); the ducks were clinically healthy at the time the blood was drawn.

**Duck plague immune serums:** Hyperimmune serum was prepared
by inoculation of attenuated virus* in susceptible, mature, Peking ducks. Following repeated challenge with a virus which is virulent for ducks,* serum from each duck was obtained and tested for serum-neutralizing antibodies. A serum pool was made from those sera that were neutralized by $10^5$ embryo lethal doses (ELD)$_{50}$ of the virus.

### TABLE II

**SOURCE OF SERUMS FROM WILD WATERFOWL THAT WERE TESTED FOR DUCK PLAGUE ANTIBODIES**

<table>
<thead>
<tr>
<th>State</th>
<th>County</th>
<th>Species</th>
<th>Number of Serums</th>
</tr>
</thead>
<tbody>
<tr>
<td>New York</td>
<td>Suffolk, L. I.</td>
<td>Ducks</td>
<td>60</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>Essex</td>
<td>Ducks</td>
<td>39</td>
</tr>
<tr>
<td>New Hampshire</td>
<td>Rockingham</td>
<td>Ducks</td>
<td>1</td>
</tr>
<tr>
<td>Delaware</td>
<td>Bombay Hook Island</td>
<td>Ducks</td>
<td>55</td>
</tr>
<tr>
<td>Maryland</td>
<td>Talbot</td>
<td>Ducks</td>
<td>44</td>
</tr>
<tr>
<td>Maryland</td>
<td>Kent</td>
<td>Geese</td>
<td>100</td>
</tr>
<tr>
<td>New York</td>
<td>Suffolk</td>
<td>Swans</td>
<td>6</td>
</tr>
<tr>
<td>New Jersey</td>
<td>The Netherlands Through Clifton, N. J.</td>
<td>Swans</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>401</strong></td>
</tr>
</tbody>
</table>

**Normal duck serum:** Serums were taken from normal, mature and clinically healthy ducks raised in confinement at the Duck Research Laboratory at Eastport, Long Island. The progenies from these ducks were susceptible to duck hepatitis virus and their sera were not neutralized by this virus.$^6$

**Diluent.** Hanks' BSS with 0.5% lactalbumin hydrolyzate and 0.02% phenol red was used for the preparation and dilution of the reagents employed in these experiments.

**Virus neutralization tests.** The "constant-serum variable-virus" method to determine virus neutralization was used. All sera were heated for 30 minutes at 56°C before use, and diluted 1:5 for testing (1 part serum and 4 parts diluent). Serial 10-fold dilutions of virus were prepared. Equal volumes of the diluted virus and sera were mixed and incubated at 37°C for half an hour. Positive and negative control sera were employed for confirmation of each test.

The tests were conducted in 8- to 9-day-old embryonated eggs inoculated via the allantoic cavity with 0.2 ml of either the virus-serum mixture or virus control. Four eggs were inoculated per dilution. Deaths were recorded daily for 8 days and the ELD$_{50}$ calculated by the method of Reed

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*Supplied through the courtesy of Professor, Doctor Jac. Jansen; hoogleraar aan de Rijksuniversiteit te Utrecht; Biltstraat 168, Utrecht, The Netherlands.
and Muench. Any death during the first 24 hours of incubation was considered nonspecific. The difference between the log 10 virus control titer and the log 10 virus-serum titer was expressed as the FLD<sub>50</sub> neutralization index.

RESULTS

Distribution of virus-neutralizing antibodies in serums of ducks from Suffolk County, New York (Tables III and IV). Serums from farm SS were collected approximately 19 days following observation of the clinical signs of disease. Those from farm VK were obtained 35 days following appearance of signs of disease and isolation of duck plague virus from the diseased flock. Blood samples were drawn from ducks on farm RP in January, 1967, and again in April. An outbreak of duck plague, confirmed by virus isolation, occurred in March. There was a low degree of mortality. Approximately 40 days later, blood samples were drawn from the surviving ducks. As indicated in the data presented in Tables III and IV, 33% to 60% of the ducks on farms where the disease was diagnosed positively had a virus neutralization index (VNI) in the range of 1.75 to >3.0. In contrast, 100% of the serums which were obtained from farm RP in January, before the outbreak of disease, were in the 0.0 to 1.5 VNI range. It is important to point out that 57% of the serums were in the 0.0 to 0.25 range and that the frequency in the 0.5 to 1.5 range decreased gradually and was 0.6% in the latter range.

Twenty-six, 4.4, and 1.8 percent of the serums of the ducks on 3 other farms (C&R, FL, HFC) had VNI between 1.75 and >3.0. All the VNI of serums from ducks on the remaining 7 farms were in the 0.0 to 1.0 range.

From the data presented in Table 3, it is evident that a low level of antibodies to duck plague virus is usually present in ducks; however, subsequent to exposure to the virus, a certain proportion of the ducks developed an immune response resulting in a VNI frequency at the 1.75 to 3.0 range (Table IV).

Distribution of virus-neutralizing antibodies in serums of domestic ducks from outside New York State. As may be seen (Table V), 100% of the duck serums from California, Illinois, Indiana, Michigan, and Ohio had a VNI in the range of 0.0 to 1.25 and approximately 90% of those had a VNI of 0.75 and less. There was no significant difference in the pattern of the distribution of the antibodies in the samples from the various states. There was no clinical evidence of duck plague infection in the flocks from which these serums were taken and tested.

Distribution of virus-neutralizing antibodies in wild ducks, geese, and swans. The pattern of antibody distribution among the wild waterfowl (Table VI) was similar to that described above for the domestic ducks that were not exposed to the disease. All serums from wild geese and swans, irrespective of their state origin, were in the 0.0 to 1.0 range except that 4% of the geese from Maryland were in the 1.25 range.

Distribution of virus-neutralizing antibodies in serums of experimen-
TABLE III

Frequency Distribution of Virus Neutralization Indices* of Serums in the
0.0 to 1.5 Range from Ducks on Long Island Duck Farms

<table>
<thead>
<tr>
<th>LONG ISLAND DUCK FARMS</th>
<th>Number of Serums Tested</th>
<th>Total Percent Frequency</th>
<th>Percent of Frequency Distribution at Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0-1.5</td>
<td>0.0-0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>SS</td>
<td>514</td>
<td>45.9</td>
<td>7.3</td>
</tr>
<tr>
<td>VK</td>
<td>106</td>
<td>67.0</td>
<td>15.0</td>
</tr>
<tr>
<td>RP (1)</td>
<td>160</td>
<td>100.0</td>
<td>57.0</td>
</tr>
<tr>
<td>(2)</td>
<td>110</td>
<td>39.1</td>
<td>6.4</td>
</tr>
<tr>
<td>C &amp; R</td>
<td>168</td>
<td>73.8</td>
<td>27.4</td>
</tr>
<tr>
<td>FL</td>
<td>136</td>
<td>95.6</td>
<td>58.0</td>
</tr>
<tr>
<td>HFC</td>
<td>113</td>
<td>98.2</td>
<td>79.6</td>
</tr>
<tr>
<td>HS</td>
<td>69</td>
<td>100.0</td>
<td>85.5</td>
</tr>
<tr>
<td>SO</td>
<td>29</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>AP</td>
<td>67</td>
<td>100.0</td>
<td>79.1</td>
</tr>
<tr>
<td>DD</td>
<td>77</td>
<td>100.0</td>
<td>97.4</td>
</tr>
<tr>
<td>JP</td>
<td>38</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>LB</td>
<td>42</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>CD</td>
<td>97</td>
<td>100.0</td>
<td>91.8</td>
</tr>
</tbody>
</table>

(1) and (2) = January and April bleedings.
*Log 10.
### TABLE IV

**Frequency Distribution of Virus Neutralization Indices* of Serums in the**

1.75 to > 3.0 range from Ducks on Long Island Duck Farms

<table>
<thead>
<tr>
<th>Long Island Duck Farms</th>
<th>Number of Serums Tested</th>
<th>Total Frequency 1.75-3.0</th>
<th>Percent of Frequency Distribution at Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.75</td>
<td>2.0</td>
</tr>
<tr>
<td>SS...</td>
<td>514</td>
<td>54.1</td>
<td>8.6</td>
</tr>
<tr>
<td>VK...</td>
<td>106</td>
<td>33.0</td>
<td>7.6</td>
</tr>
<tr>
<td>RP (1)</td>
<td>160</td>
<td>0.0</td>
<td>.</td>
</tr>
<tr>
<td>(2)</td>
<td>110</td>
<td>60.9</td>
<td>8.2</td>
</tr>
<tr>
<td>C &amp; R.</td>
<td>168</td>
<td>26.2</td>
<td>5.3</td>
</tr>
<tr>
<td>FL...</td>
<td>136</td>
<td>4.4</td>
<td>1.5</td>
</tr>
<tr>
<td>HFC.</td>
<td>113</td>
<td>1.8</td>
<td>0.9</td>
</tr>
<tr>
<td>HS.</td>
<td>69</td>
<td>0.0</td>
<td>.</td>
</tr>
<tr>
<td>SO...</td>
<td>29</td>
<td>0.0</td>
<td>.</td>
</tr>
<tr>
<td>AP...</td>
<td>67</td>
<td>0.0</td>
<td>.</td>
</tr>
<tr>
<td>DD...</td>
<td>77</td>
<td>0.0</td>
<td>.</td>
</tr>
<tr>
<td>JP...</td>
<td>38</td>
<td>0.0</td>
<td>.</td>
</tr>
<tr>
<td>LB...</td>
<td>42</td>
<td>0.0</td>
<td>.</td>
</tr>
<tr>
<td>CD...</td>
<td>97</td>
<td>0.0</td>
<td>.</td>
</tr>
</tbody>
</table>

(1) and (2) = January and April bleedings.

*Log 10.
TABLE V

FREQUENCY DISTRIBUTION OF NEUTRALIZATION INDICES* OF SERUMS IN THE 0.0 TO 1.5 RANGE FROM
DUCKS ON CALIFORNIA, ILLINOIS, INDIANA, MICHIGAN, AND OHIO FARMS

<table>
<thead>
<tr>
<th>State</th>
<th>Number of Serums Tested</th>
<th>Total Frequency</th>
<th>Percent of Frequency Distribution at Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.0-1.5</td>
<td>0.0-0.25</td>
</tr>
<tr>
<td>California</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC</td>
<td>69</td>
<td>100.0</td>
<td>55.0</td>
</tr>
<tr>
<td>WDC</td>
<td>11</td>
<td>100.0</td>
<td>81.8</td>
</tr>
<tr>
<td>ORC (1)</td>
<td>86</td>
<td>100.0</td>
<td>81.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illinois</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ID</td>
<td>104</td>
<td>100.0</td>
<td>67.0</td>
</tr>
<tr>
<td>Indiana</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GL</td>
<td>52</td>
<td>100.0</td>
<td>50.0</td>
</tr>
<tr>
<td>ML</td>
<td>45</td>
<td>100.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Michigan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>28</td>
<td>100.0</td>
<td>71.4</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>100.0</td>
<td>64.4</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>100.0</td>
<td>89.3</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>100.0</td>
<td>71.4</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>100.0</td>
<td>82.2</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>100.0</td>
<td>78.6</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>100.0</td>
<td>85.7</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>100.0</td>
<td>96.4</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>100.0</td>
<td>67.9</td>
</tr>
</tbody>
</table>

*Log 10.
TABLE V—Continued

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>100</th>
<th>96.4</th>
<th>3.6</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td></td>
<td>100</td>
<td>67.9</td>
<td>28.5</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Ohio (1)</td>
<td>14</td>
<td>64.3</td>
<td>28.5</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ohio (2)</td>
<td>13</td>
<td>61.5</td>
<td>30.7</td>
<td>7.8</td>
<td></td>
</tr>
</tbody>
</table>

(1) and (2) = First and second samples.

*Log 10.

TABLE VI

FREQUENCY DISTRIBUTION OF VIRUS NEUTRALIZATION INDICES* OF SERUMS IN THE 0.0 TO 1.5 RANGE FROM WILD DUCKS, GEESE, AND SWANS

<table>
<thead>
<tr>
<th>Source of Serum</th>
<th>Number of Serums Tested</th>
<th>Total Percent Frequency</th>
<th>Percent of Frequency Distribution at Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-0-1 5</td>
<td>0-0 25 0-50 0-75 1-00 1-25 1-50</td>
</tr>
<tr>
<td>Long Island</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild Ducks</td>
<td>60</td>
<td>100</td>
<td>51.7 30 16.7 1.6</td>
</tr>
<tr>
<td>Swans</td>
<td>6</td>
<td>100</td>
<td>50 50</td>
</tr>
<tr>
<td>Massachusetts and New Hampshire</td>
<td></td>
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<tr>
<td>Wild Ducks</td>
<td>40</td>
<td>100</td>
<td>65 22.5 5 7.5</td>
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<tr>
<td>Delaware</td>
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<tr>
<td>Wild Ducks</td>
<td>55</td>
<td>100</td>
<td>61 25 12 7.5</td>
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<tr>
<td>Maryland</td>
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<tr>
<td>Wild Ducks</td>
<td>44</td>
<td>100</td>
<td>61 11.4 15.9 11.4</td>
</tr>
<tr>
<td>Geese</td>
<td>100</td>
<td>100</td>
<td>60 20 11 5 4 4.0</td>
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<tr>
<td>The Netherlands Through Clifton, N. J.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Swans</td>
<td>96</td>
<td>100</td>
<td>80 12.5 5 2.5</td>
</tr>
</tbody>
</table>

*Log 10.
### TABLE VII

**Percentage of Frequency Distribution of Virus Neutralization Indices* of Serums from 34 Ducks Experimentally Infected with Duck Plague Virus**

<table>
<thead>
<tr>
<th>Serum Neutralization Indices</th>
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</thead>
<tbody>
<tr>
<td>Days</td>
<td>0.0 to 2.75</td>
<td>0.25</td>
<td>0.50</td>
<td>0.75</td>
<td>1.00</td>
<td>1.25</td>
<td>1.50</td>
<td>1.75</td>
<td>2.00</td>
</tr>
<tr>
<td>POSTINOCULATION</td>
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<td>42</td>
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</table>

*Log 10.

**Log 10.**
tally-infected ducks. As shown in Table VII, 100% of the preinfection (O-DPI) serums were in the 0.0 to 1.25 range. At 21 DPI, 14.7% were in the 1.00 to 1.50 range and 85% were in the 1.75 to >3.0 range. At 42 DPI, 99% had a VNI of 1.75 and higher. The antibody distribution pattern of the O-DPI serum was similar to that for the serums from ducks that were not exposed to natural infection. However, following infection, the percentage of ducks with a VNI of 1.75 and more, increased. The increasing trend was similar to that observed in the naturally-infected ducks. Comparison of the VNI of the experimentally-infected ducks (Table VIII) at 0, 21, and 42 DPI indicated that, in most cases, there was an increase of 1-2 logs in the VNI. A substantial increase in the serum antibody titer occurred at 21 DPI, but it reached a higher level at 42 DPI.

DISCUSSION

During this study, 2 important factors were considered. The first was the system of assessment of the serums for recognition of antibodies. Developing CE were chosen for conducting the virus neutralization tests using the duck plague chicken-adapted virus as the source of antigen in the serum-virus mixture. During the process of adaptation, this virus became avirulent to ducks, but was serologically identical to the virus that causes the disease and mortality in ducks. The second factor was determination of the level of antibody titer that is considered indicative of infection with duck plague virus.

The frequency distribution of the VNI of the serums tested was scored in classes that ranged from 0.0 to >3.0 (Table VII). This arrangement was advantageous in that it projected a visual horizontal scale representing the antibody levels. The classes on the scale increased in sequences of 0.25 log 10. The VNI of serums collected from ducks on Suffolk County farms, allegedly free from infection with duck plague, had shown neutralizing antibodies up to 1.5 and subsequently weighed heavily to the left of the point of 1.5 level. In contrast, serums collected subsequent to natural disease outbreaks or experimental infection tallied mostly to the right of the 1.5 point on the scale. Thus, serums with a neutralization index of 1.75 and more were considered positive.

Tests with serums of ducks from the states of California, Illinois, Indiana, Michigan, and Ohio, as well as wild ducks, geese, and swans nesting along the northeast coast of the United States, gave increased evidence of the validity of considering the 1.5 level as the maximum level of nonspecific antibodies in duck serum for duck plague virus. Thus, it was concluded that serums with VNI of 1.75 or higher indicated infection experience with duck plague virus. The results of testing paired serum samples from 34 experimentally-infected ducks revealed that the rise in neutralizing antibody titers followed exposure to the disease.

Test results on a group of 160 serums secured from a farm before the appearance of the disease signs and later, 110 serums from a flock of ducks on the same farm following diagnosis of the disease, revealed an incidence of infection experience of 60.9%.
The serologic response of ducks to experimental infection was similar to that of ducks naturally infected. The distribution pattern of serums by titer was also approximately the same.

The virus neutralization test method used indicated that this quantitative assay was highly satisfactory. Using CE-adapted virus and chicken embryos made it possible to test large numbers of duck serums by the virus

**TABLE VIII**

<table>
<thead>
<tr>
<th>VIRUS NEUTRALIZATION INDICES OF SERUMS FROM 34 DUCKS EXPERIMENTALLY INFECTED WITH DUCK PLAGUE VIRUS</th>
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<tbody>
<tr>
<td>0 DPI</td>
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<td>0.75</td>
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</tbody>
</table>

DPI = Days postinoculation.
neutralization test. When a method employing a virulent virus and duck embryo is used, the virus must be used as an antigen and the duck embryonated eggs must be inoculated on the chorio-allantoic membrane. The latter method is both more time-consuming and expensive than the former.

For serological diagnosis, in case of a suspected outbreak of the disease, it is recommended that serums be obtained from convalescent ducks. It is necessary to take blood samples in the acute stage and again, 21 days later, in order to establish the increase in virus neutralizing antibody titers to the virus.

The results of this epizootiological survey of neutralizing antibodies for duck plague virus are particularly interesting in that they point out that duck plague virus infection is, in most probability, a new infection on Long Island. It appears that the virus was introduced from outside areas to the Suffolk County duck farms. However, the nonspecific level of antibody may have been elicited by a virus of low virulence which had not been detected. It behooves duck owners to be alert to the possibility and hazards of introducing the virulent duck plague virus into their premises.

SUMMARY

A total of 2936 serums were examined for duck plague virus neutralizing antibodies. These serums originated from ducks on commercial farms in California, Illinois, Indiana, Michigan, New York, and Ohio as well as nesting wild ducks, geese, and swans on the northeastern coast of the United States and a group of experimentally-infected ducks. The virus neutralization test was performed employing chicken-embryo and chicken-embryo-adapted virus. A virus neutralization index of 0.0 to 1.5 log 10, ELD₅₀ was found to be prevalent in serums of domestic and wild waterfowl that had not been exposed to the disease. A virus neutralization index if 1.75 and more indicated infection experience with the virus. The frequency distribution patterns of virus neutralization indices were similar subsequent to recovery from experimental or natural infection. It was concluded that the virus neutralization test appeared to reflect accurately the status of the response of waterfowl to infection and, within the limits of the sample of waterfowl from which serums were tested, it appears that duck plague experience of waterfowl occurred recently in commercial duck flocks in New York State. No significant antibody titer was detected in serums of wild waterfowl that were nesting on the northeastern coast or in domestic ducks from other geographical areas in the United States.

ACKNOWLEDGMENT

The authors wish to express appreciation for the assistance of the personnel at the Animal Health Division, ARS, USDA, and the Duck Research Laboratory, Eastport, Long Island, New York, in supplying the serums used in these tests.
REFERENCES

6. Urban, W. Personal communication.
REPORT OF THE COMMITTEE ON FOREIGN ANIMAL DISEASES

N. M. Konnerup, Silver Spring, Maryland, Chairman; D. E. Detray, Beltsville, Maryland; W. T. Berry, Jr., Moulton, Arkansas; F. P. Gluckstein, Alexandria, Virginia; T. Griffin, Silver Spring, Maryland; L. Griner, San Diego, California; D. W. Johnson, Frederick, Maryland; K. L. Kuttler, College Station, Texas; F. D. Maurer, College Station, Texas; R. M. McCully, Onderstepoort, South Africa; N. L. Meyer, Hyattsville, Maryland; T. G. Murnane, Washington, D. C.; A. B. Park, Washington, D. C.; G. Poppensiek, Ithaca, New York; F. K. Ramsey, Ames, Iowa; T. Rea, Phoenix, Arizona; R. C. Reisinger, Burtonsville, Maryland; M. E. Seaquist, Gaithersburg, Maryland; K. C. Sherman, Ames, Iowa; H. G. Wixom, Sacramento, Calif.

The Foreign Animal Disease Committee met on October 17. In addition to the chairman, eight committee members were present and eleven members were absent. There were nine guests present who contributed to the discussions. Reports were presented on recent changes in the animal disease situations and the circumstances under which they were handled.

Several animal disease outbreaks have occurred since the last committee report. They are highlighted by the following situations:

Type A Foot-and-mouth disease was confirmed in the Choco region of Colombia within 20 kilometers of the Panamanian border and resulted in a request for emergency assistance from the United States. It is anticipated that such requests for assistance will increase in the future. A serious outbreak in the United Kingdom has led to the destruction of over 150,000 animals and the disease was still not contained in November 1967.

Rabies was confirmed on the island of Guam, and there is a very recent report of a possible outbreak of the disease on an island in Hawaii.

Duck virus enteritis (Dutch Duck Plague) was identified in Suffolk county, Long Island, New York in January 1967 and details will be presented in a paper following this report.

A strain of Mycoplasma mycoides, var. capri was isolated from a goat at Yale University, Connecticut. This prompted a comparative study of other Mycoplasma infections in goats, particularly those reported in the southwest. Details of this study have been reported to this assembly and will be published in the proceedings.

African Swine Fever has now been reported in thirty-two provinces in Italy and the Iberian Peninsula remains infected with the disease.

African Horse Sickness has spread to North Africa and to the southern coast of Spain. Losses in Morocco were reported heavy.

A "new" disease of public health significance was reported from
Germany in June 1967. Individuals in two laboratories associated with the production of primary cell cultures from kidney tissue of the African Green Monkey (Cercopithecus aethiops) contracted a disease reported clinically as a hemorrhagic disease syndrome. Thirty-two cases have been reported with seven fatalities. A viral or rickettsial agent, has been isolated; not identified. The animal health significance has not been established. Infected monkeys are apparently asymptomatic, and personnel handling the intact animal have not contracted the disease. This incident emphasizes that normal animals and cell cultures produced from their tissues can carry highly infectious latent viruses and provide an unusual avenue for their importation into the United States.

Other elements of discussion centered on the current inadequacies of quarantine inspection and surveillance, the recurrent lack of systems to acquaint veterinarians and livestock people at federal, state, local and institutional level with disease recognition, diagnostic and reporting techniques, the need to extend and develop training systems and training devices in foreign animal diseases to a wider range of veterinarians, the need to utilize a wide range of individual expertise from a multiplicity of agencies, institutions and organizations for routine and emergency response to arising situations, the need to prepare a priority oriented guide of foreign animal diseases for diagnostic, regulatory and research use and the imperative need to begin the organization for the revision of the USLSA Foreign Animal Disease Handbook.

Attention was called to the similarity in clinical manifestations of several diseases and the need for private practitioners, state and federal regulatory veterinarians and veterinary clinicians at schools and institutions to be thoroughly familiar with such diseases through actual clinical experience. As soon as facilities are available, a continuing training program should be organized for appropriate individuals.

The consensus of discussants was that combined federal agencies, state organizations, institutions and the private sector has not yet faced up to responsibilities for disease prevention, emergency response to new situations and planned control or eradication programming.

COMMITTEE RECOMMENDATIONS

1. Establish a task force to review committee objectives and define responsibilities.
2. Organize and extend committee communications to health officials, the livestock industry and legislative officials and the public.
3. Establish a committee function to evaluate the validity of import-export restrictions in the United States and foreign countries.
4. Augment organized regularly scheduled training in foreign animal diseases at state, federal, institutional and private levels.
5. Strengthen quarantine, inspection and disease surveillance systems.
6. Improve conditions for collection, handling, conditioning, shipping, inspection and control of laboratory, zoological and domestic animal species.

7. Develop and establish close liaison with the Council on Regulatory Veterinary Medicine of the AVMA in order to establish a system for obtaining congressional and other support for legislation and appropriation necessary to achieve more effective disease investigation and control.

8. Establish a task force within the Department of Agriculture capable of responding immediately to requests from other countries for assistance in foreign animal disease outbreaks and providing contingency funds to make such an organization operational.

9. Develop animal health policies for International Banks and International Assistance Agencies making eligibility for loans contingent upon the development of reasonable and adequate animal health control programs.

THE THREAT OF FOREIGN ANIMAL DISEASES TO THE EQUINE POPULATION

RALPH C. KNOWLES
Chief Staff Veterinarian
Equine Diseases
USDA-ARS-ANH

given at
71ST ANNUAL MEETING OF USLSA

In order for one to fully understand the disease threat to our equine population, it is desirable to take a look at the industry and its many facets.

One hundred years of equine census-taking in the USA is represented by Figure 1. It should be noted that, since 1960, no official census has been taken by the United States Department of Agriculture. Our best estimates place the present equine population at approximately 5½ million. 4-H Club enrollments in light horse projects have risen from 37,531 in 1959 to 165,617 in 1966, showing a four-fold increase in seven years. In 1964, we surveyed the horse auctions in the United States. Figure 2 represents the summation of our findings. Approximately 8% of our estimated equine population passed through auction markets in 1964. The next map (Figure 3) shows the states that conduct parimutuel race meets. Figure 4
HORSES AND MULES

Number on Farms - January 1

Figure 2

STATES WITH HORSE RACING

Figure 3
(compiled by the National Association of Racing Commissioners and published by the Blood-Horse) indicates the dynamic growth in tax revenues that have evolved. Probably the most spectacular growing facet of the equine industry is the pleasure or companion horse population.

International transport of horses has undergone notable changes: principally, one of greater speed in the transition from ocean vessels to aircraft. This gives us grave concern when flight time is measurably less than the incubation period of several exotic equine diseases.

Import quarantines are of value for those diseases in which we recognize clinical signs or serologic evidence. Unfortunately, all equine diseases are not clearly identifiable.

Let us review disease introduction experiences that have occurred in the 1960's. In August 1961, equine piroplasmosis (EP) or babesiasis caused by Babesia Caballi was first diagnosed near Miami, Florida and the tropical horse tick (dermacentor nitens) was proven to be a vector. In March 1965, Babesia equi parasitemia was disclosed in a thoroughbred at a Miami race track. I do not wish to imply that Florida has all of the critical equine disease problems in the U. S. but, has fallen heir to a great mixing bowl of our U. S. horse population (an estimated 8,000 horses move to Florida for winter racing, training, and horse shows). This, coupled with an active equine importation situation and the presence of astute equine diagnosticians, backed up by an active state regulatory agency and diagnostic assistance by laboratories, means that equine disease problems are readily identified. In late January 1963, a shipment of race horses from Argentina was received at the Port of Miami. Clinically they appeared to have "Colt influenza." They were held in quarantine, treated by private practitioners to ward off secondary infections, and later declared safe for

![Racing Revenue Graph](image)
COLLECTION AND SUBMISSION OF TICKS

1. Collect 6 to 12 ticks from an animal.

2. Make collections from 4 to 5 animals in the herd.

3. Place ticks from each animal in a vial of isopropyl (rubbing) alcohol.

4. Secure a brief history of host animal and designate body areas from which ticks were collected.

5. Submit ticks for identification to state veterinarian or federal veterinarian in charge.

6. These will be identified and reported back to the tick collector.

Figure 5

A₂ EQUINE INFLUENZA
EPIZOOTIC DISSEMINATION - 1963

Figure 6
release by our port veterinary inspectors. Soon influenza spread through a barn at Gulf Stream Park; and subsequently, it attacked 85% of the horses stabled at this race track. With the early spring movement of horses from Florida to northern states, this malady spread from thoroughbreds to standardbreds and to other types of horses. A consistent attack rate of 85% and 90% was seen. By mid-June, A₂ influenza had reached British Columbia (Figure 5). This entity probably was the greatest "eye opener" with regard to potential infectious diseases since the encephalitis outbreak of the late 1930's.

On September 7, 1960, at a wild animal compound in southern Florida, the red tick (Rhipicephalus Evertsi) was discovered. This was the first time this tick, common to Africa, had been identified in North America. This 131-acre animal compound contained 400 exotic animals representing many species including 98 Zebras and 22 Abyssinian Asses.

The literature reveals that the red tick is a vector of: bovine piroplasmosis (babesia bigemina), and East Coast Fever (theileria parva), pseudo-east fever (theileria mutans), and spirochetosis (borrelia theileri) in cattle; equine piroplasmosis (b. caballi and b. equi), and spirochetosis in horses.

This invasion by the red tick into Florida called for decisive tick eradication action. Animal treatment with tickicides required proper immobilization. Repeated applications of DDT to the premises were necessary before two successive animal inspections revealed no red ticks, the final inspection and precautionary spraying with Delnav Emulsion (Del-Tox (DLV 6 B Emulsion), William Cooper & Nephews, Chicago, Illinois and Delnav-Extra (EE-61-1 and EF-51-5), Thuron Industries, Inc., Dallas, Texas) being completed on January 15, 1962.

Since 1962, 14 species of exotic ticks have been found on imported horses or other equidae.

I appeal to you to foster tick inspection of horses with a follow-up submission of ticks found. This transparency outlines suggested tick inspection and submission procedures (Figure 6).

In closing, it seems appropriate to review and relay information on African Horsesickness (AHS) to you, historically, AHS has been known to plague the African continent since the days of Dr. Livingston's exploration. Some have related the difficulties in equine introductions and production to general lack of progress on this continent. Until 1959, AHS had not been diagnosed outside the African continent. In that year circumstantial evidence optimal vectoring conditions appears to have prevailed and AHS spread into Pakistan, Afghanistan and Iran. Within two years, horsesickness had appeared in a broad belt extending from the Middle East into India and had killed at least 300,000 horses, donkeys and mules in the affected area.

African Horsesickness was reported from Algeria in the fall of 1965 but information from that country on the disease had been limited. In mid-March of that year, the disease was reported in southern Morocco where approximately 10,000 animals died and where more than 250,000 of Morocco's one and a half million donkeys, mules and horses were vaccinated. African Horsesickness is reported to be wide-spread throughout Tunisia.
Vaccination is in progress and 108,000 animals have been vaccinated. Many animals have been destroyed. African Horsesickness was reported (October 1966) from southern Spain in the municipal jurisdictions of Los Barros and La Linea de la Concepcion, Province of Cadiz. Thirty-seven horses are reported to have died and 220 were destroyed.

In summary, it can be said that too often our backyard horses may have had exposure to world-wide disease problems. Our record of vigilance and counter-attack is good. We need to fully recognize the dangers of foreign animal diseases and parasites and to leave no stone unturned in our efforts to keep these maladies from our equine population.
REPORT OF THE COMMITTEE ON INFECTIOUS DISEASES
OF HORSES


This committee, in its third year of functioning, reaffirms that Piroplasmosis, infectious anemia, respiratory diseases and continued protection from African Horse Sickness are most significant to the horse population of the United States. The committee also reaffirms that diseases with these characteristics can be controlled only on a national level and that continued and increased efforts should be oriented under the direction of developing effective tools for a national eradication and protection program.

We recognize the need for a plan of attack against the diseases named above and propose drafting of Guidelines for uniform handling of Equine Piroplasmosis, Equine Respiratory Diseases and African Horse Sickness in addition to updating annually the prospectus with guidelines on E.I.A. This prospectus revised and updated is appended to this report.

EQUINE PIROPLASMOSIS

A total of 161 confirmed (organism demonstrated in erythrocyte) cases have been diagnosed in Florida since 1961. Of this total, 126 cases occurred in 1962; 7 cases in 1966; and 4 cases to date in 1967.

The pattern of new cases for the years 1965, 1966, and 1967 seems to reveal that vector control alone will not prevent equine piroplasmosis from becoming indigenous in the United States, or at least in Southeastern Florida.

The program has reached the point demanding an urgent need for an ample supply of antigen to conduct the complement fixation or other acceptable test and the consequent detection and eventual treatment of carriers.

It should be definitely determined if Dermacentor nitens (tropical horse tick) is the only vector of EP in the United States.

This committee urges official recognition of a diagnostic test by USDA. A complete epidemiological study of the work performed in Florida should assist in reaching this decision.

It is further recommended that the Food and Drug Administration take steps to release the following three drugs—Phenamidine, Berenil and Diamprom for the treatment of Equine Piroplasmosis (Babesia caballi and Babesia equi).
Recognizing that since 1962, 14 species of exotic ticks have been found on horses and other equidae. This Committee recommends intensification of tick inspections of all imported equidae. The Committee further supports a continuation of the National Tick Survey and wishes to emphasize the real dangers of introduction of diseases into the United States through tick vectors.

IMPORTATION OF HORSES

We recommend that a permit system be initiated on all equidae entering the United States at all air and ocean ports. Such permits should be issued from the Animal Health Division office in Hyattsville. This should facilitate more orderly handling of imports and should provide closer scrutiny in the importation of these animals.

A PROSPECTUS ON EQUINE INFECTIOUS ANEMIA WITH GUIDELINES

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1. Definitions
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   B. Health Certificates
   C. The Precipitin Test
   D. The Virus
   E. The Disease
   F. Spread of the Disease
   G. Control Measures
3. Areas of Responsibilities and Functions
   A. Control Headquarters
   B. State Veterinarians
   C. State Racing Commissions and Commission Veterinarians
   D. Race Track Management
INFECTIOUS DISEASES OF HORSES

E. Owners, Breeders and Trainers
F. Practicing Veterinarians

4. Procedure in the Event of a Suspected Outbreak

5. Research Objectives

The following prospectus and guidelines for controlling the spread of Equine Infectious Anemia particularly at America's horse racing and training tracks were formulated by representatives of the following national organizations:

American Association of Equine Practitioners
American Quarter Horse Association
American Veterinary Medical Association
National Association of State Racing Commissioners
Thoroughbred Owners and Breeders Association
Thoroughbred Racing Association
United States Department of Agriculture
United States Livestock Sanitary Association
United States Trotting Horse Association

A great deal of time and effort has been spent in evaluating the disease itself, diagnostic tests, pertinent regulatory laws and rules, current research and other factors in coordinating and developing sound ideas to serve as a basis for a national unified program of immediate control aimed at eventual elimination of Infectious Anemia.

While it is obvious that the tools at hand are less than perfect and much more needs to be known about the disease, its diagnosis and methods of controlling it—the urgency of the current situation requires the practical application of the best methods now available in order to minimize losses with the least disruption and cost to the horse industry.

Inasmuch as the majority of the nation's State Veterinarians have thoroughly reviewed, approved and endorsed the material and methods set for herein, it is fervently hoped that the remaining states will also accept them in order to establish nationwide uniform procedures which will negate the necessity for invoking unduly stringent regulations, particularly in the field of interstate movement, which would likely prove disastrous to our horse industry. With cooperative measures between the states as is contemplated herein, it is felt that immediate outbreaks can be effectively contained until an anticipated crash research program can lead us to eradication of Equine Infectious Anemia.

1. DEFINITIONS FOR THE PURPOSE OF THIS REPORT

A. Equine Infectious Anemia—a widely spread virus-caused disease of the equine family which is infectious in nature and spread by injudicious use of hypodermic needles and other instruments as well
as by insect vectors, and which may be acute, subacute or chronic in form.

B. State Veterinarian—the chief livestock regulatory official—usually an employee of the State Department of Agriculture or State Livestock Sanitary Board and responsible for the control and eradication of animal disease within his state.

C. Commission Veterinarian—one employed by the State Racing Commission or Board for the purpose of advising and assisting with veterinary matters pertaining to the Commission and to direct the veterinary functions and activities of the Commission.

D. Advisory Committee—a group of consultants composed of practicing, regulatory or other veterinarians who may be appointed by the State Veterinarian to assist him in an advisory capacity in dealing with Equine Infectious Anemia.

E. Precipitin Test—the serological test for Equine Infectious Anemia is currently performed on horse serum at Texas A & M University.

F. Horse Inoculation Test—a test whereby 200 cc of blood is transferred from a suspect horse to a susceptible test horse under the direction of a State Veterinarian following the protocol in Appendix #1.

G. Suspect Horse—a horse showing clinical manifestations of Equine Infectious Anemia and/or other evidence of the disease. (Status of the disease in the horse may be further determined by horse inoculation test).

H. Infected (Positive) Horse—a horse that shows clinical manifestations of Equine Infectious Anemia, positively reacts to an approved serological test, and is positive to an official horse inoculation test.

2. INFORMATION ACCEPTED AS FACT PERTAINING TO EQUINE INFECTIOUS ANEMIA

A. Responsibility for Control

(1) The individual State Department of Agriculture or Livestock Sanitary Board is the sole agency legally responsible for the control of Equine Infectious Anemia within the respective states.

(2) The U. S. Department of Agriculture has certain regulatory jurisdiction over horses entering the United States or known infected Animals being moved from one state to another.

B. Health Certificates

(1) A health certificate is current and valid only at the time a horse is physically examined and the certificate issued.

(2) A Health certificate at no time assures that a horse is not a carrier of the virus or has not been exposed to the disease.
C. The Virus

(1) The virus is transmitted from a carrier horse to a susceptible one primarily through transfer of minute particles of blood by biting insects and contaminated instruments such as hypodermic needles, tattoo needles, saliva collecting equipment and other articles or instruments which may penetrate or severely abrade the skin or mucous membranes.

(2) The virus has many unusual characteristics which make it most difficult to study and manage.

(3) The virus is not destroyed by the usual antiseptic and disinfecting solutions. A minimum of 15 minutes boiling is recommended to destroy the virus.

D. The Disease

(1) Is widely spread throughout the country at ranches and farms and at race tracks.

(2) Clinically it resembles many other diseases and it is impossible to make a differential diagnosis without extensive laboratory tests.

E. Spread of the Disease

(1) It is believed that the hypodermic needle in the hands of horse handlers is the biggest single cause for spread of the disease at race tracks and elsewhere.

(2) Biting insects can be the biggest factor for its spread at ranches and farms.

(3) A horse carrying the virus is of slight danger to horses stabled around it, providing: (a) biting insects are eliminated; (b) no instruments used on the carrier horse are used without sterilization; and (c) good daily sanitary practices are followed.

F. Control Measures

(1) Control depends upon the identification and eventual elimination of infected horses. This must be done in the most practicable and least costly manner.

(2) There is urgent need for a nationally coordinated control effort, along with a centralized comprehensive reporting and analyses of all cases and all tests.

(3) The suggested plans and procedures evolved in this report provide the framework for an effective control and research program.

(4) Success of any program will depend upon the cooperation and support extended by individual horsemen as well as all facets of the industry.

3. AREAS OF RESPONSIBILITIES AND FUNCTIONS

A. Responsibility for a central headquarters office for the purpose of
(1) collecting and disseminating all pertinent information and
(2) coordinating and analyzing all test and control procedures on a
nationwide basis has been assigned to:

Dr. Ralph C. Knowles
Chief Staff Officer
Animal Health Division
Agricultural Research Service
U. S. Department of Agriculture
Washington, D. C.
Headquarters Office Telephone: Area Code 202 388-8628

B. State Veterinarian or Chief State Livestock Regulatory Official (State Department of Agriculture or Livestock Sanitary Board)

(1) Responsible for the control of contagious diseases of livestock
within his state including disease among horses at race tracks.
(2) May appoint an "Advisory Committee" from practicing or other
regulatory veterinarians including the Commission Veterinarian
to assist him in an advisory capacity in case of a suspected
outbreak of Infectious Anemia at any race track.
(3) Should have available to him an isolation area including screened
stalls at or near each track suitable for isolating suspect horses
(isolation area to be provided and maintained by track manage-
ment under sanitary and isolation requirements prescribed by
the State Veterinarian.)
(4) In case of a suspected outbreak will, with the assistance of his
advisory committee determine which horses will be subjected
to further scrutiny. Will recommend which horses should submit
to the animal inoculation test.
(5) Will be responsible for conducting all animal inoculation tests
performed in his state as prescribed in Appendix #1.
(6) Will report the results of each test on each horse to the USDA
Central Office.
(7) Will maintain a list of the names of all suspect and infected
horses.
(8) Will prescribe and enforce necessary sanitary rules and isolation
rules at race tracks and elsewhere.
(9) Will declare Equine Infectious Anemia as a required reportable
disease in states where such is not already the case so that all
cases will be reported and recorded in each state.

C. State Racing Commissions and Commission Veterinarians

(1) Should see that sanitary and other protective measures pre-
scribed by the State Veterinarian are enforced at race tracks.
(2) Should insure that tattoo instruments and saliva collecting equip-
ment are adequately sterilized under the supervision of the Com-
mission Veterinarian (autoclave 15 minutes at 15 pounds or thoroughly wash clean and boil for 15 minutes (*prior to being used on any horse*).

3) Should enforce rules preventing the use of hypodermic syringes and needles on horses by other than veterinarians licensed to practice at the track.

4) Should require provision and operation of adequate isolation facilities acceptable to the State Veterinarian.

5) The Commission Veterinarian should serve on the State Veterinarian’s advisory committee and will promptly report all cases of reportable disease suspected at the tracks.

6) Should assist and support an accelerated national research program to combat Equine Infectious Anemia and Equine Piroplasmosis.

7) Should require temperatures of all horses stabled at tracks to be taken and recorded each morning and evening and such record made available, when required, to State Veterinarians. Any abnormal temperature (102°F and above) should be reported to the Commission Veterinarian.

D. Race Track Management

1) Should institute and carry out at all times the sanitary and preventive measures outlined in Appendix #2.

2) Should provide and maintain screened isolation facilities adequate to meet the needs of and be acceptable to the State Veterinarian.

E. Owners, Breeders and Trainers

1) Should understand that the disease is usually spread by the transfer of blood even in the most minute quantity from an infected horse to a susceptible horse.

2) Should realize that perhaps more horses become infected at race tracks by means of contaminated hypodermic needles in the hands of horse handlers than through all other sources combined.

3) Should realize that a perfectly normal appearing healthy horse can be a carrier of the virus for years without suspicion.

4) Should realize that if a hypodermic needle is used on a carrier horse and later on a susceptible horse the second horse is very apt to come down with the disease.

5) Should follow the sanitary and precautionary measures outlined in Appendix #2.

F. Practicing Veterinarians

1) Will immediately report any horse suspected of being infected with Infectious anemia to the State Veterinarian. If the horse is stabled at a race track he will simultaneously report it to the Commission Veterinarian.
(2) Should continue the practice of using disposable hypodermic needles and syringes (one needle—one horse).

(3) Offsize needles and other surgical and medical equipment which must be reused should be sterilized either by thorough cleaning and boiling for 15 minutes or autoclave 15 minutes at 15 pounds.

(4) It should be noted that the virus will survive boiling heat of less than 15 minutes duration and the usual sterilizing solutions.

4. PROCEDURE IN EVENT OF SUSPECTED OUTBREAK

A. Any practicing or other veterinarian suspecting a horse at a track of being affected with Infectious Anemia because of either clinical signs or tests will promptly report the case to: (1) the State Veterinarian; and (2) the Commission Veterinarian.

B. The State Veterinarian and his advisory committee will then determine which horses will be subjected to further testing. Contributing factors, as well as the amount of testing required, may vary from track to track; however, such determinations should be so practicably encompassing as to afford the necessary protection to the health of other horses stabled there.

C. Horses suspected of being infected because of clinical signs (as determined by the State Veterinarian and his advisory committee) will be moved into a suitable screened isolation area (in the case of race tracks—track management will provide facilities), subjected to such testing as may be required, and maintained at the owner's expense under the regulatory sanitary supervision of the State Veterinarian until released by him.

D. A horse which has been proven positive to an official horse inoculation test shall remain under permanent state quarantine.

5. RESEARCH OBJECTIVES

A. To establish the incidence of Equine Infectious Anemia in the U. S.

B. To develop a practical, definitive diagnostic test.

C. To further knowledge of the epidemiology and pathogenesis of the disease upon which to base improved sanitary controls.

D. To further propagate, purify and characterize the virus.

E. To study the immune mechanisms involved as a step toward vaccine development.

F. To fully evaluate laboratory tests as rapidly as possible.

HEALTH CERTIFICATES

The Committee recognizes the value of properly executed health certificates with body temperatures duly recorded.
### APPENDIX I REVISED

**INSTRUCTIONS AND PROTOCOL FOR HORSE INOCULATION TESTS FOR EQUINE INFECTIOUS ANEMIA**

<table>
<thead>
<tr>
<th>SUSPECT ANIMAL (DONOR)</th>
<th>RECIPIENT TEST ANIMAL QUALIFYING STEPS</th>
<th>ANIMAL INOCULATION TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.</strong> Draw 500 ml. whole blood into vacuum bottle containing acid-citrate-dextrose or oral Alsever's solution.</td>
<td><strong>QUALIFY BY:</strong>&lt;br&gt;1. Must be at least two years old.&lt;br&gt;2. Identify by hoof branding (hot iron).&lt;br&gt;3. Holding in test barn six weeks prior to inoculation (30-day absolute minimum).&lt;br&gt;4. Treating for internal parasites.&lt;br&gt;5. Establishing normal temperature and hemograms:&lt;br&gt;<strong>TEMPERATURE GUIDELINES</strong>&lt;br&gt;A. Obtain a temperature reading two times daily. Morning temperature taken when horse is at rest and before he is fed and watered;&lt;br&gt;B. Establish base line—consider daily temperature of individual horse, plus the temperature of his stable mates for a minimum of three weeks;&lt;br&gt;C. Eliminate variables such as new grooms;&lt;br&gt;D. Check all thermometers against a standard for accuracy, use one thermometer for one horse;&lt;br&gt;E. All routines should be the same each day.</td>
<td><strong>1.</strong> <em>Time and Inoculum</em>—Sixty days would be minimum. One recipient horse should be used. The dose of the inoculum should be 20 ml. whole blood per 100 lbs. of body weight given by any parental route.</td>
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<tr>
<td><strong>2.</strong> Submit information on donor animals as follows:</td>
<td></td>
<td><strong>2.</strong> <em>Temperature Readings</em>—Same as Temperature guidelines for recipient test animal qualifying steps.</td>
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<tr>
<td>Name__________________________</td>
<td></td>
<td><strong>3.</strong> <em>Hematology (EDTA Liquid-Preferred Preservative)</em>:&lt;br&gt;A. Perform PCV at least six times weekly following inoculation.&lt;br&gt;B. Complete blood count (CBC) at least once weekly.&lt;br&gt;C. It may be well to perform leptospirosis test and prepare blood smears in an attempt to identify Babesia during febrile periods.&lt;br&gt;D. Further hematological tests may be used to provide supportive evidence.</td>
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<tr>
<td>Identification No.______________</td>
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<td>Breed__________________________</td>
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<tr>
<td>Precipitin and/or other laboratory test results____________________</td>
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</tbody>
</table>
APPENDIX I REVISED—Continued
INSTRUCTIONS AND PROTOCOL FOR HORSE INOCULATION TESTS FOR EQUINE INFECTIOUS ANEMIA

Suspect Animal (Donor)

Clinical history

Remarks:

Miscellaneous Item
A. Before inoculation, collect and store 500 ml. of blood in ACD or Alsever's solution. This blood should be collected both from the donor and the recipient animal (in case of a mishap later). Store at 5° centigrade or in lieu of the above store frozen serum.

Recipient Test Animal Qualifying Steps

Hematology (EDTA liquid—preferred preservative)
A. Determine PCV daily (to establish a norm), three weeks minimum prior to inoculation.
6. Cross inoculation of recipients—choice of A or B below:
   A. Ring type:
   B. Pooling type:
C. Test recipients showing febrile reactions or PCV readings out of the normal range and relating to conditions not clearly differentiated from EIA should cause rejection of individuals as test candidates.

Animal Inoculation Test

Miscellaneous Items:
A. Frequency for collecting samples for serological tests (varies) weekly, if temperature rise seen, play it by ear.

Post inoculation temperatures should be interpreted relative to the base line for the individual horse. Any elevations in temperature will be considered significant if stablemates remain at their base line on that day.

Experience of investigations have shown that a temperature rise of as little as 1° above normal is indicative of infection when accompanied by significant hemograms and clinical signs.
APPENDIX #2

RECOMMENDATIONS FOR HANDLING HORSES AT RACE TRACKS AND OTHER LOCATIONS TO AID IN PREVENTION AND CONTROL OF EQUINE INFECTIOUS ANEMIA

1. Maintain at all times systematic and effective insect control, especially against flies and mosquitoes. Maintain stables and the immediate surrounding area in good sanitary condition at all times. This includes prompt disposal of manure and other refuse and good drainage to prevent vector multiplication.

2. Restrict the use of hypodermic syringes and other veterinary instruments to authorized veterinarians.

3. Prevent common use of any equipment such as bridles, bits, harness, curry combs etc., that may produce skin abrasions or absorb body secretions or excretions.

4. Clean and sterilize all types of instruments used on horses including surgical, tattooing, dental, and similar items by boiling for 15 minutes, before use on each animal.

5. Use separate sterile equipment on each animal when collecting material for the saliva or other tests.

6. Frequently clean and disinfect paddocks, starting gates, and other equipment subject to contact by different animals. Use 2% trisodium phosphate to clean, and disinfect with sodium orthophenylphenate (1 pound to 12 gallons of water used at 120°F. or higher).

7. Require incoming horses to have veterinary health examination and certificate (including temperature recording) within 10 days prior to arrival.

8. Stable horses in individual box stalls with separate feeding and watering facilities. Daily morning recorded temperatures as well as evening recorded temperatures of each animal are desirable. All horses should be subjected to careful examination by the official track veterinarian at his discretion.

9. Stable all horses presented as race entries so as to be under the health supervision of the official track veterinarian.

10. Immediately report to the State Veterinarian all horses suspected of having an infectious, contagious or communicable disease.

APPENDIX #3

MANAGEMENT OF SUSPECT HORSES NOT SHOWING CLINICAL SIGNS OF THE DISEASE

The following are conditions and regulations under which suspect horses may be allowed to work and race under approximately normal conditions:

(1) Such horses shall be maintained under conditions of adequate insect vector control.
(2) All grooming and other horse equipment used on a suspect horse will be confined to that horse and used on no other until sterilized as prescribed and controlled by the State Veterinarian.

(3) Suspect horses' temperatures will be taken and recorded each morning and each evening and reported to the State Veterinarian.

(4) Protective measures and methods by which the disease spreads should be thoroughly explained to all involved horse handlers by the State Veterinarian.
QUALITY CONTROL IN THE PRODUCTION OF VETERINARY DRUGS

C. S. Pruitt, D.V.M.
Indianapolis, Ind.

It is the responsibility of the Animal Health Industry to assure its clients, customers, and consumers that products are of acceptable quality. It is about the industry responsibility I would like to talk to you this afternoon.

A quality product is one that possesses the necessary and sufficient characteristics to assure that the product will perform its intended function. In order to more fully understand this statement, one must look to the meanings of two important words: "intended function."

The Federal Food, Drug, and Cosmetic Act defines a drug as "any substance intended to be used in the diagnosis, prevention, cure, or mitigation of a disease or to affect some body function." We immediately see that the term "intended function" enters the legal definition of a drug.

There is an economic desire to obtain the highest quality at an acceptable manufacturing cost, considering the intended product function. In order to achieve these objectives, both of which are necessary, one must be careful to recognize the quality attributes which will guarantee the intended function and not to confuse them with quality attributes which do nothing to achieve this goal.

It is paramount, therefore, to assess very carefully the many qualities characteristics and try to pick out those which are vital. These quality characteristics can be generalized into four main categories.

1. Those that have to do with the safety of the drug. These are such things as proper identity, no mix-up in labeling, freedom from harmful impurities, proper warning statements, and contraindications on the labeling, as examples.

2. Those that have to do with effectiveness of the product. Here we are dealing with attributes which will guarantee the intended function. In most cases, effectiveness is related to consistent potency, as measured by some analytical procedure. I will have more to say about this later. We are also concerned about the availability of the drug to the animal. The fact that something is present does not necessarily guarantee that it is in a form which will be usable by the animal.

There has to be a coincidence of the directions for use and the dosage statements on the label with the actual potency of the product itself. These elements have to fit together, or the product will not perform its "intended function."

3. Those that have to do with meeting regulatory requirements for the product. At first glance, one would expect that regulatory requirements would be a governmental statement concerning those quality characteristics or attributes which would guarantee safety and effectiveness. For practical purposes, the regulations must concern themselves with distribution and
assurance that industry’s customers are qualified to properly use the products. Thus, if a company is to exist in a society such as ours, it has the duty to live up to the requirements imposed by law. In that sense, this is one of the essential quality attributes that has to be met.

4. Those that have to do with consumer acceptance of the product. General characteristics which make products acceptable to the individual are taste, odor, color, shape, etc. These characteristics are necessary in order that the product may be given an opportunity to perform its “intended function.” In other words, if no one ever bought the product, it could never perform; and one has to do the things necessary to get it to the user.

On the other hand, there are other specific requirements which are placed on products by the intermediate consumer—the feed mill or the veterinarian—each of whom has specific demands to place on a product which are over and above those which are generally essential for effectiveness. It is in this area that often times a manufacturer runs into an economic problem of maintaining minimum costs. If there is a conflict between the suppliers’ values, in terms of the attributes which he controls in order to insure the “intended function,” and the attributes which are specified by the purchaser, the result is usually a cost increase.

Now what is really important is that the tablet, injectable, or feed additive perform its “intended function.” In order that it may, it must have all of the necessary quality attributes.

How does a manufacturer of animal drugs provide the assurance that a product will be safe, effective, meet government requirements, and be in an acceptable form to satisfy the customers’ needs?

All of these requirements must be built into the product. This building-in process begins with product development and carries right on through production and packaging.

It is quite obvious that a product will never reach the manufacturing stage unless it can be first developed to satisfy the quality requirements; however, it is also obvious that a product is not truly a product until it is manufactured in quantity and made available to the customer. All qualities built into the product during development must then be duplicated in the bulk production process. After production, the product must be placed into a package which offers proper protection and which has in its labeling the necessary and correct directions for the use of the product.

An elaborate and strict system of identity control is the primary system that ties development, production, and packaging together to assure quality. Each operation is performed according to written procedures. As a general working principle, the fact is accepted that any one person is subject to error; so whenever there is a quality characteristic that relies on identity, purity, strength, measurement, labeling, etc., the procedure is never to rely on the judgment of one individual, but to require that at least two positive checks be made for each key step in the operation.

For example, a requisition is submitted to a chemical stores department of a manufacturer for 100 kilograms of sodium chloride. In filling this requisition, an operation is performed which could affect the identity as well as the strength of some product. The material is provided through a
system which calls for one person to go to a given location, as directed by the requisition, and there obtain the sodium chloride in a bulk container. If the material is not in the proper location, it has failed to meet the first requirement, and therefore, the operator must report this to his management and cannot supply the material. If it is in the proper location, bears the right title, the right product number, and the right lot number, its identity has been established. This operator brings it to the second operator, who verifies the product number, the product title, and the lot number and thereby has performed a double check on the identity of the material which is to be weighed out. He then weighs out the stated quantity of the material and places it in a separate container, which is labeled with a label furnished with the requisition. The product is then handed to the next operator, who again weighs the material to verify that, in fact, there is the exact quantity of the material requested.

It is this kind of operation which is so essential in building quality into a product. Before an item leaves the supply area, they have determined whether or not it is one of several raw-material ingredients which would be used in the manufacture of a batch of a drug. If it is part of such a batch, it is brought together with the other ingredients which will be used in that batch, and a check is made to assure that all of the ingredients which are necessary for this product are present and have been weighed out with the same precautions.

There is sometimes a mistaken idea that the kind of precaution one uses for the active ingredient is not necessary for excipients or packaging components. This is not true, because the final product is a summation of all of the ingredients that go into it. Toxicities and harmful impurities can be introduced with excipients or packaging materials as well as with the active principle; therefore, the same kind of precautions must be taken with the inactive ingredients as with the active ingredients to ensure a real quality product. Many times the physical characteristics of a product which are so important in terms of availability and accessibility are a function of the inactive ingredients rather than the active principle. We cannot differentiate in our requirements for active and inactive ingredients.

The raw materials are brought to a producing area, and the operator will check to see that he has all of the raw materials which are necessary to produce a given lot of material before he will start the production of the item.

Did you ever stop to think that most of the assay procedures in the U.S.P. and N.F. start out something like this:

- Take 20 tablets and grind
- Dissolve in 500 cc. of water
- Take an aliquot portion
- Run a test

Please understand that this is not a criticism of U.S.P. or N.F. The main thrust of today's discussion about drugs seems to be: produce to a standard, quoting N.F. and U.S.P. most often, and identify by generic title. There are problems with these concepts as I will try to point out.
If the average of these twenty tablets is within plus or minus 10 percent of the stated quantity, the lot is acceptable. How many times do you as a veterinarian prescribe twenty tablets as a dose? So what does this particular test mean, in terms of the one tablet that you give to a dog or cat? The sample tested could be a mixture of ten with no active ingredient, and ten with 200 percent, and the assay would be 100 percent. Where is the assurance that the one which you use will have the desired quantity? This assurance can come only from what is done when the raw materials are placed in the mixer.

The kind of standard procedure used and the tests that are run to assure that the inactive and active ingredients are blended to a homogeneous mass are what's important. Homogeneous—not in terms of a one-pound sample but in terms of a sample the size of a dosage unit. Once this blending has been done, it is reasonable to assume that if, in the subdivision or in the tableting operation, proper weight checks are made, the product will be consistent. Without this kind of assurance, the final assay does little to give you this assurance.

A responsible manufacturer is always aware of the possibility of discrepancies that may occur even though the product may seemingly, and according to assay, meet all standard requirements. It is important to note that meeting standard requirements does not assure a quality product. I mentioned earlier that effectiveness is related to consistent potency. Let's examine a hypothetical instance where two batches of a product will meet standard requirements but do not result in a final quality product.

To aid in the consideration, we will assume that the tablet is a digitoxin tablet containing 0.1 mg. of digitoxin. The theoretical weight of such a tablet would be 68 mg. The U.S.P. tolerance for this product in terms of assay is ±10 percent. The weight variation allowable for such a product is such that no more than two tablets can be more than ±10 percent from the average of twenty and none of the twenty can be more than 20 percent from the average. It might be well to point out that there are no specifications in the U.S.P. as to the homogeneity of the granulation from which the tablets are made.

Let us examine two bottles of digitoxin tablets. In the first bottle, we find tablets having an assay which is at the high limit of 110 percent, and, in the second bottle, we find tablets having an assay which is at the low limit of 90 percent. It should be recalled that an assay is run on fifty tablets; hence, the variation between the tablets is completely masked by this large sample size. The weight variation of these tablets is run, and we find that eighteen are within ±10 percent average weight, but that two tablets are just below 20 percent above the average. Since the granulation was homogeneous and had an assay value of 110 percent, we find the two tablets actually would contain 0.13 mg. of digitoxin.

In the second bottle, we find two tablets just above 20 percent negative variation from standard. In this case, the assay is 10 percent below theoretical. We would find two tablets which contain 0.07 mg. of digitoxin.

It is immediately apparent that these two bottles pass the U.S.P. tests,
but what does this tell one of the quality of the lot? What do you know about the rest of the lot? Nothing. Assuming, however, that these two bottles do represent the extreme variations within the lot, two tablets out of forty contain twice the amount of active ingredient in two other tablets.

Let us now consider the tablets from the second product, which were made from a nonuniform granulation but whose weight variation was negligible or zero.

In the first instance, let us assume that the granulation was of such a degree of inhomogeneity that half of the tablets contained 0.2 mg. of the digitoxin, whereas the other half contained zero. This, we realize, is an almost impossible occurrence, but it is given here to illustrate that even a product as variable as this could pass the U.S.P. tests.

Since the assay results can be off by ±10 percent, any value above 4.5 mg. and below 5.5 mg. would be acceptable quality. Looking at the table, we see, then, that any sample which would be made up of twenty-five tablets containing zero content and twenty-five tablets containing 0.2 or 24/26, 26/24, 23/27, and 27/23 would have in the fifty tablets a sufficient amount of digitoxin so that the sample would pass assay. Our statisticians indicate to us that, on completely random basis, over 50 percent of the samples taken would pass the assay requirement, and if the evaluating laboratory were allowed to assay the second sample, the probability of meeting assay requirements is even greater.

This certainly is surprising, but let us take the more realistic example and assume that the tablets were not so extremely variant; but that the lot was composed of tablets containing the 0.07 mg. and 0.13 mg. of our earlier example. Under these circumstances, all samples with between eighteen to thirty-two tablets containing 0.07 mg. and thirty-two to eighteen tablets containing 0.13 mg. would pass the test. Over 91 percent of the samples taken would pass the assay, and if repeat assays were allowed, it would practically never occur that a sample would fail to pass.

I wonder how many volumes of data of this kind, guaranteeing the effectiveness and the potency of products, exist?

These facts have been recognized by the responsible companies within the pharmaceutical industry, and there are those who have been working for years to have more precise concepts adopted. It is difficult to achieve this goal primarily because of one fact—people are afraid of statistics. There is a group which has a philosophy that one should not judge the quality of a lot of material based on the assay of one tablet. But the same group does judge the quality based on the assay of the average of twenty tablets. I would propose assaying more than one tablet and then using some statistical treatment to determine acceptance and rejection standards.

I know that in this particular group today there is no need to dwell on this subject. Acceptance and rejection plans based on sound statistical approaches are the basis of most manufacturers. This has been a sound contribution by industry to total quality control.

Let me conclude, therefore, with the simple statement that to insure that a product will perform its "intended function" is not easy. In fact, this job really requires a constant surveillance. There are no short cuts.
There are no slogans, no instantly-applied philosophies, no quick way to bring about total quality control. With the assurance that the product has been designed properly, that those who have the responsibility of producing the product have, in fact, performed their functions adequately, and that the checks and tests performed along the way give the assurance that nothing unforeseen has gone wrong, then and only then is it reasonable to make the judgement that this lot will perform its "intended function."
EXPERIMENTAL AFLATOXICOSIS IN FEEDER PIGS

WILLIAM T. SHALKOP, D.V.M.; JAROSLAV N. GELETA, M.S., D.V.M.; BERNARD H. ARMBRECHT, Ph.D.; and HERBERT G. WISEMAN, B.S.

INTRODUCTION

There are a number of feedstuffs ingested by pigs that have the potential for contamination by mycotoxins. Two such feedstuffs, reported in the literature, are moldy corn and toxic peanut meal.

Aflatoxin, produced by Aspergillus flavus, is the mycotoxin usually identified with toxic peanut meal as reported in England. In this country A. flavus, Penicillium rubrum, and perhaps other companion fungi, have been isolated from Moldy Corn Toxicosis.

Loosmore and Harding in 1961 and Harding et al. in 1963 described the gross and histopathologic lesions in a natural outbreak and in experimental studies of groundnut toxicity.

Sippel et al. had a very complete pathologic description of field cases of Moldy Corn Toxicosis in pigs in 1953, and Burnside et al. were later able to reproduce a similar toxicosis by giving swine, via stomach tube, pure culture isolates prepared from corn obtained in the same area as the 1953 outbreak. Burnside used A. flavus Link, later designated A. flavus CD #5, and in another experiment used P. rubrum Stoll.

In the earlier work aflatoxin levels were not described as the four major fluorescent substances in aflatoxin, B-1, B-2, G-1 and G-2 were not demonstrated until 1962.

In our study pure cultures of A. flavus Link were fed to experimental pigs. The purpose of these experiments was to correlate the pathologic findings in feeder pigs with various levels of aflatoxin, and to make a brief comparison with the changes reported in the literature for Moldy Corn Toxicosis and Groundnut Toxicity.

MATERIALS AND METHODS

Three separate studies are included in this report. In two experiments, designated Trial I and Trial III, the pigs were on aflatoxin levels to produce a more chronic reaction. The third experiment, developed out of Trial III, produced a more fulminating toxicity and will be designated as the Acute Study.

Trial III will be considered in detail. Trial I will be referred to only in the sub-clinical results as no significant pathologic changes were observed in the pigs on this Trial. The Acute Study will be referred to briefly as this will be published in more detail at a later date.

In Trial I there were 40 pigs divided into 4 groups of 10 pigs each, and the levels for the respective groups were 0.2 ppm, 0.7 ppm, 1.1 ppm, and a Control group on basal ration only. There were 27 Yorkshire and 13 Duroc pigs in the trial, and they were about 20 weeks old at the start of the toxic feeding.
Trial III had 60 animals divided into 4 groups of 15 each, and the respective levels were 1 ppm, 2 ppm, and 4 ppm, and a Control group on basal ration only. These were cross-bred Poland-China, Duroc, and Berkshire and were about 13 weeks old at the start of the toxic feeding.

In the Acute Study 5 pigs were used and these were dosed at a rate of .3mg of aflatoxin concentrate per kilogram body weight per day, via capsule. Capsules were given daily for periods that varied from 5 to 7 days.

The aflatoxins used in Trials I and III were produced by pure cultures of *A. flavus* grown as surface cultures on rice, wheat, and sucrose, attenuated with acetone, and mixed with the regular hog ration. The aflatoxin in the Acute Study was a concentrate from a pure culture of *A. flavus*, and contained the following percentages of the B and G components: 50.2% B-1, 7.3% B-2, 5.5% G-1, and 7% G-2. The dose is expressed as the sum of B-1 and G-1 present in the material.

Paraffin sections were prepared stained with Hematoxylin and eosin, and with Gomori's reticulum stain. Frozen sections cut on a cryostat were stained with oil red O for neutral fat, periodic acid Schiff for glycogen and Gomori's method for alkaline phosphatase.

**CLINICAL FINDINGS**

*Sub-Clinical.* The most sensitive sub-clinical parameter was a loss of feed conversion efficiency, and this occurred at a per diem rate of .012 mg per kg body weight in the .2 ppm group in Trial I. A more detailed treatment of the feed conversion efficiency in these pigs is published in another paper.

*Clinical.* The animals on 4ppm, and also those on 2 ppm for prolonged time periods, had a loss of appetite, made poor weight gains, were depressed, and developed scleral icterus. A few pigs stood with arched backs and lowered heads. Others held their nose against the cage and were unresponsive to your approach, although terminal convulsive seizures also occurred that could be triggered by a sharp sound such as a handclap. Six of the pigs on 4 ppm that died within a month had bloody diarrhea. Temperatures were normal at all levels of aflatoxin throughout the experiment, with the exception of subnormal readings on some of the pigs that died.

A change in the serum alkaline phosphatase level was the earliest clinical laboratory parameter to be observed, but its fluctuations made it difficult to use as a diagnostic tool.

A loss of serum proteins developed later than the alkaline phosphatase deviation, but were a better serum evaluation because of more persistent levels. The serum albumin had a relative decrease and the gamma globulin an increase at a level of 0.03 to 0.07 mg per kg body weight per day.

Hematologic parameters showed no significant differences until the animals were near the terminal stages of the toxicoses. At this time there was an increase in the red and white blood cell counts, with changes in hemoglobin and hematocrit values. Some animals on the 2ppm and 4 ppm...
levels had a neutrophilia, a reversal of the lymphocyte-neutrophile ratio, and a marked decrease in the number of eosinophiles.

Liver biopsies were made on several animals at the start of the experiment, but were discontinued, as were bleedings from the anterior vena cava for hematologic studies, because of abnormally prolonged coagulation times.

Pathology

Mortality

<table>
<thead>
<tr>
<th>Trial</th>
<th>No deaths at any level.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial I</td>
<td>No deaths.</td>
</tr>
<tr>
<td>Trial III</td>
<td>2 deaths (killed in extremis).</td>
</tr>
<tr>
<td>1 ppm 0/10</td>
<td>2 deaths (killed in extremis).</td>
</tr>
<tr>
<td>2 ppm 2/12</td>
<td>8 deaths. 7 died between 28th and 46th day. 1 died at 5 months, but off toxic feed 2 months.</td>
</tr>
<tr>
<td>4 ppm 8/15</td>
<td>0.3 mg/kg body weight. 4 died between 7th and 14th day after initial toxic dose. 1 survived and sacrificed 12 week later.</td>
</tr>
</tbody>
</table>

Acute Study

MACROSCOPIC CHANGES

TRIAL I. None observed at any level.

TRIAL III. Most of the animals that died on the 4 ppm level had a generalized icterus, petechial hemorrhages in the subcutaneous tissue and fat, a light amber colored fluid in the pericardial sac and abdominal cavity, and the synovial fluid in the hip joint was a light yellow.

Liver. This was the primary target organ and the most obvious macroscopic change in a few of the pigs on 2 ppm and most of the 4 ppm was the development of a hard fibrous texture, and a deep yellow color of the entire organ. The gall bladder in the advanced cases was atrophic and the wall edematous; the bile in a few animals was black and had the consistency of tar.

Kidney. The changes in this organ were not consistent and when they did occur were usually petechial or linear hemorrhages in the cortex or at the corticomedullary junction. In two of the hogs on 4ppm, that died within 4 weeks, there was a bile stained fluid in the pelvis of the organ.

Mesenteric and Hepatic Lymp Nodes. Congestion and hemorrhage were not uncommon, especially in the hepatic nodes, in the 4 ppm group.

Adrenal Glands. These organs were hyperplastic in 50% of the 2 ppm pigs, in 60% of the 4 ppm pigs, and in 100% of the pigs on the Acute Study.

ACUTE STUDY. The changes in these pigs were very similar to those seen on the 4 ppm level that died, with the exception that the liver was more pliable in the acute group, which was consistent with the more fulminating course and consequent lack of fibrous tissue development.
Table I is a summary of the more important macroscopic changes seen in Trial III and in the Acute Study, along with dose levels, and number of animals affected.

**TABLE I**

**Macropscopic Changes in Trial III and Acute Study.**

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>1 ppm mg/kg</th>
<th>2 ppm mg/kg</th>
<th>4 ppm mg/kg</th>
<th>Acute mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 pigs</td>
<td>12 pigs</td>
<td>15 pigs</td>
<td>5 pigs</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Abnormal</td>
<td>8</td>
<td>11</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Gall Bladder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Atrophic</td>
<td>3</td>
<td>6</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>9</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Icteric</td>
<td>1</td>
<td>5</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>G. I. Tract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Adrenal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Hyperplastic</td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>

Figures immediately under 1, 2, and 4 ppm indicate range of aflatoxin consumed per kilogram body weight per day. Under "Liver" caption "Abnormal" refers to change in color of organ.

**MICROSCOPIC CHANGES**

*Liver.* The histopathologic description will be confined primarily to this organ. The progression of the histopathologic events in the liver depend to a great extent on the time-dose relationship.

The earliest sacrifice on the 1 ppm pigs in Trial III was at 10 weeks and at this stage there was proliferation of reticulum and collagen fibers at the periphery of the lobules. At 18 and 19 weeks these fibers were surrounding small groups of regenerating liver cells, bile duct proliferation had extended into the mid-zone of the lobules, and there were enlarged liver cell nuclei with prominent nucleoli.

The pathologic changes in the 2 ppm group were essentially similar to the nonfatal 4 ppm cases. There was a time lag in the development of the lesions, however, due to the reduced toxin intake.

At the 4 ppm level six animals died between the 4th and the 5th week and the most prominent changes were a severe fatty infiltration of all the liver cells, and complete loss of glycogen. Most of the liver cell nuclei had prominent nucleoli and only a sparse chromatin network. There was slight proliferation of reticular fibers along the septal borders.

Other animals at this level were sacrificed between the 6th and 20th
week. Up to the 5th week there was only a slight proliferation of the reticular fibers and bile duct cells along the septal borders, but between the 5th and 6th week Gomori's reticulum stain showed numerous small islands of liver cells surrounded by thin reticular fibers. The resulting rosette formation usually had a small central lumen, compared to the cellular mass, and the liver cell cytoplasm was very granular, vacuolated, or in many cells had disappeared. (Figure 1). Bile duct proliferation was moderately increased from about the 6th week.

Figure 1. Arrow points to tabular formation of liver cells with prominent nucleoli. The cytoplasm is increased relative to the nuclear mass. Pig received 4 ppm aflatoxin for 6.3 weeks, 400X.

In the 4 ppm pigs the proliferating bile duct cells could be differentiated from the regenerating islands of liver cells as the bile duct cells had a more diffuse, bluish nucleus and cytoplasm, lacked prominent nucleoli, and the lumens of the duct-like structures were relatively large compared to the cellular mass. (Figure 2).

At the 6th week, islands of degenerating liver cells were confined to the centrolobular areas and stained intensely with Oil Red O. Bands of regenerating liver cells with basophilic cytoplasm and small nuclei extended from the septum to the intermediate zone in most of the lobules. This may indicate an increased resistance to the aflatoxin for a short time at least; however, after the 7th week these normal appearing groups of cells were few in number.
Figure 2. Proliferation of bile ducts indicated by arrows. Nucleoli small and cytoplasm greatly reduced compared to nuclear mass. Pig received 4 ppm aflatoxin for 5.3 weeks. 400X.

Figure 3. Liver from control pig. Gomori’s reticulum stain. Septa and portal triads are most prominent features.
At 16 weeks there were numerous foci of lymphocytes along with a few eosinophiles.

By the 20th week large nodules of regenerating liver cells, with a thin reticular-collagen capsule extended throughout the parenchyma and above the liver surface. The regenerating liver cell nodules contained moderate amounts of glycogen, although small globules of fat were also present. (Figures 3 and 4).

Many enlarged liver cells were present at all stages of the 4 ppm level, but what is termed “megalohepatocytes” were difficult to find. It is our belief that all of the very large liver cell nuclei seen in our cases were enlarged degenerating nuclei, and not viable anaplastic cells. (Fig. 5).

In the acute cases the liver changes reflected the accentuated time-dose schedule, and showed centrolobular hemorrhage, moderate bile duct proliferation and severe fatty changes.

**DISCUSSION**

Among our groups of pigs the pathologic lesions in the animals on the Acute Study more closely approached Sippel’s findings in Moldy Corn
Toxicosis. However, he described more severe and extensive hemorrhagic lesions in his acute cases, and these would probably be considered per-acute compared to our Acute cases. The changes in our chronic group were fairly close to his chronic cases, although he reported hemorrhages in the heart and skeletal muscle that were rare and not as extensive in our pigs.

![Figure 5. Large liver cell nuclei in center of field, with prominent nucleoli and loss of chromatin. Pig on 4 ppm aflatoxin for 3.3 weeks. 400X.](image)

Moldy Corn Toxicosis can apparently produce a more per-acute reaction than that reported for toxic groundnut outbreaks. *A. flavus* is the predominant organism reported in groundnut toxicity, while in Sippel's and Burnside's work it was shown that *P. rubrum* was also an important toxin producer. Burnside notes that it required about 7 pounds of Aspergillus-corn substrate, fed over a four-day period, to produce death in swine, while only 8 ounces of *P. rubrum* substrate was lethal in less than 18 hours in one pig.
There is a very close correlation between our Chronic Study and the changes described by Loosmore and Harding in an outbreak of groundnut toxicosis. This correlation holds for both the macroscopic and the microscopic changes. Loosmore states that from field evidence piglets had to ingest the substance for at least 6 weeks before showing clinical signs, which would indicate the outbreaks were not per-acute.

We do not wish to leave the impression that the pathologic changes seen in the experimental studies completely duplicate a field outbreak of Moldy Corn Toxicosis in pigs. Only one organism was used in our experiments, but in field cases there may be several competing or synergistic organisms, not to mention numerous environmental factors that can influence the production of toxin by the fungi.

There were significant variations in the extent of the pathologic lesions in two groups of pigs on essentially similar levels of aflatoxin; namely, the 1.1 ppm in Trial I and the 1 ppm in Trial III. There is no apparent explanation for the variation in response in these two Trials. It is reported that young pigs are more susceptible to aflatoxin than older animals, and the 1 ppm pigs were about 7 weeks younger than the 1.1 ppm group. Both groups, however, were well beyond the weaning age, and it is not believed the age differential was responsible for the variation in the pathologic response.

There were two pathologic aspects of our experiments that have not been described as occurring in either field or experimental cases. These were hyperplasia of the adrenals and the reversal of the lymphocyte-neutrophile ratio with depletion of the eosinophiles.

The white blood cell changes may be a response to a terminal stress situation as a result of hypersecretion of cortisone by the adrenal cortex. There was evidence in several of the sacrificed animals that adrenal cortical hyperplasia was occurring some time before the animal would have been entering a terminal phase. The intense hemorrhagic gastroenteritis might also be related to hyper levels of serum glucocorticoids. A test was to be designed to assay some of the steroid levels in aflatoxin exposed pigs at stages considerably prior to the appearance of obvious clinical signs. Preliminary information was desired as to the reaction of swine white blood cells to higher than normal cortisone levels in the blood. Only one reference was found relative to the hog's white blood cell reaction to ACTH or cortisone and this reported a lymphocyte-neutrophile reversal, but also an eosinophilia and not the expected eosinopenia. We injected several pigs with ACTH at a dose of 1 unit per pound body weight to stimulate the adrenal cortex to produce cortisone and on taking blood samples 5 hours later had a reversal of the lymphocyte-neutrophile ratio and a reduction in the absolute number of eosinophiles. Another injection the following day produced a complete loss of the peripheral eosinophiles. Further work is planned on making steroid assays in aflatoxin exposed animals.

**SUMMARY**

1. *Aspergillus flavus* Link was fed as part of the basic ration to groups....
of experimental pigs at various levels from 0.2 ppm to 4 ppm, and a group of 5 pigs was dosed via capsule at the rate of 0.3 mg/kg body weight.

2. The mortality rate was 2/12 at 2 ppm; 8/15 at 4 ppm, and 4/5 at .3 mg/kg body weight. All other levels had no deaths.

3. Macroscopic and microscopic lesions produced at the various levels are described.

4. Neither liver cell nor bile duct tumors were seen in any of the liver sections examined.

REFERENCES

REPORT OF THE COMMITTEE ON PHARMACEUTICALS


This committee in 1966 recommended the preparation of a "Compendium on Veterinary Drugs" for use by those in the animal and poultry industries. We hereby reaffirm this proposal and believe that immediate steps should be taken by the Bureau of Veterinary Medicine, Food and Drug Administration and the American Veterinary Medical Association to develop and supervise necessary reviews to assure current information to the veterinary profession and these industries.

A "Feed Additive Compendium" is available which contains useful information on drugs and chemicals that may be added to livestock and poultry feeds. This is published by the Miller Publishing Company, P. O. Box 67, Minneapolis, Minnesota, 55440.

The contract by the Bureau of Veterinary Medicine, FDA, with the National Academy of Sciences to review all effective NDA's for animal therapy on safety and efficacy is to be completed by 1968. This review, with revisions of the NDA's covering most of the drugs in current usage, would serve as a ready source of information for the proposed "Compendium on Veterinary Drugs."

Recognizing the benefits to those industries wherein it is not economically feasible to secure effective NDA's for limited markets for certain species of pets, non-food animals, we also strongly recommend that realistic regulations be established on new drugs for such animals including horses, dogs or other pet animals.

A three-day "Symposium on the Use of Drugs in Animal Feeds" was presented by the National Academy of Sciences—National Research Council in June, 1967, in Washington, D. C. Dr. James L. Goddard, Commissioner, FDA, stated that the participants agreed that antibiotics, by reducing disease levels, have resulted in increased rates of growth. However, he pointed out that research is necessary to determine whether such usage may affect the possible transfer of the resistance phenomenon in bacteria from animals to human infections.

Again, it should be stressed that veterinarians should report any unexpected or adverse drug reactions to the manufacturer so that this can be incorporated in the new drug application file on that product. It is proposed that graduating veterinary students be acquainted with the high standards of quality control established by industry as well as their responsibilities in utilizing drugs as per label specifications. The Bureau of Veterinary Medicine has been most cooperative in presenting seminars on the intent of the Food and Drug Act.

Another area needing early emphasis is an expansion of the opportunities for graduate research training programs in pharmacology and toxicology to provide qualified, competent staffs for our Colleges, industries and
the Government. One serious and continuing problem requiring early attention is mycotoxicosis. Additional research funds should be made available to define and solve the problem in animals to reduce these losses, but equally important to determine the potential hazards to human health.

The fact that drugs, chemicals, pesticides may remain in the tissues of animals when given orally or parenterally makes it imperative that the veterinary profession realize the necessity of using these agents as per label recommendations, pointing out the recognized withdrawal periods to the owners. Finally, the significant breakthrough in the use of drugs for controlling estrus in animals will permit more widespread use of artificial insemination and improved breeding programs, with greater economic returns to these industries. Veterinarians, interested in improving the economic returns to these industries should keep abreast of new knowledge in the proper usage of these agents.
Attempts have been made to prevent outbreaks of fowl cholera in chickens ever since the classical experiments of Pasteur in which he protected chickens against fowl cholera by attenuated vaccines. Since his method could not be successfully repeated, the search for an efficient vaccine against *Past. multocida* has continued. Vaccines produced by different workers and commercial firms have been generally successful in the hands of those who described them and unsuccessful when used by independent investigators.

As early as 1920, Van Es and Martin compared the efficiency of six commercial vaccines against fowl cholera and found that no reliance could be placed on any of these vaccines. Shotwell (1967) evaluated the efficiency of eight commercial vaccines for prevention of fowl cholera in turkeys and found that only two had some protective properties. Two commercial vaccines used in Canada tested by us in turkeys and mice failed to show any protection against *Past. multocida* strains isolated from outbreaks of fowl cholera in Ontario, Canada.

In order to attempt to resolve the confusion over the value of vaccines against fowl cholera, studies were carried out on immunogenic properties and specificity on *Past. multocida* strains isolated from outbreaks of fowl cholera in Ontario.

**MATERIAL & METHODS**

Strains of *Past. multocida* used in this experiment consisted of 16 strains isolated in Ontario from turkeys which died of fowl cholera and three strains obtained from outside Canada. All strains were stored in liquid nitrogen. Frequent subcultures were avoided.

**Animals**

White unvaccinated turkeys were purchased from a farm free from fowl cholera.

White swiss mice weighing between 25 and 30 grams were obtained from the breeding colony of the Ontario Veterinary College.

**IMMUNOGENIC PROPERTIES OF PAST. MULTOCIDA STRAINS**

*Evaluation of bacterins prepared from cultures on solid and fluid media*

Before studying the immunogenicity of *Past. multocida* strains, com-
Comparative growth studies were made on various media. The most luxurient growth was obtained on Y.P.C. (yeast proteose and cystine) agar described by Namioka and Murata (1961). After finding this suitable medium, bacterins were prepared on Y.P.C. solid medium and in Y.P.C. liquid medium. Broth cultures and suspension of bacteria harvested in broth from the solid media were inactivated by 0.1 per cent beta propiolactone at 4°C and kept in this temperature overnight. Both preparations contained the same number of organisms. These two bacterins were tested for their immunogenic value in rabbits and mice. Rabbits and mice were given two doses of vaccine by subcutaneous injection. Rabbits received 2 ml. and mice 0.5 ml. at each injection. The interval between injections was 3 weeks and the interval between the last injection and the challenge was 3 weeks. The results following the challenge of these vaccinated rabbits and the passive protection which their sera conferred on mice, established that the vaccine which was prepared from cultures grown on solid media were more immunogenic than the vaccine prepared from cultures grown in Y.P.C. broth medium. Similar results were obtained in mice.

The report by Liu (1966) that sera prepared from extracts of *Pseudomonas aeruginosa* obtained from cultures grown in trypticase soy agar were able to neutralize pseudomonas toxin, but antiserum to extracts from cultures from the same substrate in liquid form failed to do so, support the observation that the physical state of the culture medium affects the immunogenicity of vaccines.

**Immunization of mice and turkeys with monovalent vaccines**

In view of better results with bacterins prepared from cultures grown on solid media, further studies on immunogenicity of *Past. multocida* strains were carried out with this type of bacterin prepared from various strains of *Past. multocida*. A group of 10 mice and 6 turkeys for each bacterin was vaccinated with two doses at an interval of 3 weeks of a monovalent bacterin and challenged with a homologous strain three weeks after the second dose. Unvaccinated mice and turkeys served as controls.

Results of this experiment suggested that there is a great discrepancy in immunogenic properties among strains. Some bacterins were able to protect nearly all vaccinated animals, while others protected either 50 per cent or not at all, in spite of the fact that all were prepared in the same manner.

**Evaluation of immunity in vaccinated turkeys by passive protection test in mice and culturing organs of passively protected mice after challenge**

After demonstrating protection by challenging vaccinated turkeys, it was decided to test the sera of vaccinated turkeys which were not infected for the presence of humoral antibodies. The mouse passive protection test was used for this purpose.

This test was found to be much more accurate in evaluating the immune status of turkeys than direct challenge. Since the degree of clear-
IMMUNOLOGICAL PROBLEMS

ance of bacteria by antiserum is a reflection of its high potency, experiments were conducted to recover Pasteurella after challenging protected mice at various intervals. In passively protected mice, no bacteria was cultured from either liver, spleen or blood 17 hours after infection, or at any subsequent time tested up to 24 hours after infection.

On the other hand, in mice that received normal turkey sera Paste. multocida was first demonstrated in the spleen 14 hours after infection and on the 17th hour after infection, the organism could be demonstrated in blood, liver and spleen and the concentration of organism increased until the 24th hour after infection.

IMMUNOGENIC SPECIFICITY

After demonstrating high immunogenic properties of some strains isolated from cases of fowl cholera in turkeys, studies were carried out on their specificity. Mice and turkeys were inoculated with two doses of a bacterin prepared from a good immunogenic strain and challenged with different strains 3 weeks after the second dose of the vaccine. The results of this experiment indicated that the immunogenicity of this strain was specific, although cross protection was observed among some strains, but was usually lower than against homologous challenge. It appears from these studies that monovalent vaccines have little value for general use in prophylaxis.

SUMMARY

The results of these studies indicated that strains of Past. multocida isolated from outbreaks of fowl cholera vary in their immunogenic activity. Bacterins prepared from cultures grown on solid media are more immunogenic than bacterins prepared from cultures grown in fluid media.

Immunity produced by a monovalent bacterin prepared from a highly immunogenic strain evaluated not only by challenge but also by passive mouse protection with sera from vaccinated turkeys is of high potency, but only against the homologous strain. Cross protection was observed among some strains of Past. multocida. Further studies on immunogenic specificity and pathogenesis of Past. multocida are still needed.

REFERENCES

REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY


The Transmissible Diseases of Poultry Committee has continued the effort of providing a philosophy of "Why Live With Disease, If It Can Be Eradicated?"

This effort has continued in the proposed Pullorum Disease and Fowl Typhoid Program. The Subcommittee for the Eradication of Pullorum Disease and Fowl Typhoid has continued its effects under the Chairmanship of Dr. Ben Pomeroy.

The following Subcommittee Report has been reported as follows: The subcommittee on Pullorum Disease and Fowl Typhoid eradication met October 16 at 2:00 PM and reviewed the recommendations of the 1966 Committee as related to the five phase program to eradicate Pullorum Disease and Fowl Typhoid.

The discussion of the subcommittee centered on several aspects of the proposed eradication program:

1. Reconfirm in principle the five-phase program and encourage the turkey industry to move ahead utilizing such a program.
2. The poultry industry has encouraged the disease control agency in each state to make pullorum disease and fowl typhoid reportable diseases. The subcommittee points out that in order to implement the five phase, one of the basic requirements is to make these diseases reportable. There are still states that have not declared pullorum disease and fowl typhoid reportable. The subcommittee recommends that all states make the diseases reportable.
3. The subcommittee recommends that there should be field follow-up of all isolations and outbreaks reported.
4. The subcommittee recommends that the uniform methods and rules for the eradication of pullorum and fowl typhoid use the terminology of surveillance, hold order or something other than quarantine and that the movement of pullorum disease and fowl typhoid infected flocks be restricted and handled in such a manner to avoid further spread of the diseases.
5. The subcommittee reviewed the report of the American Association
of Avian Pathologics report on Pullorum Typhoid Eradication, dated July 11, 1967. In regard to this report your subcommittee recommended that representatives of the various industry groups interested in the eradication program meet in St. Louis on January 7, 1968 to further discuss and implement the eradication program.

The Transmissible Diseases of Poultry Committee accepts and endorses the subcommittee report as written. The committee recommends that Dr. Ben Pomeroy continue as chairman of the subcommittee for the next year.

The committee has again attempted to highlight some of the important poultry problems:

**Gumboro Disease:**

Reports by Benton *et al.* indicate the size of the agent of infectious bursal condition is 10-50 millimicrons in diameter. The agent is fairly stable and withstands heat at 56°C for five hours. The agent was not deleteriously affected by phenol, merthiolate, Staphene or Hyamine or by a PH of 2.0. However it was inhibited by formalin, Wescodyne, and a PH of 12.0. Ether and chloroform treatment was not detrimental.

Benton *et al.* found in their studies on transmission of the infectious bursal agent that the agent could remain viable in a contaminated building for 122 days after infected birds were removed. Contaminated feed and water were capable of transmitting the disease to susceptible chickens. In this experiment, droppings and air transmission were not a factor of environmental spread.

**Infectious Laryngotracheitis:**

In opposition to previous reports indicating turkeys to be refractive to infectious laryngotracheitis Winterfield was able to infect turkeys with infectious laryngotracheitis virus producing respiratory signs and pathological changes. An age resistance was noted with the particular isolate used in this experiment. The lesions were similar to those seen in pheasants.

**Cholera:**

Sulfadimethoxine used in the drinking water exhibited a high degree of chemotherapeutic efficacy against *Pasteurella multocida* in work conducted by Mitrovic.

**Coryza:**

Infectious coryza, with complicating Pasteurella infections of the sinuses, is reported by Good and Hanley. The drug, sulfadimethoxine, is reported by Mitrovic as being of value in the treatment of coryza. Raggi *et al.* report a definite synergism between *Hemophilus gallinarium* and the bronchitis virus.
Bronchitis:

Concurrent infections with infectious bronchitis virus, and infectious laryngotracheitis virus in trials conducted by Raggi et al.\textsuperscript{12} failed to indicate any synergism between the two agents. Immunity developed when birds were inoculated simultaneously with both agents. This observation is, however, limited to the circumstances described in this work.

A means of differentiating four serotypes of infectious bronchitis virus by use of immunofluorescence was described by Lukert.\textsuperscript{13}

Work by Winterfield\textsuperscript{14} with a beta-propiolactone inactivated vaccine, as used in Great Britain, demonstrates the inability of this vaccine to produce immunity against our Massachusetts 41 and Beaudette strains.

A procedure for detecting infectious bronchitis virus neutralizing antibodies using a plague reduction cell culture method and comparing it favorably to the standard chick embryo virus neutralization test was detailed by Lukert.\textsuperscript{15}

Work by Hofstad\textsuperscript{16} again points out the importance of localized tissue response in bronchitis immunity.

Newcastle Disease:

An evaluation of the response of 6½ month old turkey hens to the MK-107 strain Newcastle Disease virus was made by Raggi et al.\textsuperscript{17} In this trial a method of tracheal challenge was described.

Coccidiosis:

Several new anti-coccidial drugs have appeared. Although first noted in 1963 as a drug with activity against coccidiosis, sulfadimethoxine, was reported this year by Mitrovic and Baurenfeind\textsuperscript{1} as having a high degree of efficacy against all pathogenic species in chickens and turkeys.

Work by Stork at al.\textsuperscript{2} and Reid et al.\textsuperscript{3} on Coyden (Meticlorpindol) and investigation of Johnson et al.,\textsuperscript{4} Morrison et al.,\textsuperscript{5} Reid et al.\textsuperscript{6} and of Engle et al.\textsuperscript{7} on buquinolate and the trials of McLaughlin and Chute\textsuperscript{8} on Novastat help introduce us to new coccidiostats that are needed in this continuing problem of coccidiosis.

The committee wishes to alert the poultry industry of the appearance of a new group of viruses of relatively low pathogenicity but potential danger when found in combination with other pathogens. These viruses belong to the influenza and parainfluenza groups. Virus isolations and serological surveys show them to be wide spread in poultry, particularly in turkeys in the U. S. and on the European continent. Diagnosticians should be cognizant of their existence in evaluating unusual respiratory and reproductive diseases.

The committee commends the USDA Animal Health Division and the New York State officials for its effort in attempting to eradicate Duck Virus Enteritis (Dutch Duck Plague) an exotic disease.

The committee also commends Dr. Louis Trehowitz the recognition
TRANSMISSIBLE DISEASES OF POULTRY

and diagnosis of the disease. The chairman of this committee and three committee members attended a study meeting in Hyattsville, Md. on September 19, 1967 and concurred in the program that vaccination should be studied through a pilot as a means to bring about organized control of Duck Virus Enteritis in the eradication program.

The committee recommended that a resolution be presented to the executive committee that the Secretary of Agriculture give serious consideration to promulgating an importation requirement involving poultry and poultry products, so that the poultry industries will be assured of uniform disease protection. This resolution was given to the Committee on Resolutions.

The committee commends the USDA Veterinary Biologics Division for its protective surveillance and encourages further expansion to assure the poultry industry of protection in the production of biologics.

The committee recognizes the Avian Leukosis Complex as a major problem of the industry, and recommends research continue to bring about more answers to the problem.

The committee recommended that a resolution be presented to the executive committee that the Secretary of Agriculture develop a program giving official recognition to the Pullorum Disease and Fowl Typhoid eradication program for turkeys in the five phase program adopted by the USLSA in 1966. This resolution has been given to the Committee on Resolutions.

The committee recognizes the need for continued improvement in mycoplasma gallisepticum antigen for stability and uniformity.

The committee further points out the need for commercial typhemurium antigen. There is no commercial product available.

RESOLUTION—POULTRY DISEASES

Whereas, the United States Livestock Sanitary Association has been vitally interested in the eradication of Pullorum Disease and Fowl Typhoid in poultry for many years, and,

Whereas, the United States Livestock Sanitary Association has endorsed uniform rules and methods for the eradication of Pullorum Disease and Fowl Typhoid, and,

Whereas, the United States Livestock Sanitary Association adopted a five phase program for the eradication of Pullorum Disease and Fowl Typhoid,

Now Therefore Be It Resolved, that the United States Livestock Sanitary Association assembled in convention this _______ day of October, 1967 request that the Honorable Secretary of Agriculture provide official recognition of the five phase program for the eradication of Pullorum Disease and Fowl Typhoid.
THE USE OF PEACEFUL NUCLEAR EXPLOSIVES
AND SOME RELATED SAFETY ASPECTS

RICHARD HAMBURGER

U.S. Atomic Energy Commission

INTRODUCTION

A Hungarian engineer conducted the first recorded experiment in the industrial uses of explosives. This was in 1627, 377 years after Roger Bacon first made his black powder, and at least 600 years after the Chinese invented gunpowder. About 22 years after the detonation of the first nuclear explosive, American Industry and the United States Government are scheduled to conduct their first experiment in the industrial uses of nuclear explosives. This program to investigate and develop the peaceful uses of nuclear explosives is called Plowshare.

Nuclear energy is one of today's facts. The taming of this force and the harnessing of this power to do man's work is one of today's challenges. Whole new techniques are being born. They have their promises and their problems. To judge their potential, we must be familiar with both the problems and the promises. The peaceful uses of nuclear explosives is one of these technologies.

The industrial use of nuclear explosives must meet three major criteria. The first is that the use of the nuclear explosive results in an acceptable engineering product. The second is that it is safe. The third is that the cost be competitive. The study, made to determine if an application meets these three criteria, is called a feasibility study. Each of these major elements may be studied independently, but in determining feasibility, they must be considered together. For example, each activity undertaken to assure safety involves a cost and this cost is, of course, a part of the total cost the application must bear. If it turns out that the safety costs are too high, then, although it is feasible to conduct the operation safely, the application is not feasible since it will not be competitive.

Costs

The nuclear explosive is a new tool. It is potentially a new tool for industry. As such, it will find a place only if its use is economically competitive with present or near future technology.

In an industrial situation, it is the effect of the explosion that is being purchased. Thus, the relation of cost to effect becomes an important consideration.

To assist industry in making estimates, the Commission has released projected charges for thermonuclear explosives (Figure 1). These projected charges are $350,000 for a nuclear explosive with 10-kiloton yield and $600,000 for a nuclear explosive with 2-megaton yield. Interpolations
may be made for intermediate yields. These charges cover nuclear materials, fabrication and assembly, and arming and firing services. Significant related services which are not covered by these projected charges are safety studies, site preparation including construction of holes, transportation and emplacement of the devices, and support. These latter costs depend significantly on the number of explosives detonated at one location, and on the location.

These projected charges are released only for use in feasibility studies and evaluations and are based on a projection to a time when explosives will be produced in quantity for routine commercial utilization. Nevertheless, the Commission believes that these projected charges are sufficiently representative of the future situation to warrant their use in feasibility studies.

The dollar-to-yield structure of these projected charges is the keystone to the use of nuclear explosives and the basis for my statement that new techniques and approaches are required. A simple comparison will illustrate
this point. In using explosives for excavation purposes, the basic commodity being bought is a linear effect, say width or depth of the excavation. Using the projected charges just quoted, it can be shown that doubling the number of dollars increases the linear effect approximately six times. If one bought twice the dollar value of TNT and then used it in the same manner,
the increase in linear effect would be 23 per cent. Or, to put it another way, it will cost you only 30 per cent more to double the crater width using nuclear explosives; but it will cost you ten times as much to do so with TNT. Further, the cost of emplacing TNT in equivalent amounts is large. For instance, 10 kilotons of nuclear explosive may be emplaced underground in a 15-inch hole and the projected charge is $350,000 (Figure 2). To emplace 10 kilotons of TNT in its compactest form—a sphere—requires a hole about 84 feet in diameter, and the TNT will cost about $5,500,000. Thus, it is apparent that both the cost of conventional explosives and their cost of emplacement becomes excessive at large yields. Quite obviously, conventional explosives would not be, and usually are not used in the same manner as is contemplated for nuclear explosives. The dollar-to-effect relationship means that the larger yield nuclear explosive is cheaper in relation to the effect produced than is the smaller nuclear explosive. This difference, in many cases, makes nuclear explosives more than just a larger stick of powder to be substituted into already standard procedures.

When you compare the cost of using nuclear explosives with the cost of doing the job by conventional means, the total energy system must be considered. For instance, conventional excavation consists of drilling, blasting, loading, hauling, and disposal. In nuclear excavation, only drilling and blasting are involved. The explosion not only breaks but also moves the broken rock. Nuclear explosives also have costs peculiar to their use, but as the yield goes up, this increment becomes a smaller percentage of total cost. Calculations based on presently available information indicate that for large excavation projects the use of nuclear explosives should be the cheaper method of excavation.

Such a comparison of total costs has been made for excavating a sea-level
canal between the Atlantic and the Pacific (Figure 3). Another estimate for two nuclear routes made in 1964 by Col. Graves of the Corps of Engineers shows more detail (Figure 4). Col. Graves' estimate shows that less

| ESTIMATED COSTS FOR CONSTRUCTION OF A SEA-LEVEL CANAL ON TWO ROUTES ACROSS THE AMERICAN ISTHMUS* |
|-------------------------------------------------|-----------------------------------|-----------------------------------|
| Route 17 (Sasardi-Morti) Panama                  | Route 25 (Atrato-Truando) Colombia |
| Phase I—Feasibility & Site Selection Surveys    | $17,000,000                       | $17,000,000                       |
| Phase II—Engineering Surveys & Design           | 13,000,000                        | 13,000,000                        |
| Phase III—Construction                          | 90,000,000                        | 130,000,000                       |
| General construction:                           | 70,000,000                        | 470,000,000                       |
| Conventional excavation & embankments           | 60,000,000                        | 80,000,000                        |
| Nuclear excavation:                              | 60,000,000                        | 60,000,000                        |
| Emplacement drilling                             | 30,000,000                        | 30,000,000                        |
| Area excavation                                  | 50,000,000                        | 50,000,000                        |
| Safety program                                   | 150,000,000                       | 150,000,000                       |
| Explosives & firing services†                   | 30,000,000                        | 75,000,000                        |
| Engineering                                      |                                  |                                  |
| Total Contingency (15% of Phase III)             | $570,000,000                      | $1,100,000,000                    |
| Total estimated cost of construction             | $650,000,000                      | $1,260,000,000                    |

† Estimate for engineering and production of a stockpile of a few hundred nuclear explosives for excavation, including services associated with firing them. This estimate is consistent with the 1964 charges announced by the Atomic Energy Commission.

Figure 4. Estimated costs for construction of a sea-level canal on two routes across the American Isthmus.

than one half the cost of either route is directly involved in the actual excavation with nuclear explosives. It is worth noting that the estimates for conventional excavation are for canals 600 feet wide and 60 feet deep while nuclear excavation would make a canal 1000 feet wide and 250 feet deep at the center.

Engineering

To understand the Plowshare concept some generalities about the interaction of the explosion with the rock in which it occurs is needed. The following is true for all explosives. Nuclear explosives differ from chemical explosives essentially in the partition of the energy and in the time of the reaction (Figure 5). When an explosion occurs underground, a number of things occur in very close succession. In the immediate vicinity all elements are disassociated and a cavity is formed. The radius of the cavi-
ty, formed by the energy of the detonation grows first by vaporization, then by melting, and finally by crushing. In the same time scale, a shock wave leaves the point of detonation and travels outward in all directions. This shock wave is a compression wave and will at some time intersect the ground surface and be refracted back towards the detonation. As the shock wave is refracted back, it places the rock in tension (its weakest failure strength). If, at this time, the energy remaining in the wave is sufficient, the rock will fail in tension from the surface downward. Depending on the relative distance between the detonation point and surface, and the strength of the rarefaction wave, this tensile failure will either proceed downwards until it intersects the cavity or stops at some lesser distance.

If the rock is broken from the surface back down to the cavity, a cratering situation will result. But if the rarefaction wave is sufficiently weak, the explosion will not crater.

In the non-cratering case, the cavity will continue to grow until the pressures inside will equal the pressure resulting from the weight and strength of the rock above. In most cases, shortly after this situation occurs,
the rock above the cavity will collapse into the cavity. This collapse will continue upward until the increase in volume of the broken rubble is sufficient to fill the void space. Just prior to this collapse, the molten rock,

![Various stages of the Sedan cratering explosion.](image)

Figure 6. Various stages of the Sedan cratering explosion.

which has lined the cavity boundary, will collect in the bottom of the cavity. In rocks containing sufficient silica, a molten glass will contain the vast majority of the fission products.

In the cratering situation the gases in the cavity continue to expand.
Since there is less resistance above the cavity, it will grow preferentially in this direction. At the same time the gases within the cavity will be cooling. The ground above the point of detonation arches and at some point breaks. Much of the material is then thrown out to the sides, while about half falls back into place. The result is a crater. Depending on how much of the upward movement is imparted by particle acceleration and how much is a result of the gases produced by the rock and its components, we have two general cases. First, in the case of a detonation in a rock producing small amounts of gas, most of the energy which throws the rock out of the crater is due to the trapped particle acceleration, and the rock moves up essentially radially and falls out very close to the crater. In the second case, if an appreciable amount of gas is produced, the free fall of the rock particles is interrupted at the time these gases break through. This gas acceleration phase starts with the added energy imparted to the rock and assists in throwing it out of the crater area. In addition to these phenomena, the crater is enlarged appreciably by the forces acting laterally on its sides. As in the non-cratering case, most of the radioactivity produced will be contained in a glass within the lower part of the crater. In addition the fall back material will scavenge some of the radioactivity. Standard measurements of the apparent crater size (the visible crater) are taken with respect to the original ground surface.

Figures 6 and 7 show the stages of development of the Sedan crater.
Figure 8. Explosively excavated ditch.

Figure 9. Drawing illustrating the concept of an explosively-excavated harbor.
The 100 kiloton nuclear explosive was buried 635 feet below the surface. The Sedan crater is 325 feet deep and about 1300 feet in diameter. The first picture of Figure 6 is equivalent to Figure 5 C. The second picture of Figure 6 is equivalent to Figure 5 D. The third picture of Figure 6 is at a time just later than Figure 5 E. Figure 7 is equivalent to Figure 5 F.

A properly spaced row of charges will produce a ditch (Figure 8). Such
a ditch may be used for a harbor (Figure 9), or a cut for a highway or railroad (Figure 10).

The chimney formed by a fully contained explosion appears usable for a number of purposes (Figure 11). In Project Gasbuggy, an experiment in stimulating a relatively impermeable gas formation, the chimney forms an oversize drill hole. Thus, instead of penetrating the formation with a hole whose effective diameter is 6 or 12 inches, one has a hole with an effective diameter of nearly 200 feet. The same type of chimney, in the proper formation, may be used at the other end of the pipeline to store gas as proposed in Project Ketch. Storage of other fluids, waste management, and mining are other suggestions for using nuclear formed chimneys.

Safety

In general, for any activity to be acceptable, the benefit from it must be large in relation to its risk. Such an equation can be maximized by keeping the risk at a low level. A great deal of work in the Plowshare program is devoted to this objective. From this point of view, the true cost of safety in Plowshare is the cost of developing and executing a plan to prevent any of the effects of the explosion from developing into a hazard. Risk is created only by the limits of our knowledge, organization ability, or
by our failure to spend the necessary money for studies, people, or equipment. The record of the AEC indicates that practically no expense is spared to minimize such risks. And with several hundred safe explosions to its credit, it already appears that the risk is much less than in almost any other type of industrial enterprise. In summary, the costs of safety in Plowshare do not arise from hazards, they arise from efforts to avoid hazards.

When using nuclear explosives, there are five effects which primarily require attention. They are: radiation; heat; air blast; ground shock; throw-out; and dust. In contained or cratering uses the potential hazard from thermal energy is so small that it does not require discussion.

Since radiation is the problem that appears to worry most people, it can be used to illustrate our safety program. In the Plowshare program, we are working to reduce this effect by several means or, in an economic sense, reducing this effect to the point that it costs less to deal with it. If a nuclear explosive is detonated at increasing depths of burial, the amount of radioactivity appearing near the crater as prompt fallout decreases rapidly since the material through which the radioactivity must pass is thicker, and thus filters and traps a larger proportion of the activity. We are also investi-

![INFINITE DOSE FROM ARRIVAL (R)](image)

Figure 12. Fallout pattern for Sedan (a), the pattern as it would be expected based on 1964 technology (b), and as it would be from future technological advances.
gating special emplacement techniques which may entrap underground even more of the radioactivity. Another route for reducing this hazard in cratering is to make explosives with less energy from fission so that less radioactive material is produced. As an example, Figure 12 a. shows the fallout pattern for Sedan. Figure 12 b. is a projection of what the fallout pattern for Sedan would have been using 1964 technology, and Figure 12 c. is our forecast of what we believe we can accomplish by future research. At the time that this future technology is reached, and for large yield detonations, throwout rather than radiation will limit the close-in area which must be controlled. The numbers contoured are the total doses which would be received if a person were standing at that location at the time radioactive particles were deposited and remained there the rest of his life. For comparison, it is reported that the average yearly dose rate from the environment for people in the northern hemisphere is between about 0.08 R and 0.17 R, of which from about 0.06 R to 0.15 R is received externally. 0.5 R per year is the Federal Radiation Council Protection Guide for an individual of a population. Further protection from radiation in a given situation can be provided by making sure that this material does not fall in harmful amounts either on people or products they use. This involves controlling conditions and times of detonation: thus, knowledge of meteorology and the transport of material become important adjuncts to Plowshare projects. There is, both within and outside the Federal Government, a large research program on the possible effects of radiation. We also apply the results of this research to minimize the risk of creating a hazard.

Based on reasonable assumptions about scavenging during the venting process, use of special emplacement techniques, use of minimum fission explosives, and employment of extensive neutron shielding, the following information can be used in planning for cratering events of useful magnitudes. For each individual nuclear explosive detonated, the sum of fission products airborne in the radioactive cloud and in the fallout can be expected to be as low as the equivalent of 20 tons. The tritium release may be less than 20 kilocuries per kiloton of total yield, and the sum of activation products airborne in the radioactive cloud and in the fallout may be expected to be as low as the amounts shown in the following table:

In addition to the avoidance of excessive external exposure to radiation, excessive internal exposures must also be avoided. This involves studies concerning the possible pathways by which radionuclides may reach man and studies delineating the effects which these may have. In every case the purpose of these investigations is to determine how to avoid creating a hazard.

The case of iodine-131 is a good example. If I-131 is released to the atmosphere, deposited on pasture and eaten by dairy cows whose milk is consumed by people, a very simple, direct path exists. This is a pathway which contains two systems which concentrate I-131; the cow and man. A portion of the radiiodine ingested by the cow in feed is secreted in milk. Approximately 30 per cent of the radiiodine consumed by people may be concentrated in the thyroid. Thus, the thyroid is the critical organ for exposure to radiiodine since it is preferentially radiated. This pathway can
be broken by rather simple means but at some cost. For instance, through selection of proper meteorological conditions, the deposition of I-131 on pasturage can be avoided. Another way to accomplish this is to control the release so that it occurs when dairy cows are not on pasture, or if deemed advisable, the dairy cows may be placed on dry feed for the short time which it takes I-131 to decay (Half-life = 8 days). A third way is to divert the milk from direct use to some other use such as cheese, again allowing for radiiodine decay.

Another option which may be available for some radionuclides is to change the design of the explosive and its environment. Thus, if fission products are the source of major problems then thermonuclear explosives can be used. We can, today, make thermonuclear explosives in many yields with no more than a few kilotons of fission energy. If tritium is a major problem, more energy can be obtained from fission and less from fusion which involves tritium.

The kind of investigations involved in safety evaluations on large projects can best be illustrated by listing the general title of the studies being conducted for the Atlantic-Pacific Interoceanic Canal Study Commission.

| Geology | Meteorology | Hydrology | Physicochemical Oceanography | Ecology | Human | Agriculture | Terrestrial | Freshwater | Estuarine | Marine |
|---------|-------------|-----------|-----------------------------|---------|-------|-------------|-------------|-----------|-----------|---------|--------|

Note: This is not a complete list, and the amounts given may be upper limits rather than best estimates.
The product of these kinds of studies is an operations plan providing the maximum assurance that the exposure of people, both internal and external, to radiation will be less than the appropriate guides.

CONCLUSION

One of the measures of man's material progress is the amount of energy at his disposal. Early man had only his muscles to work for him. Then, domesticated animals furnished additional energy which could be used for his welfare. Mechanical inventions such as the wheel, the wedge, and the pulley added efficiency to this use of muscle power. The harnessing of water power to the wheel provided man with additional energy. Today, energy from many sources is available. One family of important energy sources is explosions—not the least of which is the exploding gasoline which drove me to this meeting. Nuclear explosions are about to make their debut into this energy family.
FOOT-AND-MOUTH DISEASE IN MAN—NOTES ON A RECENT CASE

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Although there is quite a voluminous literature on foot-and-mouth disease in man (see, for example, the review by Platt, 1958), many of the reported cases do not satisfy the criteria on which positive diagnosis can be based. Early cases depended mainly on clinical description and sometimes on the appearance of antibody titres in the serum. Where virus isolation was reported, the possibility of this virus being derived from accidental contamination from infected cattle was not ruled out. The development of improved virological techniques has, however, led to better substantiation of some cases of infection of man in recent years; for example, the series described by Pilz and Garbe (1966) where high virus titres were recovered and the antibody level was followed after infection. It is of interest, however, that in the series of cases which they describe contact with the virus had been severe and prolonged. In the case to be described here, on the other hand, contact was only indirect but there are complicating factors in that the patient developed a condition involving epithelial ulceration and other damage at a time when it is believed that he was not reinfected with the virus of foot-and-mouth disease. We have, therefore, a situation in which it would appear that foot-and-mouth disease in man is a rare condition and that infection depends either on heavy exposure to infection or possibly on predisposing causes which influence the susceptibility of the patient.

CASE HISTORY
The patient was a travelling salesman for a firm of agricultural contractors. He lived on a farm with his brother but had only indirect contact with the stock. An outbreak of the disease developed on the farm, the animals were slaughtered and 4 days later the patient complained of a slight sore throat and blisters developed on the palms and dorsums of the hand at 6 days. During the ensuing period further blisters developed on the feet and a number of swellings on the sides and front of the tongue. The vesicles on the palmar surfaces of the hands were 1-2 cm. by ½-1 cm. across. The lesions regressed and had cleared up by 15 days from the time when the animals were slaughtered. Three days later, however, further blisters developed on the hands and cleared up in the ensuing week.

A further crop of blisters on the hand were observed 5 months later. Although the local lymph glands were enlarged, the illness was non-febrile. Once again the lesions cleared up and disappeared within a fortnight.

LABORATORY EXAMINATION
A suspension of epithelium collected 2 days after the first appearance of
vesicles on the hands was inoculated into roller tube cultures of bovine thyroid cells (Snowdon, 1966). Cytopathic changes were observed and the culture fluid gave a positive complement fixation reaction with FMD type O antiserum. The epithelium was also titrated and was shown to contain $10^{6.8}$ TCD$_{50}$ of virus per gram. Virus was not again isolated from the patient, although epithelial samples were collected 15 and 18 days after the animals had been slaughtered and throat swabs were taken on 5 occasions in the ensuing weeks. The antibody titres obtained with FMD antigens in neutralization tests are shown in the table, which indicates the time at which the samples were taken after the first appearance of lesions.

<table>
<thead>
<tr>
<th>Serum Samples (Days Post Infection)</th>
<th>Neutralizing Titre (Virus Type)</th>
<th>CF Titre (Overnight Fixation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>1/178</td>
<td>$\leq$ 1/6</td>
</tr>
<tr>
<td>30</td>
<td>1/708</td>
<td>$\leq$ 1/6</td>
</tr>
<tr>
<td>40</td>
<td>1/355</td>
<td>$\leq$ 1/6</td>
</tr>
<tr>
<td>120</td>
<td>1/256</td>
<td>$\leq$ 1/6</td>
</tr>
<tr>
<td>154</td>
<td>1/80</td>
<td>$\leq$ 1/6</td>
</tr>
</tbody>
</table>

Sera were also collected from 5 veterinary officers who had been in contact previously with infected cattle at the same outbreak and from 12 members of the staff of this Institute; all of these workers had come into contact with the virus of FMD, sometimes on many occasions. All samples, tested in parallel with the serum from the patient, were negative (titre less than $1/8$) except for one serum from a member of the Institute staff which showed a neutralizing titre of 1/22 against virus of type O.

CONCLUSIONS

The data presented would appear to establish that the patient had in fact been infected by the virus of foot-and-mouth disease. Whether this infection accounted for all the symptoms which were observed is rather more doubtful. The appearance of blisters 5 months after the initial incident, without any antibody rise in sera taken at this time, indicates that at least on this occasion blisters were developing which had not their origin in foot-and-mouth disease virus infection. Some of the lesions observed on the first occasion, in particular the so-called weals on the tongue, are not typical of infection with the virus and it is therefore not unlikely that the patient suffered from some undiagnosed infection of the epithelial surfaces which predisposed him to infection with the virus of foot-and-mouth disease. Once this infection had been established, it would seem possible that the more typical lesions on the palms of the hands and on the feet were in fact
true initial lesions of foot-and-mouth disease. It can therefore be suggested that the patient, although out of direct contact with infected animals, did receive enough virus to constitute an initial infecting dose for one in his state of hyper-susceptibility. The clinical signs exhibited in the initial illness were far more severe in respect of the blisters than the later episodes and did appear to be more in line with those which might be expected of foot-and-mouth disease, judging by the appearance in susceptible species.

From the point of view of the epidemiology of foot-and-mouth disease, the occurrence of cases of human infection poses some interesting problems. No spread has appeared in any of the cases known from human to human although, where a number of people have been infected in the same Institute (as, for example, in Pilz and Garbe's cases), it is possible that spread had been in this direction and not directly from the animals with which they were all working. Likewise, there is no evidence of spread from humans back to animals, although it would be wise to prevent contact between human cases and possible susceptible animals. The human case should therefore be treated in isolation.

It is interesting, too, that in common with other relatively lowly susceptible species man would appear to harbour the virus only for a short time. There would therefore seem to be little likelihood of humans being a reservoir host of the virus in inter-epidemic periods and it is almost certain that the rôle of man in epidemiology will be insignificant in comparison with the various other methods of transmission of the disease, both directly and indirectly.

The data on this case will be presented more fully in a paper by Armstrong, Davie and Hedger. I am grateful to these authors for their permission to present this preliminary note.

REFERENCES

REPORT OF THE COMMITTEE ON PUBLIC HEALTH AND
RADIOLOGICAL Fallout—1967


CLASSIFICATION OF ETIOLOGIC AGENTS

In order to minimize the conflict between the freedom of microbiological research and the safety of laboratory workers and the people at large, the Public Health Service convened a group of experts, from within the Government, including the Agricultural Research Service, and from the outside, to find a solution to the problem. The group has met several times and is expected to meet at least once more before the task is completed. They have tentatively classified bacteria, fungi, and viruses into 5 classes according to the danger involved, and have defined conditions which must be met before one may receive or work with etiologic agents. This Committee has reviewed the document and modified it in the light of existing policy concerning the zoonotic pathogens and certain other animal pathogens.

The “Basis for Agent Classifications,” “Classification of Bacterial Agents, Fungal Agents, and Viral and Rickettsial Agents,” and the “Minimal Conditions Required for Handling Class 3 and Class 4 Agents” follow. The Committee wishes to emphasize that the classifications are tentative, however they are recommended for consideration by the United States Livestock Sanitary Association, as guidelines until the regulatory agencies and the scientific community can refine them.

EXCERPTS OF REGULATIONS FOR IMPORTING ETIOLOGIC AGENTS AND VECTORS

Public Health Service

Sec. 72.25 (a)(1) For the purpose of this section, etiologic agent is defined as the causative agent of the following diseases and such others as may be prescribed from time to time by the Surgeon General: Anthrax, botulism, brucellosis, cholera, Colorado tick fever, Coxsackie diseases, diphtheria, encephalitis (arthropodborne), glands, leptospirosis, lymphocytic choriomeningitis, melioidosis, meningococcal meningitis, paratyphoid fever, plague, poliomyelitis, Q fever, rabies, relapsing fever, rickettsial pox, Rift Valley fever, Rocky Mountain spotted fever, schistosomiasis, scrub typhus, smallpox, tetanus, tuberculosis, tularemia, typhoid fever, typhus fever, and yellow fever.

(2) The provisions of this section shall not apply to specimens transmitted to laboratories for diagnostic purposes or to finished biological
products for human or veterinary use bearing the U. S. Government license number of the manufacturer.

(b) A person shall not knowingly transport or cause to be transported in interstate traffic any etiologic agent unless the agent is packaged in a minimum of two sealed containers, and each such double container is enclosed in a third container, hereinafter referred to as an individual shipping container, in accordance with the following requirements:

(1) (i) The materials shall be placed in a watertight and airtight container which shall then be enclosed in a second durable watertight and airtight container. In the case of liquid (including frozen materials), the intervening space between the containers shall be provided with sufficient absorbent material so placed as to absorb the entire contents in case of breakage or leakage. Each such double container shall then be enclosed in an individual shipping container constructed of corrugated cardboard, fiber glass, wood, or other material of equivalent strength.

(ii) If dry ice is used as a refrigerant, the individual shipping container shall be vented, and if an outside shipping container is used, it shall also be vented.

(2)(i) The maximum amount of etiologic agent which may be shipped in an individual shipping container shall not exceed one U. S. gallon provided that two or more individual shipping containers may be overpacked in a single outside shipping container.

(ii) All containers and closures are so designed and constructed of such materials that they are capable of withstanding without rupture or leakage of contents, all shocks, pressure changes, or other conditions ordinarily incident to transportation handling.

(3) The shipping documents and the manifest accompanying the shipment include statements that the shipment contains infectious material and identifies the etiologic agent involved. The shipment itself shall be appropriately labeled.

(4) The requirements of this paragraph are in addition to and not in lieu of any other packaging or labeling requirements for the interstate shipment of etiologic agents established by the Interstate Commerce Commission and Civil Aeronautics Board.

Sec. 71.156 (a) A person shall not import into any place under the control of the United States, nor distribute after importation, any etiological agent or insect, animal or plant vector of human disease or any exotic living insect, animal or plant capable of being a vector of human disease unless accompanied by a permit issued by the Surgeon General.

(b) An article or thing coming within the provisions of this section shall not be released from Customs' custody prior to the receipt by the Collector of Customs of a permit therefor issued by the Surgeon General.

Department of Agriculture

Sec. 122.1 No organisms or vectors shall be imported into the United States or transported from one State or Territory or the District of Columbia to another State or Territory or the District of Columbia without a
permit issued by the Secretary and in compliance with the terms thereof: Provided, That no permit shall be required under this section for importation of organisms for which an import permit has been issued pursuant to Part 102 of this subchapter or for transportation of organisms produced at establishments licensed under Part 102 of this subchapter. As a condition of issuance of permits under this section, the permittee shall agree in writing to observe the safeguards prescribed by the Director for public protection with respect to the particular importation or transportation.

Sec. 122.2 The Secretary may issue, at his discretion, a permit as specified in Sec. 122.1 when proper safeguards are set up as provided in Sec. 122.1 to protect the public. Application for such a permit shall be made in advance of shipment, and each permit shall specify the name and address of the consignee, the true name and character of each of the organisms or vectors involved, and the use to which each will be put.

(Sec. 122.3 sets forth stipulations regarding suspension or revocation of permits.)

BASIS FOR AGENT CLASSIFICATIONS

CLASS 1.
Agents of low potential hazard under ordinary conditions of handling. Unrestricted distribution to any bona fide laboratory, teaching, or research institution.

CLASS 2.
Agents of ordinary potential hazard. Includes agents which may produce human or animal disease of varying degrees of severity from accidental inoculation by injection or other means of cutaneous penetration, but which are contained by ordinary laboratory techniques. Distribution restricted to qualified individuals in universities, research and industrial institutions, diagnostic, and other suitable laboratories. Requests for these agents should be signed by a responsible staff member and be placed on institutional letterhead.

CLASS 3.
Agents of special hazard for which special conditions for containment are required and United States Public Health Service and/or United States Department of Agriculture permits are obligatory. Requests for CLASS 3 cultures and applications for permits to use CLASS 3 agents that continue to be restricted after importation must be signed by the chairman of the department or head of the laboratory or research institute where the work is to be carried out.

CLASS 4.
Agents that require most stringent conditions for their containment because they are extremely hazardous to laboratory personnel and/or may cause serious epidemic and/or epizootic disease. Application to use an agent that continues to be restricted after importation and request must be accompanied by a certification and appropriate documentation that the
institution or laboratory meets the conditions for facilities, equipment, and procedures specified in this guide for this class of agents. Such certification must be signed by the person responsible for the institution or laboratory where the work is to be carried out.

**CLASS 5.**
Agents excluded from the country by law and/or administrative policy or regulations.
Permit required for all international shipments.

**CLASSIFICATION OF BACTERIAL AGENTS**

**CLASS 1.**
All bacterial agents not included in higher classes by “Basis for Agent Classifications”

**CLASS 2.**
Actinobacillus—all species except A. mallei, which belongs to **CLASS 3**
Actinomyces—all species
Aeromonas salmonicida
Arizona arizonae—all serotypes
Bacillus alvei and B. anthracis
Bacterium enterocoliticum
Bordetella—all species
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diptheriae, C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyrogenes, C. renale
Diplococcus pneumoniae
Erysipelothrix insidiosa
Haemophilus ducreyi, H. gallinarum, H. influenzae
Herellea vaginicola
Klebsiella—all species
Leptospira—all species
Listeria—all species
Mina polymorpha
Moraxella—all species
Mycobacteria—all species except those listed in **CLASS 3**
Mycoplasma—all species except those listed in **CLASS 4** and **5**
Neisseria gonorrhoeae, N. meningitidis
Pasteurella—all species except those listed in **CLASS 3**
Salmonella—all species
Shigella—all species
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus agalactiae, Str. equi, Str. equisimilis, Str. genitalium, and Str. pyogenes of Lancefield's Groups A, B, C, and G
Vibrio fetus

CLASS 3.
Bartonella—all species
Brucella—all species
Mycobacterium avium, M. bovis, M. johnei, M. tuberculosis
Pasteurella multocida type B ("Buffalo")
Pseudomonas pseudomallei
Vibrio comma—all types, including El Tor

CLASS 4.
Actinobacillus mallei
Mycoplasma agalactae
Mycoplasma mycoides, Var. capri
Pasteurella pestis, P. tularensis

CLASS 5.
Mycoplasma mycoides
Pasteurella multocida type 1 (Roberts classification)

CLASSIFICATION OF FUNGAL AGENTS

CLASS 1.
All fungal agents not included in higher classes by "Basis for Agent Classifications"

CLASS 2.
Dermatophilus congolensis
Paracoccidioides brasiliensis

CLASS 3.
Blastomyces dermatitidis
Cryptococcus neoformans
Histoplasma capsulatum

CLASS 4.
Coccidioides immitis

CLASSIFICATION OF VIRAL AND RICKETTSIAL AGENTS

CLASS 1.
Influenza virus A/PR8/34
Newcastle virus, vaccine strain
Parainfluenza virus 3, SF4 Strain
Rus sarcoma virus, Bryan Standard Strain

CLASS 2.
Adenoviruses—human and animal—all types
Alcutian Disease virus
Avian erythroblastosis virus
Avian leucosis virus
Avian lymphomatosis virus
Avian myeloblastosis virus
Bovine encephalomyelitis virus
Bovine rhinotracheitis virus
Bovine virus diarrhea
Canine distemper virus
Canine hepatitis virus
Contagious ecthyma
Coxsackie A and B viruses—all types
Echoviruses—all types
Ecpoviruses—all types
Encephalomyocarditis virus (EMC)
Epidemic Diarrhea of Infant Mice virus
Enzootic bovine abortion virus
Feline pneumonitis virus
Herpes viruses—except Monkey B virus, which belongs to CLASS 4 and Allerton strain of Lumpy skin and bovine mammilitis which belong in CLASS 5
Infectious bronchitis virus
Influenza viruses—all types except A/PR8/34 which belongs to CLASS 1
K or Rat virus
Lactic dehydrogenase elevating virus
Laryngotracheitis virus
Lymphogranuloma venereum virus
Malignant catarrhal fever
Measles virus
Meningopneumonitis virus
Mouse encephalomyelitis virus
Mouse hepatitis viruses
Mouse leukemia viruses
Mouse pneumonitis virus
Mouse salivary gland virus
Mumps virus
Newcastle disease virus—except vaccine strain which belongs to CLASS 1

Papilloma viruses—all types
Parainfluenza viruses—all types, except Parainfluenza virus 3, SF 4 Strain which belongs to CLASS 1
Polioviruses—all types, wild and attenuated
Polyoma virus
Poxviruses—all types, except Alastrim, Smallpox, and Myxomatosis of Rabbits and exotic pox viruses of domestic animals which belong to CLASS 4
Rabies virus—fixed and attenuated
Reoviruses—all types
Respiratory syncytial virus
Rhinoviruses—all types
Rous sarcoma virus—except Bryan Standard Strain, which belongs to CLASS 1
Simian viruses—all types, except Monkey B virus, which belongs to CLASS 4
Varicella virus
Vole Rickettsia
Yellow Fever virus, 17D vaccine strain

**CLASS 3.**

Arboviruses—all strains except those which belong to CLASS 4 and 5, and 17D, which belongs to CLASS 2. (Arboviruses indigenous to the United States belong to CLASS 3, except VEE, which belongs to CLASS 5. West Nile virus may be classified up or down, depending on the conditions of use and differing geographical locations.)

Cytomegalovirus, human
Lymphocytic choriomeningitis virus (LCM)
Psittacosis-Ornithosis group of viruses
Rabies street virus
Rickettsia—all species, except Q-Fever, Typhus and Heartwater which belong to CLASS 4, and Vole Rickettsia, which belongs to CLASS 2 and Bovine infectious petechial fever which belongs to CLASS 5.
Rubella virus
Trachoma virus
Vesicular stomatitis virus

**CLASS 4.**

Alerstrim virus (*)
Dengue, hemorrhagic types (*)
Hemorrhagic fever agents, undefined
Hog cholera virus
Junin virus
Machupo virus
Mocambo virus
Monkey B virus
Myxomatosis virus of rabbits
Newcastle disease (exotic strains)
Omsk hemorrhagic fever virus
Q-Fever rickettsia (*)
Russian Spring Summer Fever virus and related tickborne encephalitis viruses, including Kyasanur Forest viruses
Smallpox virus (6)
Wesselsbron virus
Yellow Fever virus—wild (*)

(*) These agents may be classified in CLASS 3 for studies that involve no transmission or animal inoculation experiments.

CLASS 5.
African Horsesickness virus
African Swine fever virus
Blue tongue virus (exotic strain)
Borna disease virus
Bovine Infectious Pecchial fever virus
Bovine mammilitis virus
Foot and Mouth disease virus
Fowl plague virus
Louping Ill virus
Lumpy skin virus (neethling strain)
Lumpy skin disease virus (Allerton strain)
Nairobi sheep disease virus
Rift Valley fever virus
Rinderpest virus
VEE virus

MINIMAL CONDITIONS REQUIRED FOR HANDLING CLASS 3 AND CLASS 4 AGENTS

CLASS 3.
(1) Controlled access facility, suits, or room separate from the activities of individuals not engaged in handling CLASS 3 agents as well as from the general traffic pattern of the rest of the building or laboratory.
(2) Maintenance of negative air pressure at the site of work in a preparation cubicle or under hood. Recirculation of air is permissible only after adequate decontamination high efficiency.

(3) Recognized capability and direct supervision of specially trained and qualified technical personnel by a professional who is considered competent to handle CLASS 3 agents.

(4) Animal experiments, including cage sterilization, refuse handling, disposal of animals, etc., are conducted employing a level of precaution equivalent to conditions required for laboratory experiments.

(5) Vaccination of personnel—actually or potentially exposed—for agents having immune prophylaxis available.

CLASS 4.

(1) In working with CLASS 4 agents, all conditions for CLASS 3 agents must be met.

(2) Work areas for CLASS 4 agents must be in a facility which is in effect a separate building or be separated physically from other work areas by effective airlocks.

(3) If the work area is not in a separate building the entire area used for CLASS 4 agents must have separate air exhaust and have negative pressure with respect to other areas of the building. Exhaust air must be decontaminated by filtration high efficiency or other suitable process. CLASS 4 agents in this area should be manipulated only in safety cabinets equipped with absolute filters.

(4) Access to the work area is restricted to individuals immunized or otherwise under specific control.

(5) Protective clothing must be worn and be decontaminated before removal from the laboratory area.

(6) A CLASS 3 agent will become a CLASS 4 agent when it is exotic and is employed in entomological experiments and/or when other entomological experiments are conducted in the same laboratory area.

(7) When an agent is used in entomological experiments, the windows, walls, floor, ceiling, and airlock of the work area must be insect-proof and pure pyrethrum insecticide or suitable insect killing device must be available in the airlock.

(8) Consideration should be given to geographical location which minimizes chance of accidental establishment of an exotic agent in a susceptible ecological focus.
RABIES VACCINES MARKETED FOR USE IN ANIMALS

J. A. GOURLAY, DVM*

Ames, Iowa

When working to improve vaccines, there are two main objectives: 1) to produce greater protection levels in the vaccinates; and 2) to reduce or eliminate undesirable side effects. Work with rabies vaccine for animal use has been no exception. Over the years there has been a gradual evolving of newer and better rabies vaccines.

The veterinary rabies vaccines now available can be grouped into three categories: 1) phenolized nerve tissue vaccines, the first products available commercially. About 22% of the rabies vaccine doses produced in the United States in 1966 were in this group.5 2) Flury strain vaccines grown in chicken embryos—about 64% of the doses made in the United States in 1966 were in this category.6 3) The group of products grown in cell cultures. This group includes both live virus and inactivated virus products. This category made up about 14% of the vaccine doses produced in 1966.5 Cell culture rabies vaccines can be considered comparative newcomers.

PHENOLIZED NERVE TISSUE VACCINES

The products now being made in the United States consist of a heat and phenol inactivated 20% suspension of brain and spinal cord from sheep or goats artificially infected with the CVS strain of fixed rabies virus... the so-called Semple type vaccine.14 To demonstrate that the virus has been inactivated, rabbits and mice are inoculated intracerebrally with the vaccine. These animals must remain well for at least 14 days.17

The Habel test is used to assay potency.14 This is a constant level vaccination-increasing challenge method. To pass, a serial must protect against a challenge of at least 1000 mouse LD50's.17 Fluid thioglycollate medium is inoculated with the product to detect possible microbial contamination. To pass, there must be no growth in the medium.16

Recently, work on new products of this type has been done to improve purity, safety and efficacy. However, this work has been done on products intended primarily for human use. One innovation is the use of suckling mammalian brain material before extensive myelinization has occurred in the central nervous system of the new-born animal.7,8,9 High speed centrifugation and chromatographic fractionation to purify viral components has been used.16 Lyophilization of the vaccine is another modification that has been used with the Semple type vaccine.9 These modifications have not become available in commercial vaccines for routine veterinary use at this time, however.

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Annual revaccination is recommended when using the phenolized nerve tissue vaccines.

**CHICKEN EMBRYO PROPAGATED FLURY STRAIN VACCINE**

Statements on the efficacy of this type product in protecting dogs have almost become truisms. Licensed producers are currently marketing products that consistently exceed minimum USDA requirements for virus content. The minimum requirement for virus content is at least 2000 mouse LD<sub>50</sub>'s per 0.03 milliliter of vaccine.\textsuperscript{18} Titers exceeding 10,000 LD<sub>50</sub>'s are commonplace. All major producers have submitted at least 40 consecutive serials which meet virus content requirements when check-tested at the Veterinary Biologics Division Laboratories in Ames, Iowa. The percentage of serials unsatisfactory for potency on VBD check-tests in 1966 did not exceed 1%.

In 1966, Chicken Embryo Origin (CEO) rabies vaccine was included among the products which were required to meet minimum standards for levels of non-pathogenic organisms.\textsuperscript{18} In brain-heart infusion agar pour plates, numbers of bacterial colonies may not exceed 100 per dog dose. No tolerance for pathogens has been permitted at any time.

Flury Low Egg Passage virus vaccine, CEO is recommended for use only in dogs, preferably five months of age or older.

High Egg Passage Flury vaccine, CEO is currently marketed in the United States for use in two species...in cattle and in cats.\textsuperscript{19} The vaccine for cats contains aluminum hydroxide as an adjuvant in the water diluent.

**CELL CULTURE PROPAGATED RABIES VACCINES**

In the last decade, increased attention has been paid to cell-culture propagated virus vaccines. Kissling reported success in growing rabies virus in a hamster kidney cell system in 1958.\textsuperscript{10} The reduction in non-viral protein using this method of preparation is apparent. Kissling and Reese\textsuperscript{11} produced an inactivated rabies vaccine which contained approximately 1/200th the amount of protein found in a representative chicken embryo vaccine. Another more concentrated vaccine containing approximately 1/25th the amount of protein gave better levels of protection.

Commercial veterinary vaccine producers have also reported on the production and testing of various experimental cell-propagated rabies vaccines.

**INACTIVATED CELL-CULTURE VACCINES**

Ott and Heyke\textsuperscript{18} immunized dogs with a phenolized vaccine produced by growing the CVS strain of fixed rabies virus on hamster kidney cells. Kucera, et al. utilized an oil-in-water adjuvant in a formalin inactivated CVS virus-hamster kidney system to protect dogs. He found that at 24½ months post-vaccination, six vaccinates tested withstood a challenge which killed all eleven control dogs. Specificity of deaths from rabies was confirmed in this study using fluorescent antibody techniques.\textsuperscript{12}
ATTENUATED LIVE VIRUS VACCINES

Cabasso, et al. and Dean, et al. have reported on the effectiveness of Flury LEP virus grown in chick fibroblasts in protecting dogs. Dean compared the following: (1) resistance of dogs to challenge with street virus after being given varying dilutions of CEO and TCO vaccines; (2) dog protection results with mouse and guinea pig protection results; (3) mouse, guinea pig, and hamster infectivity with both the CEO and TCO strains; and (4) detectable antibody response in dogs at varying vaccine dilutions compared with resistance to street virus challenge. Both studies showed that while present federal guinea pig protection and mouse titer requirements provide adequate correlation with probable performance in dogs for CEO products, the regulations tended to exclude chick fibroblast propagated products which protected dogs adequately. Dean's virus infectivity studies showed that virus modification was the probable explanation rather than low virus content in the chick fibroblast vaccines. This change in virus characteristics also allowed the chick fibroblast propagated virus to be inoculated into guinea pigs in quantities approaching a dog dose without undue mortality due to the vaccine virus itself. This has not been possible with CEO propagated Low Egg Passage virus.

Cabasso discussed the possibilities of a serologic response in canine vaccinates for evaluation of response as an alternate to the guinea pig protection system. Dogs showing seroconversion could be expected to resist challenge with street virus; the converse did not seem equally valid.

Brown, et al. produced a vaccine from Flury High Egg Passage Virus grown on a permanent dog kidney cell line. Abelseth used pig kidney cells to propagate a hamster kidney adapted strain of virus.

In all dog challenge work reported, performance of TCO vaccines has compared favorably with CEO vaccines. Data in which vaccines diluted as much as 1:1000 still protected 50% or more of the vaccinates have been submitted.

Based upon the vaccine dilution street virus challenge studies with supporting data done by the producers for the TCO products, revised minimum requirements for the TCO vaccines have been issued. The minimum requirements for virus content in vaccines has been set at 1000 mouse lethal doses per 0.03 ml throughout dating for chick fibroblast propagated products and at 10,000 suckling mouse LD₅₀'s per 0.02 ml throughout dating for HEP-dog kidney vaccine.

Revisions have also been made in guinea pig protection requirements for the TCO vaccines. Because the avian cell culture adapted strains viruses are less pathogenic for guinea pigs when given at close to full dog field doses, and because vaccines providing reduced guinea pig protection at presently required levels of vaccine dilution still have shown good protection in dog vaccination-challenge trials, guinea pig protection vaccination procedures have been changed to 1/8th of a dog dose for chick fibroblast vaccines rather than the 1/80th of a dog dose required for CEO vaccines. Numbers of guinea pigs used and percentage of protection required have not been changed.
The vaccines that use pig kidney and hamster kidney cell culture systems must have mouse titers of 1000 mLD$_{50}$ per 0.03 ml of vaccine to meet present minimum requirements. No marked change in guinea pig susceptibility has been detected by passage of virus in these cells. The use of 1/80th of a dog dose to vaccinate guinea pigs has been retained for these products.

**DURATION OF IMMUNITY**

Duration of immunity data has been and is being accumulated by the producer in connection with each vaccine. In some cases, this data is, of necessity, preliminary because sufficient time has not as yet elapsed to evaluate the limits. An arbitrary one year revaccination recommendation has been tentatively set. In all likelihood, this will be extended as additional information confirms longer-lasting immunity with these products.

To provide independent information on the duration of immunity of the recently licensed TCO products, the National Communicable Disease Center in Atlanta, in cooperation with the Veterinary Biologics Division of the U. S. Department of Agriculture and the Pan American Health Organization started on May 25, 1967, an extended study using proven susceptible dogs in statistically valid numbers to test duration of immunity in eight types of vaccines. This project is designed to test immunity in test dogs up to three years after vaccination with a field dose of one of the vaccines.

**SUMMARY**

Rabies vaccines currently licensed are in three categories: phenolized nerve-tissue vaccines (22% of doses—1966); chicken embryo propagated Flury strain vaccines (64% of doses—1966); and cell culture propagated vaccines, both modified live virus and inactivated virus types (14% of doses—1966).

Phenolized nerve tissue vaccines are of the Semple type. Annual revaccination is recommended.

Chicken embryo Flury strain vaccines are in wide use. Rejections for inadequate virus content did not exceed 1% in Veterinary Biologics Division check tests in 1966.

Cell culture propagated rabies virus vaccines have reduced amounts of non-viral protein. Both adjuvanted and non-adjuvanted inactive vaccines are licensed. Attenuated virus vaccines produced in chick fibroblast and mammalian cell systems are also licensed. Reduced virulence for laboratory animals for the avian cell propagated viruses has necessitated modifications in the standard guinea pig protection test and lowered requirements in the mouse titrations. Very good dog protection against street virus challenge has still been shown in comparative studies.

Duration of immunity studies by both the producers and a NCDC-VBD-PAHO joint project are underway. Tentative recommendations are for annual revaccination with TCO products. Extension of this limit is likely as more data is accumulated.
ACKNOWLEDGMENTS

The author expresses his appreciation to Dr. Luke R. Sinclair and Dr. Donald L. Croghan for assistance in the preparation of this report.

REPORT OF THE COMMITTEE ON RABIES


This report covers the calendar year of 1966 and I wish to begin by giving credit to the National Communicable Disease Center, Atlanta, Georgia, for the following statistics on the incidence of rabies in the United States for that period.

In 1966, 4,198 laboratory confirmed cases of rabies were reported; this represented a slight increase (2%) over the previous five-year average. Forty-seven states reported animal rabies; only Delaware, Hawaii, and Rhode Island failed to report any cases in 1966. Skunks and foxes accounted for 57% of the animal rabies in the United States during 1966. The 448 rabid animals reported by Texas constituted the largest total for any state. Only 412 rabid dogs were reported in the United States in 1966, the same number reported in 1965. One human rabies death occurred; a 10-year-old boy in South Dakota died 33 days after being bitten by a rabid skunk.

A total of 970,454 individual doses of human antirabies vaccine was distributed by the two manufacturers that produce it. The number of doses of rabies vaccine given humans almost doubled in 1966, largely due to the increased requirements for military personnel.

In 1966, rabies virus was detected in 26 different animal hosts in the United States. Wild species were responsible for 2,946 (70%) of the cases in animals in the United States in 1966, and domestic animals accounted for the remaining 1,251 (30%). Skunks and foxes were again the two most frequently infected species, accounting for 2,386 cases, or 81% of all rabies in wildlife. Cattle were the third most often infected species, dogs were fourth, and bats were fifth in 1966. Prior to 1960, dogs ranked as the species with the highest incidence of rabies.

1. Rabies in Wildlife

In 1966, for the seventh consecutive year, most of the animal rabies in the United States occurred in wild species; 2,946 (70%) of the 4,197 total animal rabies cases. Skunks and foxes were most frequently infected; 2,386 (57%) of the animal rabies cases occurred in these two species in 1966. Bat rabies accounted for 377 (9%) of the total animal rabies cases. Raccoon rabies continued as a geographically restricted rabies problem, with Georgia and Florida reporting 114 of the 133 cases in the country during 1966. The remaining 50 rabies cases in wildlife were spread among 14 other species.

Thirty-four states reported a total of 1,522 cases of skunk rabies in 1966, and for the sixth consecutive year, skunks have been the species most frequently infected with rabies.
Rabies in foxes declined from 1,038 cases in 1965 to 864 in 1966, mainly due to the decrease in Tennessee where only 192 rabid foxes were reported in 1966 as compared with 394 in 1965.

The 377 rabid bats reported in 1966 were 107 fewer than in 1965, but the 1966 total was still the second highest incidence since the first case was diagnosed in a bat in Florida in 1953.

2. Rabies in Domestic Animals

A total of 1,251 cases of rabies was reported in seven species of domestic animals during the year. Cattle were responsible for 519 (41%) of these cases, dogs for 412 (33%), cats for 252 (20%), equines for 43 (4%), sheep and swine for 10 each (less than 2%), and goats for 5 (less than 1%).

TABLE I

INCIDENCE OF RABIES IN THE UNITED STATES BY TYPE OF ANIMAL
1953-1966

<table>
<thead>
<tr>
<th>Year</th>
<th>Dogs</th>
<th>Cats</th>
<th>Animals</th>
<th>Foxes</th>
<th>Skunks</th>
<th>Bats</th>
<th>Animals</th>
<th>Man</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>1953</td>
<td>5,688</td>
<td>538</td>
<td>1,118</td>
<td>1,033</td>
<td>319</td>
<td>8</td>
<td>119</td>
<td>14</td>
<td>8,837</td>
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<tr>
<td>1954</td>
<td>4,083</td>
<td>452</td>
<td>1,032</td>
<td>1,028</td>
<td>547</td>
<td>4</td>
<td>118</td>
<td>8</td>
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<tr>
<td>1955</td>
<td>2,657</td>
<td>343</td>
<td>924</td>
<td>1,223</td>
<td>580</td>
<td>14</td>
<td>98</td>
<td>5</td>
<td>5,844</td>
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<tr>
<td>1956</td>
<td>2,592</td>
<td>371</td>
<td>794</td>
<td>1,281</td>
<td>631</td>
<td>41</td>
<td>126</td>
<td>10</td>
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<td>1,758</td>
<td>382</td>
<td>714</td>
<td>1,021</td>
<td>775</td>
<td>31</td>
<td>115</td>
<td>6</td>
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<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>
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<tr>
<td>1959</td>
<td>1,119</td>
<td>292</td>
<td>751</td>
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<td>789</td>
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<td>697</td>
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<td>725</td>
<td>88</td>
<td>108</td>
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<td>594</td>
<td>217</td>
<td>482</td>
<td>614</td>
<td>1,254</td>
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<td>565</td>
<td>232</td>
<td>614</td>
<td>594</td>
<td>1,449</td>
<td>157</td>
<td>114</td>
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<td>573</td>
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<td>409</td>
<td>220</td>
<td>594</td>
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<td>1,909</td>
<td>352</td>
<td>238</td>
<td>1</td>
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<tr>
<td>1965</td>
<td>412</td>
<td>289</td>
<td>625</td>
<td>1,038</td>
<td>1,582</td>
<td>484</td>
<td>153</td>
<td>1</td>
<td>4,584</td>
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<tr>
<td>1966</td>
<td>412</td>
<td>252</td>
<td>587</td>
<td>864</td>
<td>1,522</td>
<td>377</td>
<td>183</td>
<td>1</td>
<td>4,198</td>
</tr>
</tbody>
</table>

The most significant accomplishment by this Committee in 1967 has been assisting in the organization of the National Rabies Advisory Council. From authority granted him in a resolution at the 70th annual meeting, President Kaley appointed Dr. Alexander Zeissig of Cornell University as temporary chairman to organize this Council.

In the past, rabies programs on local, state, and national levels have had no coordinated organization; a variety of agencies administered these programs. At the 1966 USLSA Rabies Committee Meeting there was a consensus of opinion that a nationally coordinated effort is necessary to achieve additional gains in control of the disease.

The initial meeting of the National Rabies Advisory Council was held
in Washington, D. C., on August 23, 1967, at which time the Departments of Agriculture, Interior, and Health, Education and Welfare of the United States Government as well as the American Medical Association, the American Veterinary Medical Association, the American Public Health Association, and the United States Livestock Sanitary Association were represented. These organizations were represented by:

Alexander Zeissig, D.V.M.
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The Council voted to invite the following to join in the national effort to eradicate rabies from the United States:

1. Farm Organizations
   a. American Farm Bureau
   b. Farmer’s Union
   c. Grange
   d. National Farmer’s Organization
   e. Agricultural Extension Service

2. Canine Organizations
   a. American Kennel Club
   b. American Field Publishing Company

3. Wildlife Organizations
   a. National Wildlife Federation
   b. Wildlife Society

4. The People
   a. Congress of Parents and Teachers Associations
5. The Conference of Public Health Veterinarians

The Council established as guidelines the following statements of fact relative to rabies:

**Rabies in Man**

1. Rabies in man is invariably fatal once symptoms of the disease have developed.
2. Man is always the accidental host; the definitive host being lower species of animals, i.e., dog, fox, skunk, bat.
3. Prevention of rabies in man may be accomplished by:
   a. Prevention of exposure—control of and immunization of domestic animals, including strays and unowned domestic animals.
   b. Control and/or eventual eradication of rabies in wildlife population.
   c. Prophylaxis of the suspected or known exposed human, according to the World Health Organization (WHO) recommendations, by use of hyper-immune antirabies serum or antirabies vaccine, or a combination of these biological agents, in conjunction with local wound treatment.
4. Thousands of persons are treated annually for exposure to rabies, however, actual deaths due to rabies in the U.S.A. are statistically few—variously estimated at from one to twenty annually. It is imperative that persons known or suspected to be exposed to rabies receive prompt medical attention.
5. The rabies problem, per se, is one involving public health, veterinary, and medical responsibilities. Elimination of the disease in its natural habitat—the animal reservoir—will be necessary for eventual control.

**Canine and Feline Rabies**

1. Rabies in dogs has decreased from over 8,000 cases in the U.S. in 1946 to only 412 in 1966. Community efforts consisting mainly of vaccination of all owned dogs and elimination of stray, unowned dogs have been responsible for this dramatic decreased incidence.
2. The incidence of rabies has never been as high in cats as it has in dogs. In 1946, there were 455 cases in cats while in 1966 there were 252 cases.
3. Dogs and cats inflict the majority of bites in people that result in post-exposure antirabies prophylaxis. An estimated 2/3 of the 30,000 human antirabies treatments each year are given following bites by dogs and cats.
4. Although concerted efforts have been successful in controlling rabies in the U.S., very little effort has been made in most communities to immunize cats. Until rabies ordinances can be changed to include
compulsory vaccination of cats, owners of cats should be reminded
that these animals should be vaccinated annually. Simultaneous
control of stray cats, as well as stray dogs, should be implemented.

5. Excellent vaccines that are potent and safe are available for use in
dogs and cats. The low egg passage (LEP) Flury strain of chick
embryo origin (CEO) rabies vaccine has proven effective for be-
stowing the longest immunity in dogs. This vaccine is not recom-
mended for any other species of animal, however, local veteri-
narians are aware of the several types of rabies vaccines available
and recommended for use in all species of animals.

WILDLIFE RABIES

1. Rabies in wildlife constitutes the major problem in the control of
the disease in the United States. Significant outbreaks of rabies in
wildlife species have occurred in almost every state and, over the
past 20 years, several human deaths have occurred as the result of
rabies contracted from the bite of a rabid wild animal. Perhaps
today the major research need in rabies control is the development
of procedures to control rabies among wildlife populations.

2. On a national basis, rabies among wildlife species now accounts for
about 70% of the rabies reported in the United States.

3. Skunks, foxes, and bats have been responsible for 20 human cases
of rabies since 1950.

4. At present, our major research need is the development of methods
to control rabies in wildlife populations.

RABIES IN LARGE DOMESTIC ANIMALS

1. Of all domestic animals, including dogs and cats, rabies now occurs
most frequently in cattle. If complete diagnosis and reporting of
rabies became possible in relation to cattle, horses, sheep, and swine,
outbreaks of rabies in new localities could be more quickly detected
through early diagnosis, and more effectively controlled.

2. Rabies in the large domestic animals is generally a self-limiting
disease. Practically the only risk of spread is through human inter-
ference related to attempts to examine, medicate, or treat the af-
fected animals.

3. Rabies in cattle in Latin America is a serious disease with losses
estimated at one million head per year. These result from attacks
by rabid vampire bats. There is recent evidence that the natural
range of the vampire bat is extending northward, and that it may
eventually invade the southern regions of the United States. If this
were to occur, an entirely new rabies threat to large domestic ani-
mal would prevail.

4. The economic factors involved preclude the universal vaccination of
large domestic animals, except in regions of serious outbreaks. A
system of indemnity payments at two levels, the lower for brains
proved negative and the higher for brains proved positive, would aid materially in rabies surveillance and eradication. Indemnities are now paid on animals dying from rabies in certain states and some other countries (i.e., Canada).

5. Diagnostic laboratory facilities are not available in some areas for examining brains of large domestic animals unless there is a possibility of human exposure. It will be impossible to launch and maintain an effective rabies eradication program until laboratory resources become available to examine all suspected cases, regardless of the species of the animal involved.

Purposes and goals of the Council were outlined as follows:

I Immediate Objectives—prevention of the disease in man and prevention and control of the disease in animals.

A. Prevention of the disease in man:
   1. Development of effective (improved) immunizing agent to prevent rabies in humans.
   2. Prompt medical attention for every individual with possible exposure to rabies.

B. Prevention and control of the disease in animals:
   1. Improve surveillance of the disease in domestic animals.
   2. Provide and maintain vaccination and stray animal control for dogs and cats.
   3. Develop methods of rabies control in wildlife. Define responsibility for carrying out the control programs in wildlife.
   4. Develop recommendations for quarantine and vaccination of exotic pets and wild animals maintained as pets.

II Ultimate Goal—eradicate rabies from the United States.

The Council plans to meet again in early 1968 to study specifically the problem of wildlife rabies.
STATUS OF STATE-FEDERAL HOG CHOLERA ERADICATION PROGRAM

M. J. TILLERY, D.V.M.*

In fiscal year 1967, the campaign to eradicate hog cholera was in its fifth year. With hog cholera incidence having been sharply reduced from that experienced prior to program activity, the major effort in 1967 was to advance from the control phases (Phase I and II) to the eradication phases (Phases III and IV).

Hog cholera remained exotic to large areas of the country. However, overall incidence increased over 1966. Even so, incidence remained well under 15 percent of the number of outbreaks estimated to have occurred in the early 1960's just prior to program initiation.

Cooperative State-Federal

Hog Cholera Eradication Program

July 1, 1967

Figure 1

In view of incidence having decreased sharply in the early years of program activity only to increase somewhat in 1967, a tendency may develop to deviate from the earlier goals for program advancement. Such a tendency must be avoided and the program must be intensified at every opportunity. To do less threatens the gains made over these five years, jeopardizes the industry-established goals for advancement which the cooperators have accepted, and seriously threatens achievement of hog cholera eradication in this country.

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The purpose of this discussion is to review progress through fiscal year 1967 as well as to note problems with which the program must reckon in the near future.

PROGRAM STATUS

On July 1, 1966, all States were engaged in the cooperative program with 29 States and Puerto Rico being in the control phases (Phase I and II) and 21 States being in the eradication phases (Phase III and IV). A year later, July 1, 1967, one State was in Phase I, 21 States and Puerto Rico in Phase II, 15 States in Phase III, and 13 States in Phase IV. Therefore, 22 States remained in the control phases, compared with 29 in 1966, and 28 States had advanced to the eradication phases compared with 21 in 1966. Of those in the eradication phases, seven had achieved hog cholera free status compared with five being hog cholera free a year earlier.

More important than numbers of States in advanced phases is the position those States occupy with each other and with the swine population. For instance, 10 Western States and one North Central State form a large block of States in the latter phases of the program. Four of these States were hog cholera free during 1967. In August 1967 Oregon and Washington became hog cholera free. Therefore, the large Northwestern area has reached the final step in hog cholera eradication. While it is recog-
nized that these States have small swine populations compared with other States, their ability to continue to maintain this large area in the advanced program phases is noteworthy.

On the other hand some States with similar swine populations and similar hog cholera incidence have remained in Phase II for two years or more. In at least one of these States a hog cholera outbreak occurred in 1967 and it spread to another State. Although hog cholera outbreaks occurred in three of the Western States in 1967, Arizona, California, and Wyoming, as well as in North Dakota, no spread to other States could be demonstrated. Therefore, it appears that advanced phases carried out by these States can effectively stamp out hog cholera while maintenance of Phase II procedures will not effectively carry out the aims of this program.

Considerable progress was made over the year by States with larger swine populations. Michigan, 15th in swine production, became hog cholera free. Three outbreaks were experienced within the following six weeks. However, no association could be established between the outbreaks; each was stamped out without further spread; exhaustive surveys were conducted to search out suspicious illnesses not reported (none were found); and Michigan retained hog cholera free status.

Illinois and Missouri were in Phase III throughout the year and Kentucky reached Phase III in August 1966. By July 1967, Iowa, Arkansas, and Louisiana had reached Phase III. Therefore eight States in the Central part of the country have reached advanced states. Aside from the geography involved, the importance of this progression lies with the swine trade channels in these States, with the Southern States and Wisconsin furnishing large numbers of feeder pigs to the heavier populated Hog Belt.

Consequently, the Hog Belt feeder may be assured that he is now in the best position ever experienced in this country to import feeder pigs from these States with slight risk to his operation.

Numbers of States in advanced phases which are involved in this swine traffic must be increased. With 62 percent of the country's swine being located in States which have reached the eradication phases, it is imperative to program success that the gains made in these States be protected. A major procedure for safeguarding these gains is program advancement in those States yet in Phase II which ordinarily supply feeder pigs to the States in more advanced phases.

Achieving a certain program level, for instance Phase II or Phase III, requires considerable effort. On reaching that level a tendency may develop to be content to remain there for extended periods of time. This tendency must be overcome, particularly when Phase II has been attained. We must remember that Phase II does not utilize any eradication procedures. Further, Phase II is designed to reduce hog cholera incidence and is not meant to maintain a reduced incidence over prolonged periods of time. There is ample evidence, in the experience of several States that Phase II will sharply reduce hog cholera incidence. There is also a good evidence that Phase II will not continue to suppress hog cholera over long periods of time. Rather,
the trend has been for hog cholera incidence to be sharply reduced in some States after operating in Phase II for several months, only to creep up in the next year or two, if maintenance of Phase II is attempted.

In view of these experiences, Phase II should be abandoned in favor of Phase III as soon as incidence is sufficiently reduced to make this step economically feasible. To remain in Phase II for prolonged periods invites the possibility of losing precious gains, resulting in a more expensive eradication program over the long haul.

A similar consideration should be made in Phase III. Inasmuch as Phase III involves salvage procedures, we must remember that there is risk of perpetuating the virus through garbage. Although the risk is slight, it should not be tolerated any longer than necessary. Therefore, when Phase III has served its purpose, Phase IV should be entered without hesitation. Experience has shown that progressive intensification of this program has led or is leading to eradication of hog cholera, while other experience has shown that some gains have been lost in static programs.

INCIDENCE

Hog cholera incidence increased in fiscal year 1967 compared to 1966. In 1967, 689 of 2230 suspicious outbreaks reported were confirmed. In 1966, 583 of 1427 suspicious outbreaks were confirmed. While there was an increase in 1967 over 1966, incidence continues to be at least 85 percent less than the estimated 5000 to 6000 outbreaks occurring annually from 1960 to 1962.

In reviewing the incidence of earlier years, the 1967 increase should
not be surprising. Hog cholera was sharply reduced from 1963 to 1966. To expect that the sharp decline could be maintained uneventfully would not be realistic. However, we must not take comfort in this thought, but we must examine some of the factors which appear to be involved in the 1967 increase. These include:

MAINTENANCE OF PHASE II OVER PROLONGED PERIODS

Seventeen States had been in Phase II for two or more years at the end of fiscal year 1967. In nine of these hog cholera increased, lending substance to the proposition that Phase II will not suppress hog cholera indefinitely.

Of the remaining eight States, three had a decrease in outbreaks and five reported no change in incidence since 1966. The net increase in Phase II States which had occupied this position for more than two years was 138 outbreaks. The national increase from 1966 was 106 outbreaks.

PROGRAM ADVANCEMENT

Paradoxically, program intensification usually results in an apparent increased incidence which is then overcome. In 1967 eight States advanced to Phase III. In five of these, hog cholera confirmations increased. This

![Diagram of percent of suspicious reports confirmed as hog cholera.](image-url)
increase is likely due to the announcement of program advancement, more intensive information distribution, and renewed attention to the reporting of suspicious outbreaks. In four of these five States, incidence was declining by the end of the year in line with earlier experiences of advancing States.

In the 20 States which had been in Phase III throughout 1967 or longer, eight had increased confirmation, two had decreases, and 10 reported no incidence in 1966 or 1967. The net decrease in these States in 1967 was 28 outbreaks. This decrease coupled with the 138 outbreaks increase in States in Phase II for two years or more largely accounts for the increased incidence in 1967.

CHANGE IN VACCINATION PROCEDURES

Nebraska eliminated usage of modified live virus vaccines in January 1967. In the next six months, hog cholera incidence increased sharply with

66 outbreaks confirmed compared with 19 during these months in 1966. In 35 of the Nebraska outbreaks, hog cholera followed usage of inactivated vaccines. In over one half of these, or 25, the vaccine was that of one producer who later withdrew these serials from the market.

While the Nebraska experience caused widespread concern, the program
appears to have gained valuable information. Those involved with hog cholera eradication are now alert to the possibility of inactivated vaccines causing difficulty, and this was not the case earlier. Therefore, more stringent production standards have been adopted so as to reduce any risk of this nature in the future.

DIAGNOSIS

Reporting of suspicious outbreaks appears to have improved in 1967, with 2230 suspicious reports compared to 1427 in 1966. This represents a 56 percent increase in reporting while confirmations increased 18 percent. Therefore, it appears that reporting systems continue to be improved.

The confirmation rate continued to decline in 1967, reaching 31 percent compared with 41 percent in 1966 or 70 percent in 1965. This decline in confirmation rate must be maintained. The British experience in their recently completed program was similar to ours so far. In the final 18 months of her program, Great Britain's investigators examined more than 250 herds in which suspicious illness was reported to identify four hog cholera outbreaks.

Likely, we must follow a similar course. Therefore, reporting systems must be constantly appraised and methods must be implemented to increase reporting. We must be particularly alert to confirmation rates leveling out or increasing. If this should happen, a valid conclusion would be that reporting systems are failing or have not been fully activated in the areas involved.

Diagnostic procedures have steadily improved since program initiation. Prior to the program, laboratory examination was utilized in about one third of the hog cholera confirmations, increasing to 60 percent in 1964, to 80 percent in 1965, and to 86 percent in 1966. In 1967, laboratory support was utilized in 93 percent of the confirmations. This represents excellent progress. However, a goal of laboratory support in all confirmations is necessary. Further, a goal of laboratory examination for hog cholera of all swine specimens, regardless of the reason for submission appears in order. At least two Phase III States have adopted this policy and both report that hog cholera is sometimes detected which may not have been identified otherwise; or, at best, a delay in diagnosis would have occurred.

Field diagnosticians continue to report more discreet outbreaks of hog cholera. Obscure forms of the disease are reported. Together with pregnant sow transmission, examples of obscure forms include outbreaks in which morbidity is light, others in which the swine recover, and other instances in which the historically reported lesions, such as splenic infarcts and petechiae are absent. Likely, hog cholera will follow the trend experienced in other animal disease eradication programs in that frank outbreaks will decline earliest and even more obscure forms will be seen prior to program completion. Therefore, it appears that laboratory examination for hog cholera of all swine specimens submitted is reasonable.

Although difficult to measure, field diagnosticians are becoming increas-
ingly skillful in detecting hog cholera. This is supported by their identifying pregnant sow transmission in the field, identifying hog cholera carriers, and identifying other obscure forms which were seldom recognized prior to program activity. In view of the skills gained through program experience, it appears reasonable that these highly skilled diagnosticians should investigate all suspicious outbreaks reported. To do otherwise provides opportunity for misdiagnosis and the consequences which may follow.

**EPIDEMIOLOGY**

Virtually all confirmed outbreaks of hog cholera were investigated by cooperating veterinarians in 1967, maintaining the level achieved in 1966. Apparent sources of infection were identified in 81 percent of the confirmed outbreaks compared with 68 percent in 1965 or about 10 percent just prior to the program initiation. That the field epidemiologists have increased this skill eight-fold in five years is excellent progress particularly in view of epidemiology becoming more complex with program progress.

Even so, we must not overlook that source of infection remains unknown in 19 percent of the outbreaks confirmed in 1967. Likely, we will never be able to identify the source of all outbreaks. However, we must continue to improve the ability to locate sources of infection, otherwise, we cannot discount the possibility of unknown sources, or sources now thought to be of little consequence, assuming more importance in the years to come.

Hog cholera outbreaks were confirmed in 373 counties in 34 States and
Puerto Rico in 1967. This was an increase of 30 counties and six States reporting confirmations in 1966. Hog cholera was reported in four Phase IV States and one hog cholera free State in 1967. No Phase IV States or hog cholera free States reported confirmations in 1966.

Outbreaks associated with vaccination increased in 1967, and accounted for 33 percent of the infected herds. This is a significant increase over the 1964-66 average of 13 percent. Numbers attributed to this course increased to 227 outbreaks compared with 114 in 1966 and 89 in 1965. This source is the only one which showed increase in numbers in 1967, and this increase caused considerable concern among those engaged in hog cholera eradication.

Therefore, a recommendation was made to the Secretary of Agriculture’s Hog Cholera Eradication Advisory Committee that a group be chosen to study the state of the knowledge concerning hog cholera biologics and report its recommendations to the Committee. This group reported to not only the Secretary’s Committee but also to the appropriate committees of United States Livestock Sanitary Association and Livestock Conservation Incorporated, at a joint meeting in June. The study group’s recommendations have resulted in these actions:

1. Amendments to Part 76, Title 9, Code of Federal Regulations to provide:
   (a) That serum alone treatment be permitted for swine to be moved into States which provide for this type treatment.
   (b) That only modified live virus vaccines which have been approved by the Director of the Animal Health Division be used to qualify swine for interstate shipment.
   (c) That official vaccination with modified live virus vaccines be recognized for two years.
   (d) That official vaccination with two doses of inactivated vaccines be recognized for one year following the second dose, if the two doses were administered at least two weeks but not more than six months apart.

2. A timetable for discontinuing hog cholera vaccination:
   (a) By January 1968—all vaccination with modified live viruses be official.
   (b) By January 1969—all vaccination with modified live virus cease.
   (c) By January 1971—all vaccination cease.

3. Tissues other than blood have been withdrawn from inactivated vaccine production procedures.

4. Only pigs negative to serum neutralization tests are utilized for vaccine safety testing.

5. Inactivated vaccines must be sterile.

Hog cholera vaccines have been a vital factor in the earlier stages of the eradication program when the major effort was directed toward Phase II, or the reduction of incidence. With a large segment of the swine popu-
lation being resistant, hog cholera control was no doubt achieved much more economically then would have been the case if the vaccines had not been available. However, it has been recognized that certain vaccines no longer provide the margin of safety desired as the program has advanced. Therefore, the restrictive actions regarding these vaccines appear in order. Further program experience is needed to determine whether vaccine safety is now aligned with program advancement.

Little change in sources attributed to movement of swine is noted in 1967 as compared to 1964-66. Source of infection due to area exposure in-

![Sources of Hog Cholera Outbreaks](image)

**Figure 7**

creased slightly; sources due to intrastate swine traffic decreased slightly; and sources due to interstate traffic have never been of consequence in this program.

However, the movement of infected or exposed swine continues to account for 35 percent of the hog cholera outbreaks. The quarantine procedures of Phase II are designed to limit transmission through these means. Therefore, it appears that we cannot expect this proportion of outbreaks to be lowered until such time as administration of these procedures becomes more efficient.

Raw garbage feeding caused little more hog cholera in 1967 than in 1964-66. However, we should not take comfort in this situation. In theory, no hog cholera should be caused by this practice inasmuch as authority exists throughout the country to prohibit such feeding. That raw gar-
bage feeding continues to be a source of hog cholera demonstrates that better application of these authorities is needed. This is not only important to hog cholera eradication but is also vital to the defense of this country from the establishment of diseases now exotic. This surveillance is now under added burden inasmuch as African Swine Fever spread to Italy during 1967.

The remainder of the identified sources are grouped as miscellaneous. As yet, none of these are thought to be of consequence individually.

COMMENTS

In 1967, hog cholera incidence continued to be suppressed to a low level, retaining the bulk of the gains made in the earlier years. With substantial advancement in program status, most areas of the country were involved in consolidating the earlier gains.

This period of transition from hog cholera control to eradication is incomplete. From the experiences of certain States, we should not linger in Phase II as incidence will likely creep up and make inroads on early gains. The most effective programs have been those in States which have completed this transition and brought more pressure to bear on hog cholera in relatively short periods of time. No program for hog cholera eradication should be regarded as complete until such time as it has satisfied all the standards recommended by this Association.

**Hog Cholera Eradication**

**STATE PARTICIPATION-GOALS**

**JULY 1, 1967**

![Diagram showing progress towards hog cholera eradication from 1964 to 1972.](image)
These standards have been demonstrated to be effective and practical. When carried out without delay, each phase of the program has accomplished the intended purpose. As the program advances nationally, we must be alert to propositions to deviate from these standards because of the inconvenience of their application at the moment. Deviations must be carefully considered, and, if the proposals will not satisfy the principals of hog cholera eradication in our judgment as veterinarians, they must not be utilized. To do otherwise unnecessarily adds risk to the hard earned gains we have made and adds burdens which are not warranted.

The swine industry established a series of goals for hog cholera eradication in February 1964. This has been one of the stronger indications of confidence in this program to date and the goals have been accepted by this Association as realistic and attainable. Those remaining and the degree of accomplishment is as follows:

1967—All States at least to Phase III. By July 1967, the goal was 56 percent achieved. By October 1967, 31 States (62 percent) had achieved Phase III.

1969—Practical eradication of hog cholera (All States to Phase IV). By July, the goal was 26 percent achieved. By October 1967, 16 States (32 percent) had achieved Phase IV.

1972—Official declaration that the United States is hog cholera free. Nine States (18 percent) have achieved hog cholera free status.

The immediate need is attainment of the 1967 goal. With almost four years in which to carry out control measures after the goal was established hog cholera incidence is no longer an obstacle to achievement of Phase III. Therefore, the disease, hog cholera, cannot be looked upon as providing interference in this respect. Further, this being the first eradication step, it should be reached as soon as possible in order to protect gains already made.

The time allotted to us by the industry for attainment of the goal is but two months away. Success or failure in attaining Phase III by December 1967 will demonstrate our ability to respond to swine industry's faith in this cooperative eradication program. Further, attainment or failure will largely govern the time necessary to attain the remaining goals. The choice is ours; the time is upon us to make the decision. In view of the gains made to date in hog cholera eradication we must accept responsibility for the outcome, whatever our decision may be.
DETECTION OF HOG CHOLERA VIRUS IN NORMAL PIGS SALVAGED FROM INFECTED HERDS AND HYPERIMMUNE PIGS FROM BIOLOGICAL COMPANIES

E. A. CarbreY, V.M.D., M.S.; W. C. Stewart, D.V.M.; and J. I. Kresse, B.S.

During 1966 some of the major swine-producing states entered Phase III of the hog cholera (HC) eradication program. Although this phase required total depopulation of all hogs on a HC infected premise, the regulations had been revised to permit the salvage of any pigs not having signs of disease. These exposed pigs were carefully inspected for clinical evidence of HC by a regulatory veterinarian and promptly shipped to the abattoir for slaughter under supervision. All carcasses from pigs having lesions of HC were condemned. However, carcasses from pigs passing inspection brought close to normal market prices and were released for distribution as fresh meat. It was estimated that overall indemnity costs had been reduced as much as a third by this procedure.¹

In spite of the economic advantages of this policy, there was some cause for concern. Although clinical signs and lesions are coincident with the multiplication of virus and ensuing viremia, some of the carcasses from these salvaged pigs were certain to contain HC virus. Through normal channels of trade some of this meat might eventually reach susceptible pigs as raw pork scraps in garbage. For this reason, it was decided to determine what proportion of these salvaged carcasses were potential spreaders of HC virus.

A survey was designed to investigate this problem and the results are reported here.

Another source of concern to the veterinarians responsible for the hog cholera eradication program was the operation of biological companies engaged in the production of anti-HC, hyperimmune serum. In this procedure pigs are inoculated intravenously with 5.0 ml. of virulent HC virus blood per pound body weight and bled out for serum 15 to 30 days later. The fresh carcasses of these hyperimmunized swine are salvaged for meat at a nearby slaughterhouse. The question was raised as to whether pigs receiving such massive inoculations of HC virus and coming directly from a virus contaminated premise were carrying virus in their tissues at the time of slaughter. Spleen specimens were obtained from a representative number of serum plants and examined for the presence of HC virus.

Materials and Methods

The diagnosis of HC infection was made by the field veterinary diagnostician on the basis of clinical signs, necropsy lesions, total white blood cell counts, and laboratory findings. However, the only infected herds selected for this survey were those from which HC virus had been isolated by the fluorescent antibody, tissue culture technique (FATCT).³
Spleens were collected from 25% of the pigs salvaged from the herd except when the number was 10 or less in which case spleens were collected from all of the pigs. No spleens were taken from pigs condemned for HC on ante-mortem or post-mortem inspection. The spleens were shipped packed in dry ice and stored in the frozen state at the laboratory.

Two procedures were employed to detect HC virus in the spleens.

Survey I. In the first procedure a 33 1/3%, clarified suspension of each spleen was inoculated into PK-15 cell cultures and examined by the FATCT. The concentration of HC virus in the suspension was determined as previously described and expressed as virus plaques per ml. The coverslip cell cultures were fixed, stained, and examined at 48 hours after inoculation.

Spleen suspensions found to contain HC virus were stored frozen and confirmed by pig inoculation. First or second generation specific-pathogen-free HC susceptible pigs weighing approximately 40 pounds (18 kg.) were inoculated intramuscularly and maintained in isolation until death occurred. If the pig survived, it was given 1.0 ml. of virulent HC virus suspension intramuscularly containing at least 10,000 plaques/ml by the FATCT.

The HC virus strains were roughly classified as follows on the basis of the inoculation of one pig: high virulence, sickness and death with recovery of HC virus; low virulence, chronic sickness with protracted course; and avirulent or immunizing, little or no reaction with the development of immunity.

Survey II. The second procedure was developed to permit screening of a larger number of spleens for HC virus by pooling the tissues and inoculating the suspensions directly into pigs. The entire collection of spleens from an infected herd was thawed out and divided into lots of 20. Two grams of each of the 20 spleens in a lot was placed in a Waring blender and triturated with 120 ml. of Eagle's medium plus 0.5% lactalbumin hydrolysate and antibiotics. The pooled suspensions were clarified by centrifugation at 1860 g in a refrigerated centrifuge. Each pig was given 40 ml. of suspension intramuscularly and subcutaneously in different locations to avoid tissue necrosis. All of the pigs inoculated with suspensions from one herd were kept in the same animal room. Observations and temperatures were recorded in an effort to differentiate the pigs which were initially infected by inoculation from those infected later by contact. Tissues from pigs which died were cultured for HC virus by the FATCT. If all of the pigs in the room remained healthy throughout 21 days post-inoculation (DPI), their immunity to HC was challenged by the inoculation of virulent virus as described above.

The number of spleens pooled for pig inoculation was different in two cases. Herd RR accession consisted of 87 spleens which were all pooled together and inoculated into 4 pigs. Another accession with 35 spleens was pooled in smaller lots.

Survey III. The purpose of this survey was to attempt to detect virus in the spleens of hyperimmunized hogs. Arrangements were made to
DETECTION OF HOG CHOLERA VIRUS

obtain at least 100 spleens in lots of 10-20 each from different serum producers and culture each spleen for HC virus on PK-15 cell cultures employing the FATCT. To further check for HC virus some of the negative spleen suspensions were inoculated into susceptible pigs. These spleen suspensions were not pooled, but each inoculated into one pig.

Results

In Survey I spleens were cultured for HC virus by the FATCT from 34 lots of pigs sent to slaughter for salvage. Virus isolations were made from 8 of the 34 herds sampled. A total of 982 spleens were examined, and HC virus was isolated from 13 (Table 1). The virus plaque counts per ml. of spleen suspension ranged from 2 to 2200. The percentage of spleens containing HC virus was 1.3%. On the basis of a sample size of almost 1000, it was determined from a table of 99% confidence intervals by interpolation that the true percentage of infected spleens was between 0.3 and 2.3%. In other words, there was only one chance in a hundred that the percentage of pig carcasses carrying HC virus exceeded 2.3% as determined by this survey.

The virulence of the HC virus strains isolated from each herd and determined by the inoculation of one susceptible pig is recorded in column 5 of Table I.

The positive tissue culture isolation from herd RVM was not confirmed by pig inoculation; however, there was some evidence that an error had occurred in labeling the individual spleen suspensions. Tissue suspensions from herds BEC and DRW were not inoculated into pigs. Of the remaining 5 positive cases inoculated into pigs, 2 virus strains were of high virulence, 1 of low virulence, and 2 avirulent or immunizing strains.

With the assistance of the veterinary diagnosticians in the field, it was possible to obtain information as to the immune status of the pigs under risk of exposure in the salvage groups. These data are presented in column 6 of Table I and will be reviewed in detail with the same information on the herds from Survey II.

The results of Survey II in which suspensions of the spleens from the salvage pigs were pooled and inoculated directly into susceptible swine are presented in Table II.

A total of 8 accessions from infected herds was processed, and HC virus was detected in the spleens from 5 herds. Although it was planned to inoculate a pool of 20 spleens into one pig, some variations were introduced. The 87 spleens from herd RR were made into one composite pool and inoculated into 4 pigs. It was estimated that each pig received approximately 0.5 ml. of suspension from each of the 87 spleens. However, all 4 pigs became sick simultaneously on 7 DPI and died of hog cholera a week later. The 35 spleens from herd MJL were divided into pools of 7 spleens each and inoculated into 5 pigs.

Information as to individual pig infection was lost by this pooling procedure. However, from the information obtained by observation of the pigs and compiled in column 4, “Pigs Infected by Inoculation,” it was concluded that 418 of the 589 spleens examined by this procedure did not
contain HC virus. There was a strong element of judgment in separating the pigs infected by inoculation from those infected by contact.

**TABLE I**

**HOG CHOLERA VIRUS ISOLATIONS BY TISSUE CULTURE OF INDIVIDUAL SPLEENS, SURVEY I**

<table>
<thead>
<tr>
<th>Herd</th>
<th>Spleens Cultured</th>
<th>Positive HC Virus (FATCT) Plaques/ml</th>
<th>Pig Inoculation</th>
<th>Status of Herd of Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVM...</td>
<td>35</td>
<td>2</td>
<td>7, 68</td>
<td>Negative</td>
</tr>
<tr>
<td>RW....</td>
<td>54</td>
<td>0</td>
<td></td>
<td>Immune</td>
</tr>
<tr>
<td>BEC....</td>
<td>33</td>
<td>2</td>
<td>110, 400</td>
<td>Immune</td>
</tr>
<tr>
<td>OS....</td>
<td>30</td>
<td>0</td>
<td></td>
<td>Immune</td>
</tr>
<tr>
<td>JH....</td>
<td>11</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>DRW....</td>
<td>112</td>
<td>2</td>
<td>5, 2200</td>
<td>68% Susceptible</td>
</tr>
<tr>
<td>HEM....</td>
<td>66</td>
<td>0</td>
<td></td>
<td>Immune</td>
</tr>
<tr>
<td>W &amp; RG.</td>
<td>59</td>
<td>0</td>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>KA....</td>
<td>16</td>
<td>2</td>
<td>2, 3</td>
<td>Immunizing</td>
</tr>
<tr>
<td>JMF....</td>
<td>63</td>
<td>0</td>
<td></td>
<td>Immune</td>
</tr>
<tr>
<td>VP....</td>
<td>22</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>W V....</td>
<td>16</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>CVS....</td>
<td>13</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>JB....</td>
<td>53</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>GW....</td>
<td>11</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>CJB....</td>
<td>21</td>
<td>1</td>
<td>2</td>
<td>Immunizing</td>
</tr>
<tr>
<td>BLR....</td>
<td>8</td>
<td>0</td>
<td></td>
<td>Immune</td>
</tr>
<tr>
<td>EA....</td>
<td>31</td>
<td>0</td>
<td></td>
<td>Immune</td>
</tr>
<tr>
<td>JCB....</td>
<td>50</td>
<td>0</td>
<td></td>
<td>Immune</td>
</tr>
<tr>
<td>WPT....</td>
<td>7</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>QP....</td>
<td>10</td>
<td>0</td>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>HC....</td>
<td>13</td>
<td>0</td>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>RH....</td>
<td>13</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>GG....</td>
<td>12</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>JSZ....</td>
<td>10</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>PH....</td>
<td>21</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>JJH....</td>
<td>13</td>
<td>1</td>
<td>900</td>
<td>High Virulence</td>
</tr>
<tr>
<td>WH....</td>
<td>8</td>
<td>0</td>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>W &amp; RK.</td>
<td>33</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>HCM....</td>
<td>52</td>
<td>0</td>
<td></td>
<td>Immune</td>
</tr>
<tr>
<td>LLB....</td>
<td>11</td>
<td>1</td>
<td>820</td>
<td>Low Virulence</td>
</tr>
<tr>
<td>LL....</td>
<td>66</td>
<td>2</td>
<td>8, 1200</td>
<td>High Virulence</td>
</tr>
<tr>
<td>LRK....</td>
<td>4</td>
<td>0</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>JWS....</td>
<td>5</td>
<td>0</td>
<td></td>
<td>Unknown</td>
</tr>
</tbody>
</table>

All of the virus strains isolated in Survey II were found to be of high virulence.

The status of the herd of origin was tabulated in the last columns.
DETECTION OF HOG CHOLERA VIRUS

Of Table I and Table II. Of the 42 herds represented in the two surveys, the presence or absence of immunity in the salvaged pigs could not be determined for 6 of them. Salvage lots containing less than 20% immune pigs were considered susceptible. A group of pigs was considered to be immune if the field diagnostician reported a history of vaccination with attenuated live or killed virus vaccine. For the purposes of tabulation, herd DRW was considered susceptible although 32% of the pigs salvaged had been vaccinated with attenuated live virus vaccine.

The 36 herds with a known status of immunity are tabulated in Table III as positive or negative according to whether HC virus was isolated from the salvaged pigs.

The finding that 4 of the 13 herds from which virus was isolated were reported to be of immune status was noteworthy.

**TABLE II**

**HOG CHOLERA VIRUS ISOLATIONS BY PIG INOCULATION OF POOLED SPLEEN SUSPENSIONS (APPROXIMATELY 20 PER PIG), SURVEY II**

<table>
<thead>
<tr>
<th>Herd</th>
<th>Number of Spleens</th>
<th>Pigs Inoculated</th>
<th>Pigs Infected by Inoculation</th>
<th>Virulence of Positive Accessions</th>
<th>Status of Herd of Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>JS</td>
<td>22</td>
<td>3</td>
<td>0</td>
<td>....</td>
<td>Susceptible</td>
</tr>
<tr>
<td>MB</td>
<td>60</td>
<td>3</td>
<td>0</td>
<td>....</td>
<td>Immune</td>
</tr>
<tr>
<td>HH</td>
<td>64</td>
<td>3</td>
<td>1</td>
<td>High</td>
<td>Susceptible</td>
</tr>
<tr>
<td>MJL</td>
<td>35</td>
<td>5</td>
<td>1</td>
<td>High</td>
<td>Susceptible</td>
</tr>
<tr>
<td>DN</td>
<td>117</td>
<td>6</td>
<td>1</td>
<td>High</td>
<td>Immune</td>
</tr>
<tr>
<td>VES</td>
<td>170</td>
<td>8</td>
<td>0</td>
<td>....</td>
<td>Immune</td>
</tr>
<tr>
<td>EJT</td>
<td>34</td>
<td>2</td>
<td>2</td>
<td>High</td>
<td>Susceptible</td>
</tr>
<tr>
<td>RR</td>
<td>87</td>
<td>4</td>
<td>4*</td>
<td>High</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

*The 87 spleens from this herd were not divided into lots of 20 but were inoculated into the 4 pigs as a composite pool.

**TABLE III**

**COMPARISON OF HOG CHOLERA VIRUS ISOLATIONS WITH IMMUNE STATUS OF THE HERDS OF ORIGIN**

<table>
<thead>
<tr>
<th>Status of Herd of Origin</th>
<th>Hog Cholera Virus Isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Susceptible</td>
<td>9</td>
</tr>
<tr>
<td>Immune</td>
<td>4</td>
</tr>
</tbody>
</table>
The results of Survey III were quite uniform in that no HC virus was detected in the 93 spleens of hyperimmunized hogs collected at the biological plants. Suspensions of 22 spleens were each inoculated into susceptible pigs. All 22 pigs remained healthy throughout 21 DPI and were found susceptible to HC following inoculation with virulent virus. The 8 lots of spleens were obtained from three companies at different times and ranged in size from 10 to 19 in a lot.

Discussion

The low percentage, 1.3%, of HC virus isolations from the spleens of healthy, exposed swine salvaged from infected herds gave a clear indication of the low risk involved in this procedure. Careful clinical examination of the pigs selected for salvage by the veterinarians was certainly related to this finding.

The method of direct pig inoculation of pooled spleen suspensions followed in Survey II gave some confirmation of the findings of Survey I in that 418 of 589 spleens inoculated did not contain virus. If it is assumed that each of the spleen pools which infected a pig contained 2 infected spleens, then 12 of the 589 spleens would have contained HC virus. The percentage of positive spleens by this estimate would have been roughly 2%.

The isolation of HC virus from pigs reported to be immune by vaccination was quite unexpected. Some thought had been given during the planning stage of the survey to exclude herds of immune status since the possibility of HC virus isolations would be remote. However, 4 of the 13 salvage lots from which HC virus was isolated contained vaccinated hogs. Herd DN was “long-term vaccinated” by a veterinarian and herd JJH was vaccinated by the owner. Part of herd BEC was vaccinated with killed virus vaccine and the remainder with attenuated live virus vaccine.

There seems to be little justification for assuming that the immunity of a group of slaughter pigs will guarantee the absence of HC virus in their tissues. Of course, the most probable explanation is the difficulty of obtaining 100% vaccination under average farm conditions. Occasionally there would be a few susceptible pigs in each herd that were either too young or too close to slaughter weight for vaccination. However, HC virus was detected in the tissues from only 4 out of 15 immune herds, as against 9 of 21 susceptible herds. A degree of immunity in the salvage swine reduced the virus isolation per herd by about one half.

The negative results of Survey III on the spleens of hyperimmunized hogs were not unexpected. Since there was a considerable residue of antibody in the tissues of the pigs, it was considered of value to inoculate individual spleen suspensions into pigs from a significant number, 22, of these spleens. No HC virus was recovered from these tissues.

Summary

Spleens were collected from 1,571 healthy, exposed pigs sent to slaughter for salvage as fresh carcasses from hog cholera (HC) infected
DETECTION OF HOG CHOLERA VIRUS

herds undergoing depopulation. Culture for HC virus on PK-15 (pig kidney) cell cultures by the fluorescent antibody, tissue culture technique was performed on 982 spleens. Virus was isolated from 13 spleens, or 1.3%. The remaining 589 spleens were pooled in lots of 20 and inoculated directly into pigs. Six of the pools contained HC virus, but 25 pools representing 418 spleens were negative.

Spleens from 93 hyperimmunized pigs used for the production of HC antiserum were examined for virus with negative results.

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The authors acknowledge the assistance of the Swine Diseases Staff and field veterinarians of the Animal Health Division, ARS, USDA, and members of the Veterinary Biologics Division, ARS, USDA, in the completion of this project.

REFERENCES


1967 REPORT OF THE COMMITTEE ON THE NATIONWIDE ERADICATION OF HOG CHOLERA

The year 1967 has seen increased outbreaks of hog cholera apparently attributable to improper vaccine usage. Additionally, evidence points to certain modified live virus and inactivated vaccines as being the source of numerous hog cholera outbreaks. These cases have resulted in action on the part of the Veterinary Biologics Division of the U.S. Department of Agriculture having withdrawn its approval from certain of these products, an action which is highly commendable. Albeit much of this unapproved vaccine still remains in the field and continues to be the source of hog cholera problems. Immediate action should be taken by the states to remove such products from circulation or field use so as to eliminate this most imminent threat.

The Committee has reviewed the recommendations of the Agricultural Research Service Hog Cholera Biologics Study Group, and in the main endorses them. In light of the cases of hog cholera which continue to occur throughout the nation, however, the Committee feels that there is justification in correlating phase out goals with the status of program progress. To unequivocally require that January 1, 1969, should be the date of discontinuance of manufacture or use of modified live virus vaccines and that January 1, 1971, be recognized as the shut-off date for inactivated vaccines could bring about a situation disastrous to the swine interests of the country. Recent limited studies have indicated immunization deficiencies, as well as actual hazards, as heretofore stated, with both types of products, and many states are at this time giving serious consideration to all product phase out far in advance of the recommended time table. Such action should of course be in accord with disease incidence in these states and the states from which they secure their swine replacements.

Because of the inherent danger of all modified vaccines and the limited immunizing properties of single doses of inactivated products, the Committee feels that product usage will be self limiting. In this connection, the Committee wishes to make the observation that when states are contemplating advancement from Phase III to Phase IV, it is more desirable to make the transition from an attenuated vaccine program to a no vaccination program; however, if the use of vaccine seems a necessity, with the evidence now before us that single dose immunity levels of inactivated vaccine are inadequate, we would recommend that in cases where this product is used, two doses should be administered 30 days apart. We recognize that this is practical only in cases of retained breeding stock, and thus our conclusion that product usage will be self limiting.

The Committee notes that hog cholera diagnosis has seemingly become more difficult as the program has progressed. With these difficulties it becomes apparent that we must apply increased skill to diagnostic techniques. In view of this, the Committee has reexamined the standard diagnostic procedures finally evolved in 1963 and find that these procedures continue to be sound. We wish to emphasize that in all instances of
suspected hog cholera, a positive diagnosis be made which fits the minimum requirements of these procedures.

Many states are routinely applying cholera diagnostic measures on all samples of swine tissue being submitted to their laboratories for varied disease analyses. We wish to recommend this practice as being foresighted in the eradication effort and would urge all states to adopt similar procedures.

Again, all states are urged to carefully review their laws and regulations to determine that the necessary legal authorities do exist for meeting program requirements as they advance through the various phases.

The Committee has once more reviewed a request for consideration of partial depopulation in Phase III, and it is felt, as indicated in previous reports wherein the standards were established, that immediate partial depopulation of infected droves of swine cannot in the interest of sound disease principles be condoned. The Committee recognizes in some peculiar situations that complete depopulation of certain units and retention of other units might be feasible because of given physical characteristics. If these situations arise, the entire circumstances should be carefully considered by the cooperating officials, and if this procedure is to be followed, it should be only after mutual agreement has been reached without sacrificing the principles above referred to.

The Committee recommends the following changes in program standards:

1. Under Phase IV, an additional requisite should be made for states entering this phase. “The use of any living hog cholera virus vaccine within the State is prohibited, provided, however, that the appropriate federal and state authorities may authorize the use of these products in supervised research and biologic production.”

2. In the Free State category, the requisite surveys applicable herein need not be “statistically sound” in order to reliably demonstrate the absence of hog cholera. All the earlier criteria incorporated in the program standards, including laboratory examination of all samples submitted, garbage fed swine inspection, stock yards inspection, and the reporting of suspicious illness, should be maintained at optimum levels to provide a survey of contemplated reliability.

3. In disposing of infected or exposed swine in Free States it has been noted that an omission was apparently made when these standards were first devised, and therefore, a provision for disposal by supervised rendering techniques should be added to this section.

4. With respect to the requirements relating to importation of swine into Hog Cholera Free States, the following recommendations are made:
   a. Swine may be imported into a Free State without vaccination from any Free State, provided that they are accompanied by a proper veterinary health certificate and are maintained in isolation at destination for a period of at least 21 days.
   b. In such cases where Free states feel that there are not sufficient sources of swine available, notwithstanding such sources as other Free
states or animals treated with inactivated vaccine or with serum alone, swine which have been properly vaccinated with modified live virus and serum may be imported provided they are isolated for at least 21 days and meet the remaining conditions now existing with respect to this method of importation.

The Committee recognizes that as more states advance to the final phase of the eradication effort commercial hog cholera virus production within these states represents a possible hazard, and as such should be constantly reviewed by the Veterinary Biologics Division in order to preclude any disaster from this source.

Respectfully submitted,

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U.S. Livestock Sanitary Association
Phoenix, Arizona
October 20, 1967
THE EFFECT OF 5 ESCHERICHIA COLI SEROTYPES ON GNOTOBIOTIC SWINE

R. C. MEYER, H. E. RHOADES and S. P. SAXENA

INTRODUCTION

There can be little doubt that innumerable factors both microbial and physiologic are or can be involved in the diarrheas of baby pigs. As to causes, the microbial (bacterial and viral) are the most important. While the possible role of *Escherichia coli* in enteric diseases of young pigs was suspected as early as 1899 by Jensen,\(^1\) the isolation of various strains of *E. coli* from cases of young pigs with diarrhea does not necessarily establish any etiologic relationships.

Attempts to reproduce enteritis in baby pigs experimentally with different isolates of *E. coli* has met with varying degrees of success\(^2,8\) and it has only been in the past few years that several investigators have been able to reproduce enteric diseases in conventional\(^4\) and colostrum deprived baby pigs\(^5,6\) and more recently in newborn gnotobiotic swine.\(^7\)

The role and value of germfree swine is obvious in the study of enteric infections for they provide: (a) the means to control and manipulate the microbial environment and, (b) a test animal with a uniformity of susceptibility not generally obtainable with conventional nursing young swine.

MATERIAL AND METHODS

Germfree Swine:

Only gnotobiotic (germfree) newborn swine obtained by hysterectomy and maintained in germfree isolators were employed in this study. Methods of animal procurement and rearing were as previously described.\(^8\) A total of 42 piglets were used in this study.

Serotypes of *E. coli*:

All strains of *E. coli* employed were isolated in Illinois from young swine exhibiting diarrhea in the field or from animals submitted to the Illinois State Department of Agriculture Diagnostic Laboratory and the College of Veterinary Medicine, University of Illinois, Urbana.

Isolates of potentially pathogenic *E. coli* were typed initially as to the O grouping until its pathogenicity could be established and then in more detail employing the procedures as outlined by Gossling and Rhoades.\(^9\)

Five different serotypes were employed in this study; two strains of 08 and one each of 03, 09 and 0138. The seed cultures of each serotype used were lyophilized cultures. The inoculum for each pig consisted of 1cc of an 18-hour brain-heart infusion broth culture administered orally by means of a 5cc syringe and blunt 18 ga. needle.

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In the initial studies with these serotypes only 1 day old piglets were used. If enteric disease and death did not result in the inoculated pigs within 6 days post infection, the animals were killed and necropsied.

When a pathogenic *E. coli* serotype (as determined by productions of clinical disease or death) was found, the experiment was repeated two additional times employing litters with different genetic backgrounds. In these studies some members of the litter were challenged orally at one day of age as before and others at three days of age.

**Bacteriology:**

All necropsies were performed in an aseptic manner. Specimens collected for bacteriologic exam consisted of heart blood, lung, spleen, liver and segments from the gastrointestinal tract. These specimens were cultured on blood agar and MacConkey's agar and incubated at 37° C. Smears of such specimens were also prepared on glass slides and stained by Gram's method for direct microscopic examination.

**Pathology:**

Tissues for histologic examination were fixed in neutral 10% formalin and consisted of brain, lung, liver, spleen, segments of gastrointestinal tract, kidney and mesenteric lymph nodes.

**RESULTS**

**Clinical Signs:**

Of the 5 serotypes examined for pathogenicity only one proved pathogenic and capable by itself of inducing a diarrhea and death.

The 03: strain proved non-pathogenic when fed to 1 day old germfree swine. These pigs remained active, alert and healthy during the 6 day length of the study. At time of necropsy, there were no signs of enteritis. The only bacterium isolated from these pigs was the specific serotype administered at the start of the study and was isolated only from the intestinal tract of these pigs.

Studies with the 09: serotype gave results similar to those of 03.

The serotype 0138: employed also failed to produce clinical signs of disease in the six day period. However, at the time of necropsy the entire intestinal tract of the piglets infected was extended (inflated is a better word) with gas. There were no gross signs of inflammation however, and the pigs appeared otherwise quite healthy.

The first of the 08: serotypes to be tested also gave results similar to that obtained with the 03 and 09 serotypes.

The second 08: serotype (08:K87, K88ac:H19) however, proved to be highly pathogenic for newborn swine, and consistently killed 1 and 3 day old piglets; generally in less than 24 hours after the oral administration of the bacterial culture.

The clinical signs were in order of appearance (1) loss of appetite,
depression, (3) a greenish-yellow watery stool generally clear in appearance and with flecks of coagulated milk, (4) marked and rapid dehydration followed by (5) coma and death.

The loss of appetite could be noted early, generally 6-8 hours after the challenge with the pathogenic E. coli.

Pathology:

Upon gross examination the piglets infected with E. coli 08:K87, K88ac:H19 showed what could be construed only as a mild enteritis with little hyperemia, or congestion of the mesenteric lymph nodes or the small and large intestine. The intestinal tract contained small gas pockets and the contents were generally of a clear greenish-yellow liquid flecked with particles of coagulated milk. More marked was the inflammation of the stomach and the congestion of the gall bladder. These observations relative to a gastritis were consistently made in the 1 day old piglets and in more than ½ of the 3 day old piglets.

Histological examination of fixed tissues is still in progress.

Bacteriology:

Specimens collected at necropsy for bacteriologic examination were positive only for the specific serotype employed in that study. The 08:K87, K88ac:H19 unlike the other serotypes was consistently found in the heart blood, liver, and spleen, as well as stomach and small intestine. The other strains were not found outside the gastrointestinal tract.

DISCUSSION

Many investigators have considered E. coli to be involved in gastroenteritis and septicaemia of young pigs. However, it has remained until quite recently for proof of their involvement. In many ways the observation with E. coli 0:8K87, K88ac:H19 appear similar to E. coli infections of newborn swine involved in natural outbreaks of colibacillosis. These findings are in general agreement with other reported work4,5,6,7 which indicate that specific serotypes of E. coli when fed to newborn and young pigs could induce a diarrhoea in a short period of time with a high mortality rate.

Recently Stevens10 in Great Britain estimated that diarrheal diseases due to specific causes such as Clostridium, Salmonella, T.G.E. virus, toxic substances such as arsenic and various nutritional disorders only account for approximately ¼ of the total enteric and diarrheal disease observed in the field. The majority of the remaining were believed associated with E. coli in one way or another. Whether his estimates are valid is difficult to assess but are worthy of consideration when trying to establish the relative importance of various diseases and research priorities as they relate to swine.

The diarrheal diseases of young swine are not an easy problem to solve for as stated it is but one syndrome with many causes. Where the
cause is microbial, however, the gnotobiotic pig should prove a useful and valuable research tool. The use of such an animal should afford the means to more accurately identify pathogenic serotypes as well as provide the opportunity to explore the interactions of selected bacteria, viruses and diet in the etiology of diarrheal diseases.

From the University of Illinois, College of Veterinary Medicine and Agricultural Experiment Station.

This study was supported in part by the Illinois Department of Agriculture's Swine Disease Research Program.

REFERENCES

FACTORS INVOLVED IN THE SPREAD OF PSEUDORABIES AMONG SWINE

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There is historical evidence that pseudorabies virus (PrV) infections have occurred in the United States for more than 100 years although the first record of its infectious nature was published in 1902 from Hungary. Despite these well known facts not much attention has been paid to the disease in swine in the United States because until late 1962 it had been a benign or silent infection with few exceptions. The principal distress arose from losses of cattle, and sometimes of sheep, dogs or cats which in most instances had been in contact with infected swine. The suggestion that the disease was widespread but silent was derived from an extrapolation of the finding that significant virus neutralizing antibodies were present in a high percentage of a number of lots of anti-hog cholera serum which presumably represented large numbers of swine from a large number of farms. In a serological survey we found that while a high percentage of the anti-hog cholera serum lots from commercial sources contained significant values of anti-pseudorabies virus antibodies while very few swine on farms had them. Only 4 sera from 2 herds were found to contain anti-PrV antibodies and none were found in 267 serum samples from 102 herds representing 54 counties in Indiana. Similar results were obtained in England. It seems more likely that there is, as has been reported in Eastern Europe, a low percentage of swine that become carriers and shedders of the virus. When carriers are included during the assembly of swine to be used in the preparation of anti-hog cholera serum, the infection spreads rapidly by contact. The anti-hog cholera serum produced from them also contains antibodies against PrV. Only one of the source herds contributing swine to the enterprise would have had to have been infected.

The growing attention to PrV infections is associated with the appearance in the U.S. beginning in the late months of 1962 of viral populations that are virulent for swine. Since that time we have records of or have been informed of virulent infections of swine in Massachusetts, Pennsylvania, Michigan, Georgia, Florida, Missouri and California. There have been important outbreaks in Indiana in both summer and winter months annually since 1962. Concern about these increasing losses in swine and domestic animals associated with them—cattle, sheep, dogs and cats, draws interest to factors involved in the spread of the disease among swine.

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Experimental exposure of swine to PrV by intramuscular, intragastric, intratracheal, and intranasal routes has demonstrated that while infection may occur by exposure by any of these routes, intranasal exposure clearly results in the disease as it is seen in the field. The primary site of viral replication appears to be in the upper respiratory tract followed 48 to 72 hours after exposure by its appearance in the olfactory bulbs, pons and medulla. Apparently the virus travels in the epineural lymph spaces of the first (olfactory), fifth (trigeminal) and ninth (glossopharyngeal) cranial nerves. It passes to the olfactory bulbs and thence caudad in the cerebrum, to cells of the Gasserian ganglion at the pons, and to the nerve cells of the solitary nucleus in the medulla and rapidly, thereafter, spreads through the rhombencephalon (hindbrain), mesencephalon (midbrain) and spinal cord. (See Fig. 1) Viremia appears to be rather transient as the virus is not consistently obtained from the samples of parenchymatous organs or the blood during the disease and reports were not found of isolations after the ninth day. The virus has been found in washings of the naso-pharynx during the infection and in some instances for varying periods.

Figure 1

Schematic representation of extravascular portals of entry of pseudorabies virus to the brain of swine under natural conditions of exposure.
after signs of disease have subsided and antibodies are present in the serum. Virus has been recovered by some from urine but not in feces or rectal swabs. The suggestion is strong that carriers eliminate virus from the upper respiratory tract and nasopharynx and by their habits at the trough and/or contact activities, spread infection to susceptibles in a short period of time. Contact transmission probably occurs by the inhalation of virus directly from the moist nasal orifices and exhalations of a carrier or indirectly from food or fomite on which virus has been deposited from the nasopharynx of the carrier.

Contributions of Molecular Biology

Much has been learned of the physical and cell cultural characteristics of PrV as well as its molecular biology. It is a member of the Herpes group of viruses. The nucleic acid of PrV is double stranded DNA having molecular weight of $70 \times 10^6$. The capsid has cubic symmetry with 162 capsomeres and an average diameter of 186 mu. The virus is ether sensitive as infectivity is lost in 30 minutes at 37°C. In cell culture plaques appear at 24 hours and are 5-10 mm in diameter. The latent period is about 5 hours in monolayer rabbit kidney cells. The cytopathic effect is syncytial and Type A Cowdry intranuclear inclusions are formed. The chemical composition of PrV nucleic acid is similar to that of herpes simplex virus (HSV) in that both contain about 73 moles % of guanine and cytosine. After infection of a cell occurs there is a progressive decrease in the rate of synthesis of cellular DNA, and by 7 hours after infection it has stopped. Viral DNA, conversely, is actively synthesized in the nucleus. A protein has been found to be responsible for inhibition of the synthesis of cellular DNA. Viral proteins are probably synthesized in the cytoplasm and find their way to the nucleus of the infected cell. The capsids of the virus are assembled within the nucleus. The viral antigens first appear in the nucleus and spread later to the cytoplasm. The virus acquires the outer membrane or envelope as it leaves the nucleus. Progeny viral DNA controls the formation of proteins which regulate the leakage of virus and other cellular constituents from the cells during late stages of the infective process. All components of the virus are not produced in equal amounts which results in many incomplete viral forms within the cell. About 10% of the viral particles produced are whole; the remainder are defective in some fashion. The infection proceeds until death of the animal or until recovery is brought about possibly by a combination of elevated temperature, lowered pH, lowered oxygen tension, activation of an interferon system or unknown factors. Usually by the 7th day, antibodies can be found in the serum for the first time. Recovery begins prior to the presence of significant amounts of neutralizing antibodies in the serum. Interferon suppresses formation of new virus particles. Thus it does not inactivate them or their nucleic acids directly, nor does it prevent attachment and penetration of cells by virus.

The mechanism of interferon action is unknown and relatively little work has been reported on its effects on DNA virus replication. One interesting report suggests that a translation inhibitory protein is induced
by interferon which binds to ribosomes permitting normal translation of cellular m RNA but inhibiting that of viral RNA in its role as m RNA for an RNA virus and inhibiting viral m RNA in the case of a DNA virus.\textsuperscript{24} Although PrV has been found to stimulate only low values of interferon both \textit{in vivo} and \textit{in vitro}\textsuperscript{16} large amounts may not be required directly for effectiveness, or the turnover rate of interferon may be such that not much is detectable at any one time.\textsuperscript{46} Thus this type of resistance is best described as an interferon system having several steps and probably some alternative pathways involved. The importance in animals is that the system operates early in the infection to aid in progress towards recovery. Infrequently a balance is reached between resistance factors and virus production resulting in chronic release of whole virus or free viral nucleic acid and other viral components. An oversimplified explanation of the role of interferon systems in persistent infections as suggested by several investigators is that the persistent infections are dependent upon them. In a persistent infection, as the production of virus increases within a cell, interferon synthesis is induced and reduces viral replication. As a consequence, as virus particle numbers decline, interferon activity declines reducing inhibition of viral activity.\textsuperscript{46} Similarly the balances may result in latent or persistent viral infection from which unknown circumstances precipitate exacerbation and release of virus to the environment. Herpes viruses are well known for this feature.

Some interesting information has been obtained relating to latency and viral reactivation. Experimental results suggest many controls over the appearance, levels, and disappearance of deoxyribonucleotide kinases necessary for the replication of viral DNA in animal cells.\textsuperscript{14} It has been demonstrated that lethal damage to PrV in chick cells by ultraviolet light may be reversed by exposure to light of longer wave length.\textsuperscript{32} Such evidence suggests that interferon system and viral replication cycles in balance in an intact animal would respond to as yet unknown effects of forces such as body temperature extremes, acidity, oxygen tension, steroids, emotional stress and solar radiation.\textsuperscript{34} The existence of latent, recurrent, or persistent infections, which are manifested by periodic or chronic leakage of whole virus from cells, may be a feature of the inefficiency of viral replication among members of herpes group of viruses.

\textit{Environmental Conditions Favoring Viral Transmission}

The observation that swine are more resistant to PrV infections than are cattle, sheep, dogs, cats and rats is probably important to an understanding of how the disease is perpetuated in nature.\textsuperscript{7,8,12,38,4} These are the animals that are most often in contact with the diseased swine in the field. Other domestic animals may be susceptible but are only very rarely involved in an outbreak. If there is another species involved as a potential reservoir it may be one or more among wild animals including wild rodents. Humans do not seem to be involved as infected carriers. Laboratory workers, animal handlers, livestock producers who have at least been in contact with infected animals and in some cases have very likely been in an aerosol of the virus have remained asymptomatic and without neutralizing antibodies in their
serums. Cattle, sheep, dogs, cats and rats are highly susceptible and those infected with virulent strains succumb to the infection in a short time. Available evidence suggests that infections among these species may be dead ends for the virus. This has, of course, not been the case among swine and virus isolations from immune recovered animals have been recorded in Europe. No records of asymptomatic immune shedders of the virus among swine in the U.S. of A. have been found. Nevertheless growing circumstantial evidence suggests their existence.

The risk involved in assembling swine from many sources for redistribution has recently been illustrated. Early in 1967 a company selling feeder pigs shipped unknown numbers of 60 to 80 lb. swine to garbage-feeding establishments near 3 large cities and retained smaller swine on a contract farm away from the assembly and shipping premises. The disease appeared simultaneously at all four locations. In one case approximately 200 of 2500 died, in a second 35 to 40 of 300 died; in the third 38 to 250 died and 47 of 500 died at the fourth location. The swine had been imported from two other states over an unknown length of time prior to distribution. In California, chronic losses among swine on a garbage feeding establishment are reported due to PrV infections. Several outbreaks in Indiana have occurred on farms where purchased feeder pigs have been included in the environment. It appears that bringing swine together from many sources for redistribution is dangerous as well as is operating a continuous importation, feeding, and sale for slaughter enterprise.

The problem of closed herd infection and infections among cattle or sheep not in contact with swine remains difficult to explain. If human activities involving fomites are not a factor then a free living form of animal life such as rats may be involved but little information of this possibility was found in the available literature.

The Role of Artificial Establishment of Resistance

Herpes simplex infections in man constitute an example of the propensity of at least several members of this group for initiating latent, persistent, or recurrent infections. Recurrence of herpes labialis ("fever blisters") under various stresses which affect contiguous cells until fever, acidity, hypoxia and/or cell mediated resistance limit the extent of lesions while humoral antibodies prevent generalized distribution of the virus, are a common circumstance in man. The possibility that the use of an attenuated live PrV vaccine may provide appropriate conditions for establishing some form of inapparent infection which permits spread of PrV virus through continuous or intermittent shedding of the virus should be carefully scrutinized.

The information available on pathogenesis suggests that the primary site of viral replication is in the upper respiratory tract including the pharynx and associated lymphoid aggregates and that resistance to the severe effects of the disease stem from protection of the cells of the CNS, perhaps by an interferon system, after the virus gains access. Evidence concerning the protective value of specific antiserum suggests as does the timing of the rise of virus neutralizing antibodies in the blood in response to infection
that such antibodies have a rather modest value in protecting swine against virulent virus exposure by natural means. Comparisons of the results following exposure of swine to the virus by various routes suggests that the intranasal route might be an effective one for the modified live virus vaccine since it provides extravascular access to the CNS in addition to tonsillar lymphoid tissue resulting in induction of cell associated resistance as well as circulating antibodies. Modified live vaccines are available and used in Eastern European countries. The nagging question is why there are so many outbreaks of PrV infections among swine if the vaccine is not contributing to the problem. Presumably the answer is that in part at least the problem was there previously. Having found that inactivated vaccines were inadequate to protect swine, sheep and cattle investigators in Eastern Europe have developed attenuated live virus vaccines. Some investigators found that by subcutaneous inoculation the rise of antibodies was slow and of low titer requiring a second inoculation to achieve higher antibody titer. However, others have not found this to be necessary. In both cases antibody response was low and slow to rise. Attenuated PrV strains have been studied in sheep and cattle with moderate success reported. In all cases subcutaneous inoculation provides both antibody titers and protection against challenges other than intranasal and intracranial, which pathogenetically seems crucial.

Alternatives to live vaccine are not especially attractive at the present. They are antiserum, inactivated virus vaccine, and chemotherapy. Antiserum may be used during a quarantine period at points of assembly or at the beginning of an outbreak. Even so its value is limited. An effective killed vaccine is not on the present horizon and chemotherapy seems to be only a theoretical possibility at this time. Because of these facts interest in a modified live vaccine may become so strong that it cannot be resisted and create another factor in the spread of pseudorabies among swine which is the establishment of persistently infected swine which shed virus.

Conclusions

1. The crucial importance of the limitation of significant pathological changes to the CNS is underscored by differences in response following exposure to PrV by various routes and suggests a somewhat unique pathogenetic pattern. Consequently it seems likely that intranasal application of vaccines would be indicated to establish maximum effectiveness of artificially induced resistance by stimulating cell associated resistance as well as circulating antibodies.

2. Circumstantial evidence in the U.S. and reports from Europe point to the existence of asymptomatic virus-shedding carrier swine which endanger the mass of susceptible swine that under common methods of commerce are brought together for redistribution to farms and "open-end" swine feeding operations.

3. The occurrence of disease in clearly closed-herd swine operation and among cattle and sheep not associated with swine suggest the existence of free-living virus shedding birds or animals which threaten all.

4. Careful study should be made of the dangers of the use of modified
live virus vaccines because of the possibility of contributing to the development of virus shedders.

REFERENCES


A POOLED SAMPLE METHOD FOR POST-SLAUGHTER DETECTION OF TRICHINIASIS IN SWINE

W. J. ZIMMERMANN*

Trichiniasis has been a public health problem in the U. S. for at least a century, but with the exception of the regulations of the Meat Inspection Division (MID), Consumer & Marketing Service, United States Department of Agriculture for preparing ready-to-eat pork products, no specific control or eradication program has been formulated to eliminate the disease problem. Now, however, there are indications of a major awakening to the problem. The swine health workshop, dealing with trichiniasis eradication at the 1966 National Pork Industry Conference, unanimously adopted a resolution that supported and encouraged a program of increased research toward eradication of the disease. Earlier in 1966, Livestock Conservation, Incorporated, established a task force study committee to evaluate the trichiniasis problem and to determine ways that the disease could be eradicated in the U. S. Reports presented at the 1966 USLSA meeting by the Committee on Transmissible Diseases of Swine and the Committee on Public Health and Radiological Fallout also helped to focus attention on the problem. With the full cooperation and attention of the swine industry, regulatory officials and public health authorities, hopefully an eradication program may soon be formulated and activated.

The trichiniasis problem is decreasing rapidly in the United States even without the evolvement of a specific control program. During the 1930's the prevalence of *Trichinella spiralis* in farm-raised (predominantly grain-fed) swine was 0.95 percent. This decreased to 0.63 percent during 1948-52. Since that time, the prevalence has steadily declined so that more recent studies (1961-65) revealed a prevalence of 0.12 percent. Current studies by the author in cooperation with MID indicate that this downward trend is continuing.

A similar decrease in prevalence is apparent for garbage-fed swine. During 1950, Schwartz obtained a prevalence of 11.0 percent in swine fed raw garbage. The advent of vesicular exanthema with subsequent passage of garbage cooking regulations reduced the prevalence to 2.2 percent in 1954-59. The prevalence then increased to nearly 6 percent in 1961 after vesicular exanthema had been eradicated. Since evolvement of the hog cholera eradication program with increased emphasis on proper cooking of garbage, the prevalence has now decreased to 0.5 percent. A recent estimate, based on 1961-66 findings, indicated that about 105,000 trichinac-infected swine are marketed yearly in the U. S., with about 99,000 of the infected swine being in the farm-raised classification. Formerly the problem was considered to be related primarily to garbage-fed swine. With more efficient cooking of garbage and a decrease in the number of

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garbage-fed swine, farm-raised swine are now the primary source of human infection in the U. S.

The decreasing prevalence of the disease in swine to a more readily eradicative level, along with the desires of the pork industry for an eradication program, has now focused attention on the development of diagnostic tests suitable for detection of the disease in swine. The trichinoscopic method which has widespread usage in other parts of the world, has been deemed too expensive for adoption in the U. S. Since the trichinoscopic method utilizes a diaphragm sample of 1 gram or less, questions have also arisen in the U. S. as to the efficacy of the method. However, Germany utilizing the trichinoscope routinely for examination of all pigs slaughtered, has reduced the prevalence in swine to about .0001 percent.8 Serological tests have often proved unreliable in swine, although some of the more recently developed serological methods show promise of efficacy.8 However, more study is necessary before these are adaptable to packing house or field usage.

A pooled sample, post-slaughter method of detecting trichinae infections in swine was developed as a safe and relatively inexpensive diagnostic test adaptable to packing house usage. After cooperative preliminary evaluation, the MID has now approved the method in principle for official use. The method proposed is a modification of the artificial digestion technique developed by the National Institutes of Health9 and utilized by the Veterinary Medical Research Institute (VMRI), Iowa State University for the examination of over 70,000 samples, of which about 45,000 have been swine diaphragms or pork products.

**Method:**

The basic procedure, as outlined schematically in Fig. 1, is a modified form of that used at the VMRI for routine examinations. Modifications are the pooling technique, sample size, and pepsin-HCl concentration. The basic procedure is as follows:

1. The procedure is initiated by dividing the slaughter into lots of 20 consecutive carcasses, with each carcass identified as to lot.
2. A portion of a pillar (crus) of the diaphragm is removed from each hog in the lot. These are taken to the laboratory for processing.
3. The pillars are trimmed to weigh 5-6 grams. The capacity of the system is such that this range could possibly be increased to a range of 7-8 grams, thus giving an increased safety factor.
4. The pooled sample, consisting of 20 individual pillar portions, is finely ground by a mechanical food chopper. A separate grinder unit is used for each pooled sample.
5. The ground pooled sample is then placed in a 3 liter beaker which is identified as to lot. The beaker is filled with a digestive fluid containing 1 percent pepsin and 1 percent hydrochloric acid. The digestive fluid is prewarmed to 37°C. The beaker is then placed in an incubator where the sample undergoes digestion at 37°C for
12 hours with constant agitation. The liquid is then allowed to settle for 1 hour at 37°C after which about 2/3 of the supernate is siphoned off.

6. The remaining supernate and debris is then poured through a 60 or 80 mesh screen into a 250 mm funnel closed at the bottom with rubber tubing and a clamp. The beaker is rinsed with sufficient prewarmed water to add to the funnel until the screen is covered. The fluid is allowed to settle 1 hour at 37°C.

7. The clamp is then opened to allow fluid from the 250 mm funnel to fill a 125 mm funnel which is similarly closed off. This is also allowed to settle 1 hour at 37°C.

8. A portion of the fluid is then drawn into a ruled Syracuse watch glass or a ruled petri dish for microscopic examination. Microscopic examination is made at 27x although other magnifications will give satisfactory results. Possible modification of the trichinoscope may allow use of a projection type examination at 80x magnification.

9. The finding of a trichina larva indicates a positive lot. If a positive lot is obtained, an additional 45-50 gram portion of the pillars

Figure 1. Pooled sample trichiniasis diagnostic technique (schematic outline).
from each hog in the lot is collected and examined individually. The infected swine carcasses are then processed to kill the trichina larvae by methods prescribed in Meat Inspection regulations.

The entire procedure, from collection of samples to microscopic examination of the dishes, would utilize approximately 17 hours. The 12 hour digestion period is the predominant time factor. This would necessitate two shifts of employees (see discussion).

Fig. 2 shows one of the trichina digestion incubators utilized at the VMRI. With the completion of the front row of funnels, this incubator would have a capacity of 640 pigs per day. The use of a separate incubator for digestion only would increase this capacity to 960 or even possibly 1280 pigs per day, if scheduled properly.

Efficacy of Method:

A series of trials was carried out to determine the efficacy of the proposed pooled sample method. A 100 or 125 gram sample of pork was ground and digested as indicated in the previous procedure. Infected rat meat was digested in a separate beaker. Trichina larvae were then counted in a Syracuse watch glass and added to the digestive fluid before
pouring in the large Baermann funnel. The remaining procedure was then followed.

The results of these studies are shown in table I. Only one of the 22 beakers to which trichinae were added gave negative results. The

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<th>Number Larvae Recovered Per Beaker</th>
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finding of trichina from 5 of the 6 beakers to which a single larva was added is of significance. Eight of the 16 beakers to which more than one larva was added yielded 100% recovery. The complete recovery of 8 and 9 larvae, respectively, also points out the efficacy of this method.

In earlier trials, using lower concentrations of pepsin and hydrochloric acid, a larva was recovered from eight of ten beakers to which a single trichina was added. All beakers containing more than 1 larva were positive. At the lower concentrations, however, a fine sediment resulting from incomplete digestion occasionally interfered with the efficacy of the procedure.

Pilot Study:

In order to evaluate the method further, a 3 day pilot study was carried out with the assistance of USDA personnel.* Diaphragm samples were obtained through the cooperation of the George Hormel and Company plant, Ft. Dodge, Iowa. MID personnel obtained the samples during normal inspection procedures. The samples were transported to the VMRI for examination.

During the three days, the diaphragms of 2,730 swine were examined. All samples were free from trichina as determined by the pooled sample

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DETECTION OF TRICHINIASIS

Four positive control samples were determined as positive by test. One nematode larva of unknown genus was detected by the test.

The pilot study showed that by using this technique, every hog processed in a packing house could be examined for trichina without interfering with normal work flow of the slaughtering department. Results of each day's examinations were obtained before cutting operations started the next day.

Discussion:

In considering any method for detection of trichiniasis in slaughtered swine, three factors must be considered. One is the efficacy or safety of the method; the second is the cost of labor, supplies, and equipment necessary to carry out the method; and the third is the adaptability of the method to packing house procedures.

The digestion method is generally recognized as the most accurate method for detection of infective trichinae in man or animals. A recent report indicates that some slaughter houses in Poland now utilize the digestion procedure for examination of swine from endemic areas or when the pork will be utilized in raw or semi-raw products. The trichinoscopic method has generally proved safe as indicated by its widespread usage. The trichinoscopic method uses a sample weighing one gram or less. Extensive studies conducted at the VMRI on U. S. swine indicate that more than 80 percent of the positives from farm-raised swine contain less than 1 trichina per gram. Thus more than 80 percent of these positives may not be detected by the trichinoscopic method. Those not detected, however, are generally not considered capable of causing clinical trichiniasis in man.

Since the pooled sample method utilizes a minimal sample of 5 grams in contrast to the 1 gram or less utilized by the trichinoscopic method, the pooled sample method would detect a greater number of infections. The pooled sample method would not detect all infections but the intensity of the infections would be such that they would not cause clinical trichiniasis in man, and it is unlikely that most would cause any infection whatsoever in man. The theoretical limit of efficacy is 1 larvae per 5 grams or 23 larvae per 115 grams (0.25 lb.). Since the concentration of trichinae in the pillars of the diaphragm is generally 2-5 times higher than other muscles, the maximal possible infective dosage would be decreased even farther.

As shown previously, the pooled sample method was highly efficient in detection of known numbers of larvae. In trials where only a single larva was added, positive findings were made in 5 of 6 cases. All trials in which more than 1 trichina was added were positive with 100 percent yield in 8 of the 16 trials in this category. The finding of a single larva would constitute a positive lot.

Implementation of the procedure in any packing house would necessitate the use of two shifts of employees to allow examination of diaphragm samples before the swine carcasses are cut up. The day shift would be
responsible for identifying lots, collection of samples, grinding of samples, and initiating digestion procedure. The night shift, working approximately from midnight to 8:30 AM, would be responsible for examination of samples after the 12-hour digestion period. The labor crew for a plant with a capacity of 4000 hogs per day, as indicated by experience at VMRI and by the pilot study, would consist of 9 or 10 employees. The relative simplicity of the procedure is such that highly trained personnel would not be required for other than supervisory positions. The labor cost would be less than 6 cents per pig. The cost of the pork utilized for examination would be only a fraction of a cent.

The equipment and supplies necessary to process the samples would include chemicals, incubators, grinders, microscopes, beakers, and funnels. The cost of the pepsin and hydrochloric acid would be less than 2 cents per pig. A cost estimate for equipment and supplies would indicate a cost of less than 1 cent per sample when depreciated over a 3 year period. Thus the total cost of labor, equipment, and supplies would be less than 9 cents per pig slaughtered. Even when the plant does not run at full capacity, the cost should not exceed 10 cents per pig.

The pooled sample method is adaptable to packing house conditions as indicated by the pilot plant study. The results of the examinations would be available within 24 hours of slaughter, a period which is generally the minimal cooling period before processing of carcasses. Only when a positive lot is found would this period have to be expanded, and then only to a maximum of 48 hours. Based on current prevalence findings, the number of retained lots and necessary rechecks would be minimal and should not interfere with normal plant operation.

There is much to be gained by adoption of a method allowing examination of pork for trichiniasis in a packing house. The proposed method would assure that the inspected pork would not cause clinical infection in man and would also markedly decrease the number of sub-clinical infections. Most infections in the U.S. are traced to locally processed, non-inspected pork sausage products. The pooled sample method would also reduce this problem. In Iowa, for example, two outbreaks were reported involving 18 people in 1961 and 29 in 1966. Both were traced to locally processed sausage utilizing fresh pork from MID inspected plants. Had this pork been obtained from a plant using a trichina inspection procedure, these outbreaks would not have occurred.

Also related to the reduction of the public health significance of the disease would be the corresponding reduction of the prevalence of T. spiralis in swine. The detection and destruction by heating or freezing of trichinae in infected pork would reduce the possibility that pigs or wildlife reservoirs would consume garbage containing infective pork. Thus the prevalence in swine would also decrease markedly.

Another possible gain could be marked expansion of the export markets, since the minimal sample size of 5 grams should give greater assurance of freedom from T. spiralis than does the trichinoscopic method. There has long been a stigma of trichiniasis on U.S. pork in European countries. Examination of pork for T. spiralis would do much to lessen
this concern. During 1964, over 60,000 swine were examined by trichinoscopic method in the U. S. for shipment to France and Portugal. This indicates the market potential as well as foreign concern over the trichiniasis problem in the U. S. The domestic consumption of pork would also increase once the populace of the U. S. can be assured that pork is trichina-free and overcooking of pork is not necessary.

Another major gain would be the ability in many cases to trace the infected animals back to the originating farm. During the previously cited trichinoscopic examination of pork for export to France and Portugal, 3 swine of a lot of 482 were found infected. One diaphragm contained 4561 larvae per gram as indicated by subsequent examination at the VMRI. This was the highest trichinae count obtained from any natural infection in the VMRI studies. An extensive followup investigation by the Animal Health Division, Agricultural Research Service, United States Department of Agriculture included visits to 35 farms. Extremely poor sanitation and swine management practices were evident on one farm. Subsequent examination of 8 additional swine from this farm revealed three infected hogs with trichinae per gram counts of 2,400, 1,451, and 190. This demonstrates that an effective diagnostic method will not only result in immediate detection of hog carcasses that could endanger human health, but will also aid in pinpointing problem herds as a possible additional source of infection. Adoption of a swine identification system would facilitate tracebacks.

Summary:

A pooled sample method utilizing a digestion procedure is proposed as a new approach to the diagnosis of trichiniasis in slaughtered swine. The method has been approved in principle by the Meat Inspection Division, Consumer and Marketing Service, United States Department of Agriculture. The method proposed is relatively inexpensive, safe, and adaptable to packing house operations.

REFERENCES


UNITED STATES LIVESTOCK SANITARY ASSOCIATION
COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE

REPORT FOR 1967

Phoenix, Arizona October 19, 1967


Discussions of swine diseases by members provided information of progress on some disease problems confronting the industry, need for continuing support and effort in established programs, and gave attention to some which should receive greater emphasis. While the report may not in some specific areas repeat positions that have been held in previous years, it should not be taken that the positions have been abdicated. The spectrum of attention of the committee is so broad that it is not feasible to present issues that remain stable more often than seems to be prudent. The following items are commended to the Association for adoption as the report of this committee:

1. TRICHINOSIS

Trichinosis remains an important zoonotic disease which has had an adverse impact on domestic and foreign acceptance of pork. The disease is very costly to producers because of unrealized income and to the public because of the expense of special processing requirements. It is of sufficient importance to warrant renewed efforts to find better means of diagnosis, natural sources of infection, and modes of spread in order to make significant strides toward eradication possible.

Allocation of limited public funds in 1967 has made it possible to initiate studies to evaluate a pooled sample method of diagnosis of the disease in carcasses through examination of chemically digested diaphragmatic muscle tissue. There is need for a means of detecting the presence of trichinae in living animals as well as the evaluation of the pooled sample method at the time of slaughter.

The relationship between effective enforcement of garbage cooking laws and the decline of trichinosis among swine fed that garbage seems clear but should be established through survey efforts. The relatively greater prevalence of trichinosis in farm raised swine has been thought to be due to the ingestion by swine of wild ver-
tebrate tissues and/or fresh pork scraps. An investigation of these theories seems warranted. All laws and regulations in all political divisions requiring the cooking of garbage used as feed for swine should be vigorously enforced.

2. **TRANSMISSIBLE GASTROENTERITIS**

The report of the Sub-committee on Transmissible Gastroenteritis (TGE) is accepted and is included as an addendum to the report of the committee. Current and accepted methods of diagnosis are reviewed; the controversy surrounding the etiologic agent is briefly discussed and relevant comments are made on a commercially produced TGE vaccine. The Sub-committee was requested to continue to evaluate progress that is being made in the study of TGE in the coming year.

3. **JOWL ABSCESSES**

Economic losses due to condemnation of swine or parts of carcasses because of abscesses continue at a high rate (more than $12,000,000 annually) as presented in the 1966 report. During the past year some research groups have been organized and funded. It is believed that an increased rate of research progress return could be achieved if such groups were larger and more numerous. It is recommended that private enterprise of various kinds re-examine the possibility of making substantial commitments to the resolution of the problem. Help is needed to add impetus to the public supported effort to find means of preventing losses due to abscesses in swine.

4. **PSEUDORABIES**

Pseudorabies spreads rather easily among swine. In view of the more virulent nature of some virus strains since 1962, the disease is gradually becoming a more serious problem among swine which are collected from farms for redistribution as feeder pigs. Experiences with the disease at assembly points in Indiana and feeding premises in California, Massachusetts, Pennsylvania, Florida, and Georgia within the past year (October 1966 to October 1967) have emphasized its growing importance.

Initial reports of the value of antiserum in such outbreaks are not highly reassuring since morbidity was not diminished though mortality was significantly reduced but not eliminated. Efforts should be made to consider measures to reduce the hazards involved especially when the broadness of the species susceptibility spectrum is considered. All regulatory and diagnostic laboratory officers are urged to provide adequate preparation for the diagnosis of this disease which seems to be more prevalent than commonly considered.
5. **MASTITIS-METRITIS-AGALACTIAE**

It is clearly apparent that the so-called mastitis-metritis-agalactiae (MMA) syndrome must be given greater research attention. A significant segment of the swine industry considers MMA to be a problem of paramount importance. Research support by public and private agencies should be given to determine the cause or causes of the syndrome so that effective means of avoiding the problem may be found.

6. A Symposium on Embryo and Fetal Abnormalities, Death, and Abortion was held in Chicago, Illinois, on October 2 and 3, 1967. It was sponsored jointly by this committee, the Animal Health Division of the Agricultural Research Service, USDA, and the National Academy of Sciences. Proceedings of the Symposium will be published and therefore available to all who are interested. The response to the meeting was excellent and the Committee recommends that Symposia of this nature be encouraged.

**REPORT OF THE SUB-COMMITTEE* ON TRANSMISSIBLE GASTROENTERITIS OF SWINE**


In respect to the criteria that may be used for the diagnosis of TGE in swine, only a few additional or clarifying comments need be made on the report of 1966. These are:

1. Bentonite agglutination (BA) test for the serologic diagnosis of TGE. This test was briefly referred to in the 1966 report, although reservation was expressed as to its value. Since then, additional information has become available casting further doubt on the value of this test as a means of diagnosing TGE. The neutralization test, however, has remained a most reliable method for the serologic diagnosis of TGE.

2. Etiology of TGE. In last year's report, reference was made that some investigators believed that the true etiology of TGE was a noncytopathogenic virus which was different, antigenically and otherwise, from the TGE virus that has been described and characterized by several workers in Japan, England, and the U.S.A. The published evidence, at present, indicates that there is only one

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*This is a sub-committee of the Committee on Transmissible Diseases of Swine. Three members are from this Committee and three from the Conference of Veterinary Laboratory Diagnosticians.
virus causing TGE and, further, that the strains studied have been antigenically very similar or identical.

(3) TGE in piglets from immune or partially immune sows. An atypical syndrome has been described in which severe diarrhea among 1½ to 4 week old piglets was the principal problem in some herds. The sows of these herds had previously been infected with TGE virus. In addition, a continuous farrowing program was in operation, allowing for a rather continuous exposure of piglets to TGE virus. In all probability, sufficient immunity was transferred to the newborn piglet to provide temporary protection. More attention should be given to this syndrome, as it is sufficiently different from the usual course of TGE in a herd so that it is difficult to make a clinical diagnosis.

As stated in the 1966 report, the following methods are considered to be of great value in diagnosing TGE:

(a) Clinical signs and history
(b) Villous atrophy in the jejunum or ileum
(c) Serologic method, using the neutralization test
(d) Detection of virus by pig or cell culture inoculation
(e) Fluorescent antibody test. However, not much information is yet available on this test.

In respect to the commercially available TGE vaccine (Diamond Laboratories) the following comments are in order:

(1) The material used for preparing the vaccine was found to contain a virus that is antigenically similar or identical to the cytopathogenic strains of TGE virus.
(2) Neutralizing antibodies against the cytopathogenic strains of TGE virus were not demonstrated in serum from vaccinated sows in the studies conducted, nor have they been reported by others.
(3) Conflicting reports have been received from the field as to its efficacy.
VESICULAR STOMATITIS IN THE UNITED STATES DURING THE LAST FIVE YEARS (1963-1967)

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Introduction

Vesicular stomatitis (VS) is frequently propagated in laboratories because of its dramatic lesions and its adaptability to many host systems. There appears to be a wide range of host susceptibility to VS in nature. This may be one reason so little is known about its epidemiology. Vesicular stomatitis epidemiologic studies have been rare, of short duration, or intermittent and carried out by small groups of workers. The reservoir in nature may involve arthropods, plants or fungi.

Vesicular stomatitis has debilitated thousands of horses during military campaigns, caused loss of milk production and contributed to mastitis in dairy herds. Vesicular stomatitis has caused loss of weight gains in swine, has required constant differentiation from foot-and-mouth disease, and has produced illness in people. Vesicular stomatitis has existed for 100 years, yet its epidemiology is not understood.

Some of the first reports of VS in the United States concerned concentrations of military horses during the Civil and First World Wars. Since then, it has occurred irregularly in the bovine, equine, and porcine population of our country, as well as in other countries of North, Central, and South America.

Hanson wrote on the early history of VS virus. A paper presented before the United States Livestock Sanitary Association in 1960 gives some VS incidence for the years 1952 to 1960.

Vesicular Stomatitis Incidence and Distribution, 1963-1967

During 1963, Georgia and Alabama had an unusually large epizootic of New Jersey type VS (Map 1). Two hundred ninety-two confirmed positive herds were found in Georgia, (Map 2), 64 in Alabama, five in South Carolina, four in Arkansas, and one in Florida. Fifteen diagnoses were made in horses, the remainder in cattle. A positive diagnosis, as used in this paper, means by a positive complement fixation (CF) test on vesicular epithelium with or without virus isolation in tissue culture, a positive CF test on serum, or by the serum neutralization (SN) test performed on convalescent serum. Frequently VS is confirmed in a herd by using two or three of these methods. Some doubtful cases with positive neutralization tests on single serums are pointed out. The first map for each year shows infected counties, the second the incidence by county, and the third map the outbreak date for each county. During 1963, the highest concentration of VS was found in Meriwether County, Georgia, with 74

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INCIDENCE OF CONFIRMED NEW JERSEY TYPE VS HERDS IN ALABAMA, FLORIDA, GEORGIA, AND SOUTH CAROLINA BY COUNTY - 1963

MAP 1

MAP 2
OUTBREAK DATES OF VS IN ALABAMA, FLORIDA, GEORGIA, AND SOUTH CAROLINA BY COUNTY - 1963

M A P 3

DISTRIBUTION OF VESICULAR STOMATITIS INFECTED COUNTIES - 1964
confirmed infected herds, and in Upson County with 64. Talbot County, where VS was first observed on June 24 (Map 3), had 35 infected herds. The figures are those of suspected vesicular conditions investigated by regulatory veterinarians and confirmed by laboratory diagnosis.

During 1964, after not being found in the United States for six years, Indiana type VS was diagnosed in Maverick County, Texas, on May 6 (Map 6). From five counties near the Rio Grande River, VS spread through Central Texas. A few cases occurred in five counties of Oklahoma, Arkansas, and Missouri and then caused extensive infections in eastern Colorado (Maps 4 and 5). One hundred twenty positive herds
were found in Fremont County and 35 in Pueblo County. Five cases of VS in man were diagnosed in Colorado, and more had the clinical pattern of illness but no samples were taken.

New Jersey type VS was diagnosed in one herd in northeast Texas and another one in southeast Oklahoma during 1964 (Map 4). In the Southeast, 11 herds in Georgia were infected, 11 in South Carolina, eight in North Carolina, 10 in Florida, one in Alabama, and one in Mississippi.

The New Jersey VS incidence was much lower during 1964. During 1963, 74 positive herds were found in Meriwether County, Georgia, and
seven in 1964. Four scattered counties had one each. During 1964, New Jersey VS virus was widely distributed from North Carolina to northeast Texas, but no large epizootic was diagnosed.

The VS pattern for 1965 (Map 7) had two unusual features. New Jersey VS was not diagnosed along the Atlantic Coast. Five serums containing antibodies were received from separate Alabama counties; because these were positive by the serum neutralization test in the absence of complement fixation titers, the antibodies in these serums may have been due to infection in 1963. One herd in Mississippi, two herds in McCurtain County, Oklahoma, and two herds in Arkansas had the only other New Jersey VS diagnosed during 1965.

The other unusual feature of the VS diagnosed during 1965 was the recurrence of Indiana VS near Espanola in Rio Arriba County, New Mexico. This was the area in which it was first diagnosed during the epizootic of 1956. This epizootic (Maps 8 and 9), first observed in early July, involved New Mexico, western Colorado, two counties in Arizona, and one in Utah.

Seventy herds in Rio Arriba County (Map 8), 25 in San Juan, and 26 in Taos made up the concentration of infection in New Mexico. Colorado had 48 infected herds in Montezuma and 25 in La Plata County. Two serum samples submitted from Fremont County, Colorado, were positive by SN only, which indicates that these may have been the result of infection the previous year. These and two similar serum samples from Bell County, Texas, are the only overlap detected between the 1964 and 1965 epizootics of Indiana VS virus. Scattered cases occurred into September in New Mexico, Colorado, and Utah.
After several human cases were reported in the Farmington area of San Juan County, New Mexico, and adjacent Colorado, state public health officials invited an investigation by the National Communicable Disease Center.

**INCIDENCE OF INDIANA VS HERDS IN ARIZONA, COLORADO, NEW MEXICO, AND UTAH BY COUNTY - 1965**

During January 1966, New Jersey VS was diagnosed in Webb County, Texas, near the Rio Grande River. In mid-April (Map 10), it spread along the Rio Grande border counties and swept northward across the Edwards Plateau of Central Texas into Oklahoma and Arkansas (Maps 11 and 12). New Jersey VS was also diagnosed in one county of eastern Mississippi and from one herd in South Carolina. For the second consecutive year, only occasional isolated New Jersey cases appeared in the eastern United States.
Further west, both New Jersey and Indiana types were found in Colorado and New Mexico (Map 13) with seven cases of New Jersey VS in Emery County, Utah. The highest incidence of VS was in Mesa County, Colorado, where 48 herds were infected with the New Jersey type, five with Indiana type, and there were 18 cross-type reactions. Cross-type reactions were those in which the serum reacted the same to both New Jersey and Indiana types of antigen so that the type could not be determined. The cross reactions were obtained by CF or by SN tests. The 33 herds in Webb and 32 in Bexar County made up a sizable portion of the 409 herds in which New Jersey VS was diagnosed in Texas. Colorado had 138 VS infected herds and New Mexico 37.
Through August 1967, only one well established case of VS has occurred (Map 14). This was a severe infection involving a herd of hogs in Catahoula Parrish, Louisiana. The owner first observed illness on August 3. New Jersey neutralizing antibodies have been detected in about six other widely separated herds of Texas and South Carolina, but these were with single serum samples or paired samples lacking antibody increase.

**Epidemiological Investigations**

Reports of several cases of VS in man, among owners and handlers of infected cattle in San Juan County, New Mexico, and adjacent La Plata County, Colorado, caused the State Health Departments of New Mexico and Colorado to request assistance. The National Communicable Disease Center responded by sending teams of epidemiological investigators. In addition to the cases in man, their studies included attempts to isolate the virus from mosquitoes, rodents, and rodent ectoparasites. One isolation of Indiana VS virus was made from *Aedes* mosquitoes.

During the epizootics from 1963 through 1966, the Bovine Virus Unit at the National Animal Disease Laboratory attempted to isolate virus from
arthropods trapped on VS infected premises. New Jersey VS virus was isolated from a pool of eye gnats, *Hippelates pusio*. The virus was apparently of low concentration. Efforts to reisolate the virus were not successful, although there was a similar pattern of deaths in the baby mice which unfortunately were eaten by their mother. More collections will be necessary to confirm or eliminate *Hippelates* as a vector.

**INCIDENCE AND OUTBREAK DATES OF CONFIRMED VESICULAR STOMATITIS HERDS IN COLORADO, NEW MEXICO, AND UTAH BY COUNTY - 1966**

**VIRUS TYPE - NUMBER OF POSITIVE HERDS - DATE OF OUTBREAK**

In 1966, SN antibodies were found in the serum samples of three of 13 Texas pigs. These swine were on premises where only cattle had been observed to be clinically infected. All known clinical infection in
swine within the United States has been of the New Jersey type. During the 1950's, New Jersey VS was frequently diagnosed in swine in the Coastal states from Mississippi to North Carolina.

One of 79 deer serums obtained from the Southeastern states during 1966 contained New Jersey VS virus neutralizing antibodies; none contained antibodies to Indiana VS virus.

During June, 1966 and February, 1967 blood samples were taken from seven study herds in Georgia; these herds had been designated VS study herds during the 1963 studies. Nearly half the adult animals in these herds were vaccinated with a New Jersey VS vaccine during 1963.

New Jersey VS virus may have been present during 1966 at a subclinical level in three or four of the herds under study; these herds show cattle under three years of age with VS neutralizing antibodies. Ages were not obtained on 63 head from which blood samples were obtained during February 1967; because these were four to eighteen months of age, some of the three positive reactions in this group may have been from passive antibodies.

Discussion

The distribution for the past five years has been presented. Vesicular stomatitis is a perplexing, yet challenging disease. For many years, two types of vesicular stomatitis were recognized. These appeared to be stable. Recently, new strains related to the Indiana type have appeared outside of the United States. Cocal virus which is a subtype of the Indiana serotype
was isolated in Trinidad in the British West Indies and later in Belem, Brazil, during 1961 and January 1962. Both of the original isolations were made from mites removed from terrestrial rice rats (Oryzomys); later one isolation was made from rat tissues. Another Indiana type variant strain, which appeared in horses in Argentina during 1963, appears to be identical by serological tests to Cocal but very different from another variant which appeared in Brazil during 1964. All of these were considered subtypes of the Indiana serotype.

<table>
<thead>
<tr>
<th>Age</th>
<th>Positive</th>
<th>Total Tested</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/3-3 yrs.</td>
<td>11</td>
<td>117</td>
<td>9</td>
</tr>
<tr>
<td>4 yrs.</td>
<td>10</td>
<td>43</td>
<td>23</td>
</tr>
<tr>
<td>5 yrs.</td>
<td>46</td>
<td>100</td>
<td>46*</td>
</tr>
<tr>
<td>6 yrs.</td>
<td>69</td>
<td>94</td>
<td>73*</td>
</tr>
<tr>
<td>7 yrs. and up</td>
<td>86</td>
<td>130</td>
<td>66*</td>
</tr>
<tr>
<td>Totals</td>
<td>222</td>
<td>484</td>
<td>45†</td>
</tr>
</tbody>
</table>

*Nearly half the adult animals in the study herds were vaccinated with a New Jersey VS vaccine during 1963.
†Average.

An important fact about the Cocal virus isolations seems to be their involvement with rodents. The 1961 epizootic was explosive and involved about 65 percent of the rodent population of the forest floor.

Four isolations of Indiana VS virus were made from Phlebotomus sand flies collected in a tropical rain forest of northeast Panama. Vesicular stomatitis virus was isolated during encephalitis studies, and no attempt has been made to isolate more VS virus from Phlebotomus. These insects exist in our southern states and are important vectors of disease in many parts of the world.

A paper by Glazener, Cook, and Trainer reported finding New Jersey VS antibodies in 36 of 122 wild turkeys sampled at the Welder Wildlife Refuge in southeast Texas; eight serum samples neutralized Indiana VS virus, but six of these also neutralized the New Jersey serotype. The antibodies to New Jersey VS were formed during 1964 while Indiana type VS was prevalent in the western part of the state. During 1963, 26 turkeys were tested for VS virus, and none were considered reactors. During 1964, two of 17 turkeys tested reacted; during 1965, 34 of 79 (43 percent) were reactors. New Jersey VS was diagnosed in only one county of Texas during 1964 and in one adjacent Oklahoma county.

Considering the large numbers of mosquitoes collected during VS
epizootics and in VS endemic areas by different workers without isolating VS virus, the two scattered mosquito isolations of VS virus at Trinidad and New Mexico do not support the hypothesis that commonly collected mosquitoes are the main vectors.

Shelokov and other workers\textsuperscript{18,1} in Panama have published two papers. One associates the isolations of Indiana VS virus from \textit{Phlebotomus} and a 25 to 35 percent incidence of Indiana VS antibodies in the human population with their leishmaniasis endemic areas where \textit{Phlebotomus} abound.\textsuperscript{18} Leishmaniasis is transmitted by \textit{Phlebotomus}. Jonkers has a paper in press on VS epidemiology.\textsuperscript{10}

The Cocal virus isolations from mites recovered from rice rats, the isolations from \textit{Phlebotomus}, and the VS antibodies in wild turkeys give added evidence of host-vector cycles in wildlife. Studies by Hanson and his co-workers also gave evidence of wildlife involvement.\textsuperscript{7} If we want to discover this cycle, we must intensify our investigations.

\textbf{Summary}

Very little is known about the epidemiology of vesicular stomatitis. The occurrence of this disease is unpredictable. It appears to be endemic in some areas. Both New Jersey and Indiana types occur in the southern United States, usually starting in spring or summer and spread northward to involve as many as 600 or more herds affecting cattle, horses, swine, and occasionally people. It appears that a host-vector cycles exists in nature.

\textbf{REFERENCES}

A REVIEW OF THE CARRIER STATE IN FOOT-AND-MOUTH DISEASE

PAUL SUTMOLLER, GEORGE E. COTTRAL, and JOHN W. McVICAR

Ever since foot-and-mouth disease (FMD) has been studied, many workers have been convinced that virus carriers are an important factor in the epizootiology of the disease. There has been little agreement, however, on the importance of the role of carriers. One of the first investigators, Burgi, surveyed a number of outbreaks in Switzerland from 1920 to 1927. In his opinion, about 3 percent of recovered cattle remained carriers and excreted FMD virus intermittently for at least 5-6 months and probably up to one year. Others agreed with this. Fluckiger pointed out the possible role of carriers in other species such as goats.

During the severe outbreak of FMD in the United Kingdom in 1922-24, the traditional slaughter policy was partially relaxed to permit retention of 105 infected herds in isolation. Later, some of these animals were introduced into other herds which had never had FMD. The Ministry of Agriculture report of this period states, "At Maghull (near Liverpool) on the 11th August, 1924, in this outbreak, two animals had been purchased from premises where Foot-and-Mouth Disease had occurred about eight months previously and in which the animals were isolated. It is possible, therefore, that in this instance, the disease was introduced by one of these animals which, though making a normal recovery, nevertheless was a "carrier" of infection. The great majority of recovered animals are not infective, but a very small proportion of them is believed to be capable of infecting others. It is believed that this occurs through the release of infective material held in the horn structures of the hoof which, in course of time, allows the escape of infection."

The report of 1925 states, "Seven cattle were found infected out of a total of 15 cattle and 14 pigs. A bull and a heifer were purchased on 9th July, 1924, from certain premises in Cheshire where an outbreak was confirmed in November, 1923, and in which isolation was adopted. During that outbreak both the bull and heifer had passed through an attack of Foot-and-Mouth Disease, the bull recovering in early January, 1924, and the heifer about the middle of January, 1924. It would appear possible, therefore, that one of these animals was a "carrier" of infection. No further cases occurred in this area . . ."

Contrary to this information was the experience at the outbreak of FMD among the cattle at the National Dairy Show in Chicago in 1914. In this instance, some 700 show cattle were quarantined. Approximately 4.5 months after the start of the outbreak, 50 young susceptible cattle were placed in contact with the recovered cattle. Susceptible animals were also inoculated with saliva, feces, urine, vaginal discharge, and scrapings from the interdigital spaces of the recovered animals. Two weeks later, 50 pigs were exposed to and fed on milk from the recovered cows. Approximately 7 months after the start of the outbreak, the recovered cattle were released.
to farms. Neither the experimental exposure nor the later contacts on the farms resulted in the appearance of FMD.

During the last 60 years, there were numerous reports both for and against the theory of the spread of FMD by carriers and excellent reviews were published by Ramon and by Fogedby. However, one example should be cited as circumstantial evidence of carriers being involved in the introduction and recurrence of the disease.

In October, 1945, a consignment of Zebu cattle arrived in Mexico from Brazil where FMD was enzootic. A second consignment of 327 cattle arrived in May, 1946. The cattle were quarantined for several months on an island and then transported to the mainland. Foot-and-mouth disease broke out in November, 1946, on the ranch where the Brazilian cattle were introduced. A recurrence of FMD of the same virus type was found in May, 1953, 22 months after the last known infection was eradicated.

There was no evidence that the disease was reintroduced during that time and it was assumed that the virus was retained in a carrier animal that had recovered from, or had been vaccinated against FMD.

Several experiments have attempted to confirm field evidence that biological carriers of FMD do exist. In 1925-26, a USDA commission studied FMD, including carriers, in Europe. Selected suspect cattle were isolated in contact with susceptible cattle and pigs. After exposure for 50 to 80 days, the suspect carriers were killed and bile and suspensions of hoof material inoculated into susceptible cattle. No clinical FMD resulted.

In another experiment, guinea pigs were inoculated intradermally in the footpad with suspensions of hoof material from cattle recovered from experimental FMD. Lesions of FMD were produced in one guinea pig with material collected from a heifer 34 days after infection (DPI).

In 1931, Waldmann and co-workers reported on the isolation of FMD virus from recovered guinea pigs and cattle. Acetone precipitation was used to concentrate virus from plasma and from organ infusions. Carbon adsorption was used for the concentration of virus from urine. These workers had to rely on guinea pig footpad inoculation for virus detection as it would be another twenty years before unweaned mice or tissue cultures became common tools. They found virus in the plasma of guinea pigs up to 60 DPI and in kidney and urinary bladder up to 94 DPI. Virus was detected in the plasma of cattle up to 58 DPI.

Waldmann's group examined cattle for virus excretion in urine by testing 8 oxen used for producing hyperimmune serum. Previously, virus had been isolated from the blood plasma. Table I summarized the results. Six cattle excreted virus in the urine, and virus was detected more than once in 4. In 1, virus was isolated 5 times from 185 to 246 DPI.

Waldmann and his co-workers observed that virus might lose virulence after persisting in the animal. Most isolates produced distinct vesicles, many with generalization, but others had to be passaged to produce clear lesions of FMD in the guinea pigs.

From 1931-37, British workers attempted to confirm Waldmann's findings. They found that charcoal readily absorbed the virus under experimental conditions, but it was difficult to elute. There is no record of inocu-
lations of guinea pigs with charcoal, as had been done by the German workers. Attempts were then made to recover virus from blood by precipitation with acetone. In their experiments, virus was added to normal guinea pig or cattle serum prior to the acetone precipitation. They could not demonstrate concentration of virus by this method. Apparently no attempt was made to treat the plasma of recovered cattle or guinea pigs.

### TABLE 1. SUMMARY OF THE VIRUS ISOLATION BY WALDMANN, et al. FROM BLOOD PLASMA AND URINE SAMPLES OF 8 STEERS USED FOR THE PREPARATION OF HYPERIMMUNE SERUM

<table>
<thead>
<tr>
<th>STEER NO.</th>
<th>DAYS POSTINFECTION WITH VIRUS ISOLATED FROM BLOOD PLASMA</th>
<th>DAYS POSTINFECTION WITH VIRUS ISOLATED FROM URINE</th>
<th>VIRUS TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1091)</td>
<td>6, 36</td>
<td>185, 209, 223, 230, 246</td>
<td>C</td>
</tr>
<tr>
<td>2 (2746)</td>
<td>27</td>
<td>44, 55, 145</td>
<td>C</td>
</tr>
<tr>
<td>3 (2535)</td>
<td>32</td>
<td>47</td>
<td>C</td>
</tr>
<tr>
<td>4 (2537)</td>
<td>32</td>
<td>41, 47, 66</td>
<td>C</td>
</tr>
<tr>
<td>5 (2811)</td>
<td>58</td>
<td>71</td>
<td>C</td>
</tr>
<tr>
<td>6 (2786)</td>
<td>30</td>
<td>NEG.</td>
<td>C</td>
</tr>
<tr>
<td>7 (2548)</td>
<td>10</td>
<td>18, 24, 35</td>
<td>C</td>
</tr>
<tr>
<td>8 (2944)</td>
<td>17, 71</td>
<td>NEG.</td>
<td>A</td>
</tr>
</tbody>
</table>

These differences in techniques might account for the different findings of the German and British groups.

The British workers did report some interesting results when kieselguhr was used as an absorbing agent in urine. They found that the virus could be absorbed and then later eluted by suitable adjustment of the pH of the system. Unfortunately, they did not attempt to use this method to explore the findings of Waldmann's group. They observed that the urine of cattle recently recovered from infection was acid and "attempts to recover virus from urine as acid as pH 4.8 to 6.0 are futile as the virus is not stable at hydrogen-ion concentrations within these limits." 14 To our knowledge, no further attempts were made to concentrate and isolate virus from late convalescent cattle with neutral or alkaline urine.

Later, the attention of most FMD research workers was largely directed toward the cultivation of the virus in vitro and the development of suitable vaccines. The question of virus carriers remained speculative until 1959
when the pioneer work of van Bekkum and co-workers,\textsuperscript{15} in The Netherlands was published. In a preliminary experiment, pooled samples of urine, of feces, and of saliva collected from experimentally infected cattle were tested by inoculating susceptible cattle. Virus was isolated for several months from saliva, but no indication of the proportion of positive animals could be obtained.

The term "saliva" may need some more explanation at this point: the samples to be tested are obtained from the oral pharynx and anterior portion of the oesophagus with a cup probang. (Fig. 1.) The material obtained con-

![Figure 1. Instrument for the collection of oesophageal-pharyngeal fluid from cattle.](image)

sists of mucus, desquamated epithelium, and often, foot particles diluted with varying amounts of salivary secretion. We prefer to call this material oesophageal-pharyngeal fluid or simply, O-P fluid.

When van Bekkum's group tested a number of cattle in a more systematic manner, using unweaned mice for virus detection, the original findings were confirmed. Virus was recovered from the samples of O-P fluid of some cattle for more than 5 months. Infective O-P fluid was also collected from vaccinated cattle exposed to diseased animals and occasionally carriers were found without clinical signs of the disease.

Five years later, the subject captured the general interest that resulted
in the initiation of research at the Pan-American FMD Center in Rio de Janeiro, Brazil, the Animal Virus Research Institute in Pirbright, England, and the Plum Island Animal Disease Laboratory in the United States of America. In Brazil, Sutmoller and Gaggero isolated FMD virus from approximately 50 per cent of cattle tested 4 and 6 months after an outbreak of FMD on a farm near Rio de Janeiro. Some of the virus strains isolated from these carrier cattle were studied in relation to their virulence for cattle and pigs. The results indicated that virus, after its persistence in cattle, might be capable of starting an epizootic among susceptible animals, especially pigs.

Other work at the Pan American FMD Center was concerned with modified live virus vaccines. Carriers were found among 12-to 16-month-old progeny of cows immunized with rabbit-adapted FMD virus vaccine. These calves had not been vaccinated. The virus isolated from the O-P fluid was of the same serotype as the vaccine strain. Foot-and-mouth disease virus antibody was not demonstrated in the serums of a majority of the carrier cattle.

These findings were confirmed by Auge de Mello and co-workers. Rabbit-adapted type C virus was detected in samples of O-P fluid from vaccinated cattle and unvaccinated contacts up to 180 days postvaccination. Chicken embryo-adapted type A virus was detected at 90 days postvaccination. These data do not represent the endpoint. There did not seem to be a relationship between the absence or presence of antibody to FMD virus, type C, and the recovery of the virus from the O-P fluid. The cattle without significant antibody levels and from which virus had been isolated showed some resistance to contact exposure with type C virus.

The same investigators were able to detect FMD virus in the blood, tonsils, kidneys, pancreas, bone marrow and skin of cattle for 20-60 days after vaccination with modified live virus vaccine.

Burrows, in England, studied the site of virus persistence and multiplication in cattle. He considers the dorsal surface of the soft palate and the pharynx as the chief sites of virus multiplication. Occasionally, virus could be recovered from animals up to 15 months after infection. He also demonstrated that a high percentage of sheep became carriers after exposure to FMD virus, but that virus was not usually detectable 4 months after infection. Assay of postmortem specimens showed that the virus in sheep was present in the epithelium of the pharyngeal and tonsillar regions.

Studies of FMD virus carriers at Plum Island were initiated in 1966. These studies concern virus isolation techniques and factors regarding the virus-host relationship such as the amounts of virus in the O-P fluid of carriers, the state of the virus in the fluid, and the development of both local and circulatory antibody in carriers. Experiments were designed to study the probable routes of infection, the number of infective virus particles needed to establish carriers in both susceptible and immune cattle and the transmission of virus from carrier to contact animals.

Five-liter culture bottles containing a bovine kidney cell monolayer
were used for the detection of minimal amounts of FMD virus in the O-P fluid specimens. These bottles could be inoculated with relatively large amounts of material. Virus recovery was improved by treating the O-P fluid with a fluorocarbon prior to assay. This treatment eliminates bacterial and fungal contaminants and apparently breaks down the union of the virus with antibody or other inhibitors. Usually, there is sufficient infective virus in fluorocarbon-treated O-P fluid of carriers to be detected by standard plaque assay methods.

The results of experiments, which involved the inoculation of both susceptible and immune cattle with variable doses of FMD virus by oral or nasal routes, are recorded schematically in Table 2. Three groups

**TABLE 2. SCHEME OF FMD CARRIER CATTLE DEVELOPMENT**

<table>
<thead>
<tr>
<th>PRE-EXPOSURE STATUS OF CATTLE</th>
<th>REACTION AFTER EXPOSURE (CLINICAL SIGNS)</th>
<th>POST EXPOSURE STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUSCEPTIBLE</td>
<td>LESIONS TONGUE AND FEET, VIREMIA, FEVER</td>
<td>FMD CARRIER</td>
</tr>
<tr>
<td></td>
<td>NO LESIONS, VIREMIA AND FREQUENT FEVER</td>
<td></td>
</tr>
<tr>
<td>PASSIVELY IMMUNIZED</td>
<td>OCCASIONALLY SMALL LOCALIZED LESIONS, SEDOM VIREMIA, NO FEVER</td>
<td>CARRIER</td>
</tr>
<tr>
<td>ACTIVELY IMMUNIZED (VACCINATED)</td>
<td>NONE (NO LESIONS, NO VIREMIA, NO FEVER)</td>
<td>CARRIER</td>
</tr>
</tbody>
</table>

of cattle were studied. They were susceptible animals, animals passively immunized with type specific antiserum, and animals immunized by vaccinating with an inactivated virus vaccine.

Practically all infected cattle became carriers. As expected, there was a great difference in clinical reaction of the cattle according to their immune status. Most susceptible cattle went through a stage of overt disease prior to becoming a carrier. Some susceptible cattle became carriers without
showing signs of vesicular disease, particularly when exposed to minimal amounts of virus. All susceptible cattle that became infected had a viremia and frequently fever. In immunized cattle, the establishment of asymptomatic carriers appeared to be the rule. Only occasionally were small localized lesions observed in the passively immunized group.

These results include two facts of interest to the epidemiologist:

1. Foot-and-mouth disease virus can infect cattle and multiply in the pharynx regardless of their immune status.
2. This infection and multiplication can occur in the complete absence of clinical signs.

Now that carriers can be produced at will, studies will be undertaken to determine the role of the carrier in the epizootiology of the disease. It does seem appropriate at this time, however, to discuss some of the consequences of the newer knowledge of the carrier state in relation to control measures. The control measures used in dealing with FMD depend on the conditions in each area and vary from the stamping-out policy in FMD-free countries to vaccination in countries where the disease is endemic or which are exposed to infection from their neighbors.

**FMD-Free Countries**

These investigations on carriers support the view that stamping out of the disease by slaughter of all infected and contact animals of susceptible species is the most effective way of dealing with FMD outbreaks. They show the wisdom of those who were responsible for establishing and maintaining this policy in this country at times when convalescent animals generally were thought to be safe a few weeks after recovery. Sometimes a vaccine barrier around outbreaks is used in FMD-free countries that for many reasons are not able to apply the slaughter policy to its full extent. It appears from the work at Plum Island that normal-appearing carriers are likely to be established among vaccinates if exposed to virulent virus. This finding means that the animals in the vaccinated areas would have to be kept under quarantine and close observation for, as it appears at this time, an unknown number of years.

The success of stamping out depends largely on early diagnosis. An FMD outbreak in cattle, sheep, or pigs with classical vesicular lesions would attract attention and be easily diagnosed. The difficulty occurs when nontypical lesions appear, which may happen if the virus has very high or very low virulence. An example of high virulence is the recent outbreak in Tierra del Fuego, Argentina, where about 300 of 900 cattle died in a short time. Even though the Argentine authorities have had extensive field experience with FMD, they did not at first recognize this outbreak as FMD. Low virulence may be found in strains of virus that have persisted for long periods of time in animals. Such strains may produce only mild infections in the first passages even in susceptible cattle. It is likely that strains of this kind are the ones with the best chance of being introduced into an FMD-free country. They may then spread unrecognized because of the nontypical clinical signs.
Recognition of the carrier problem greatly complicates the procedures to be followed in movement of susceptible species from endemic areas to FMD-free countries. The usual measures consist of quarantining the animals during which time diagnostic serologic tests are performed. The testing of samples of O-P fluid has been included in the requirements for the importation of cattle from France to Canada. Negative results obtained after testing these samples does not necessarily prove the absence of virus. Work at Plum Island showed that samples which were negative on direct testing contained virulent virus when submitted to a treatment whereby the virus is separated from antibody or growth-inhibitors.

Endemic Areas

In FMD-endemic areas, control measures usually consist of mass vaccination of the cattle population and, in case of outbreaks of the disease, restrictions of movements of animals and animal products. In some European countries, these measures are supplemented by destroying diseased animals and their healthy susceptible contacts, which usually are young cattle or pigs.

At Plum Island, we were able to demonstrate that vaccinated cattle remained clinically healthy after exposure to virus in spite of a subsequent active virus multiplication in their pharynx.24 We also have detected fully virulent virus in droplets produced by the coughing of these animals for as long as one month after the first exposure to the virus.26 It is concluded that movement of such cattle could be a vital factor in the spread of FMD.

The multiplication of the virus in immune animals possibly favors antigenic “drift” of the virus. Workers at Pirbright, England, showed that virus variants could be produced in the laboratory by growing virus in tissue culture in the presence of FMD antiserum27 or by serial passages of the virus by tongue inoculation in partially immune cattle.28,29 Burrows22 found that a distinct antigenic variation in a virus strain occurred between 14 and 17 weeks after the infection of the animal. One can postulate, therefore, that variants or different subtypes could emerge from a vaccinated population in which virus is actively spreading. If such variants are sufficiently different from the prevailing virus, they might break the existing immunity in the population and cause a new wave of clinical infection. The spread of virus by healthy-appearing, but infected carrier cattle might also explain the occasional outbreaks in pigs or unvaccinated young cattle.

At present it is not possible to present a very optimistic picture of the permanent success of the vaccination campaigns now being carried out. Under present conditions it appears that the best that can be achieved is a certain percentage of cattle protected against clinical FMD, but with some virus in permanent circulation within the cattle population. However, after the recognition of a problem, research has often been able to provide new and better tools. For years we have been vaccinating against FMD without really knowing how we were affecting the virus-host-environment relationship. As Wedman30 suggests, “It is time to recognize that environ-
mental factors affect the agent, the transmission, and the host, and that this in turn affects the results, disease or infection." We have probably been thinking in too simple terms, like "when a virus meets a susceptible host, disease is produced, but when a virus meets an immune host, nothing happens."

It is a challenging task for field and laboratory workers to study the factors which determine how the virus perpetuates itself. The outcome of this work may enable us to find the weak links in the chain of virus transmission and may result in more effective action.

ACKNOWLEDGMENT

The library assistance of Mr. B. Balassa and Mrs. F. Demarest is gratefully acknowledged.

From the Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, USDA, Greenport, Long Island, New York 11944.

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Inactivated foot-and-mouth disease (FMD) vaccines in current use in the world today are inactivated, for the most part, with formaldehyde. It has been established that the kinetics of inactivation of FMD virus (FMDV) with formaldehyde deviate considerably from a first order reaction and that the prediction of residual infectious virus in such a vaccine is difficult.10

In 1959, Brown and Crick4 described a new FMDV inactivant, acetyl-ethyleneimine (AEI), which appeared to be superior to formaldehyde. Their original observation was verified by further work5 and work of Fellowes.67,8 They indicated that 0.05 percent by volume of AEI and a reaction temperature of 37°C resulted in rapid inactivation of viral infectivity with preservation of immunizing properties. This report presents some pertinent findings in use of AEI for the preparation of experimental inactivated FMD vaccines over the last five years.

MATERIALS AND METHODS

Virus: Foot-and-mouth disease viruses of immunological classifications A-1, O-2, and C-3 obtained from field cases in Argentina in recent years, and A-119, a British strain, were used in these studies. Virus was obtained from various sources such as primary bovine kidney (BK) cells or baby hamster kidney (BHK-21) cell line grown in tissue culture or vesicular fluid from fresh lesions on infected guinea pig footpads. Some experiments were done with clarified harvest material and others with viruses processed to a high degree of purity by methods previously described.

Infectivity Assay: Infectivity was assayed in primary BK cell tissue cultures by plaque formation of decimally-diluted virus suspensions. Assay cultures were grown in four-ounce prescription bottles and assays performed as previously described.2

Inactivation Procedures: Acetyleneimine was added as 0.05 percent of the volume of the virus suspension under study except where otherwise noted. Suspensions were heated to 37°C in a serological water bath before the inactivant was added with a pipette or microliter syringe. The suspension was thoroughly mixed for 30-45 seconds. A sample was collected at this point and was considered the O-time inactivation sample. Inactivation was continued without stirring at 37°C in a water bath for the appropriate time.

EXPERIMENTAL AND RESULTS

Inactivation Kinetics: The reduction of infectivity with minimal damage to the antigen immunizing properties is the goal of vaccine inactivation. The margin of safety of an inactivated vaccine is dependent upon
the kinetic function of the inactivation reaction and the length of time the reaction is carried out. The parameter of interest (infectivity) can only be assayed within the limits of the sensitivity of the infectivity assay system. This means that the probability of the presence of infectious virus is a predicted value based upon the measurement of the initial portion of the inactivation curve. If this is linear, it is assumed that the reaction proceeds in a linear manner beyond that point at which it can no longer be measured by the assay system. Figure 1 is the graphic function describing the inactivation of FMDV in which samples were taken every 15 minutes from 0 to 240 minutes of inactivation. The reaction is first order at a rate of 0.0215 log plaque forming units (PFU) infectivity loss per minute. If inactivation is continued for 24 hours, under these conditions, a level of infectivity of about $10^{-25}$ PFU per 1-ml vaccine dose is reached.

Based on this study, a routine assay method for use in AEI inactivation of vaccine lots was developed. Four samples were taken, one each at 0, 30, 60, and 90 minutes of inactivation. The samples were diluted decimally and 0.1 ml of each dilution added to each of two prescription bottles. Overlay was added and plaque titer determined at 48 hours. This four-point assay has been programmed for a desk top computer* and Figure 2 shows the computer print-out. The log plaque count and coded time are

*Olivetti-Underwood Programma 101.
The results of Table 1 show a highly significant drop in toxicity.

Table 1: All preparations were injected for intoxication tests by phage assay. After two hours, the volume of each tube 0.5% percent of the volume of each tube. An additional amount of phage was added to each of each pair as

3.7, C. The addition of phage would then increase the amount of phage by 3.7, C.

The following experiment was done to study this point more accurately. The following experiment was done to study this point more accurately.

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Parameter: the drop in toxicity caused by phage addition.

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Parameter: the drop in toxicity caused by phage addition.
of preparations containing AEI but no effect because of presence of varying protein concentrations. This was accepted as an indication that AEI is not noticeably reactive with protein.

Reaction with viral ribonucleic acid (RNA). Studies were done to determine the effect of AEI on infectious viral RNA. Pure FMDV, A-119 was incubated for five hours at 4°C in phosphate buffered saline in the presence and absence of one percent AEI w/v. At the end of the incubation period, sodium thiosulfate was added to a final concentration of two percent

### TABLE I

<table>
<thead>
<tr>
<th>µg BSA</th>
<th>0</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AEI</td>
<td>6.60*</td>
<td>6.73</td>
<td>6.73</td>
<td>6.60</td>
<td>6.73</td>
</tr>
<tr>
<td>AEI...</td>
<td>6.59</td>
<td>6.76</td>
<td>6.74</td>
<td>6.48</td>
<td>6.75</td>
</tr>
<tr>
<td>With</td>
<td>4.08</td>
<td>4.18</td>
<td>3.90</td>
<td>3.70</td>
<td>4.04</td>
</tr>
<tr>
<td>AEI...</td>
<td>3.09</td>
<td>4.23</td>
<td>4.11</td>
<td>3.95</td>
<td>4.23</td>
</tr>
</tbody>
</table>

**Analysis of Variance**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>D. F.</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of AEI</td>
<td>1</td>
<td>36.9920</td>
<td>36.9920†</td>
</tr>
<tr>
<td>Levels of BSA</td>
<td>4</td>
<td>0.4469</td>
<td>0.1117</td>
</tr>
<tr>
<td>Interaction</td>
<td>4</td>
<td>0.1299</td>
<td>0.0324</td>
</tr>
<tr>
<td>Residual</td>
<td>10</td>
<td>0.5706</td>
<td>0.0571</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>38 1394</td>
<td></td>
</tr>
</tbody>
</table>

*Log_{10} PFU/ml.
†p > .01.

w/v to neutralize the AEI, and the RNA extracted with sodium dodecylsulfate (one percent) at pH 5.1 as previously described. Two ml of the RNA preparation was applied to a 30-ml, five- to 25-percent, linear sucrose gradient and centrifuged for 17 hours at 24,000 rpm at 2°C. The gradient buffer was 0.01 M sodium acetate, pH 5.1. Ultraviolet absorption at 260 mμ as the centrifuged gradient passed through a flow cell is shown in Figure 3. There is no significant difference in the absorption profiles of the two preparations, except a change in absorbance at the top of the tube from reduction of sodium thiosulfate concentration as a result of AEI destruction. Virus RNA profiles containing no sodium thiosulfate have no absorbance peak at the top of the tube. It is noted, however, that only the 37 S RNA from untreated virus remained infectious. No plaques
were formed in cultures inoculated with RNA from AEI-treated virus. This indicates that the inactivation takes place at an RNA site not causing an observable change in the structure of the RNA.

Figure 3. Viral RNA profiles before and after treatment with acetyleneimine and sucrose density gradient centrifugation.

Effect on virus mass and size. Analytical ultracentrifuge studies of inactivated purified A-119 FMDV showed a single schlieren peak of 141.9 S (Svedberg units) which is not significantly different from the uninactivated virus. This indicates no significant change in mass or size has occurred from inactivation.
Effect of AEI on serological antigen. Several trials have shown that the complement-fixing (CF) antigen of FMDV is not significantly affected by inactivation of the virus with AEI. Table II shows results of a representative CF test of type O-2 antigen before and after inactivation at 37°C with 0.05 percent AEI. This preparation was inactivated to an estimated $10^{-10}$ PFU/ml. The difference in CF titer is not significant.

This apparent lack of effect on CF antigen is also shown in experiments where cattle have been immunized with AEI-inactivated virus. Vaccine antigen was excellent as evidenced by vaccinated animal antibody response and resistance to infection with virulent virus.9

Other Factors: In addition to the effect of AEI on the vaccine virus, it is advantageous to know the effect of certain environmental variables

<table>
<thead>
<tr>
<th>Antigen Dilution</th>
<th>Cells Lysed</th>
<th>Antigen Dilution</th>
<th>After Inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before 37°C, 24 Hours, 0.05% AEI</td>
<td></td>
<td>After Inactivation</td>
</tr>
<tr>
<td></td>
<td>Inactivation</td>
<td></td>
<td>37°C, 24 Hours, 0.05% AEI</td>
</tr>
<tr>
<td>1/1200</td>
<td>21%</td>
<td>1/1200</td>
<td>26%</td>
</tr>
<tr>
<td>1/1600</td>
<td>54%</td>
<td>1/1600</td>
<td>63%</td>
</tr>
<tr>
<td>1/2000</td>
<td>77%</td>
<td>1/2000</td>
<td>82%</td>
</tr>
<tr>
<td>Computed Titer</td>
<td>1/1550</td>
<td>Computed Titer</td>
<td>1/1450</td>
</tr>
</tbody>
</table>

on the inactivant itself. Two of these were studied. They were comparison of inactivant from different commercial sources and the effect of storage temperature on potency.

Comparison of different lots of AEI. Acetyleneimine from two different manufacturing sources was compared by adding 10 lambda of each of the preparations to two 20-ml portions of the same virus stock preheated to 37°C. Samples were taken from each preparation at 30, 60, and 90 minutes of inactivation and titrated by plaque assay. The results were analyzed by methods described by Bliss° for vitamin potency comparisons. The results are shown in Table III which notes a significant inactivation with both preparations but no difference between the two lots.

Effect of storage temperature on AEI potency. Ordinarily, AEI is stored at temperatures below freezing to prevent polymerization of the compound. The effect of room temperature storage was tested by adding 0.05 percent of AEI stored at 4°C to one portion of a lot of virus and 0.05 percent of the same AEI stored 42 days at 23°C in the dark to an equal portion of the same virus. Table IV notes that, after storage under these conditions, the AEI at 23°C had only 31 percent of the potency of that
TABLE III

COMPARISON OF POTENCY OF TWO DIFFERENT LOTS OF ACETYLETHYLENEIMINE

<table>
<thead>
<tr>
<th>Original virus titer</th>
<th>6.95*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time minutes</td>
<td>30</td>
</tr>
<tr>
<td>Imperial Chemical Industries (ICI)</td>
<td>5.67</td>
</tr>
<tr>
<td>Dow Chemical Co.</td>
<td>5.36</td>
</tr>
</tbody>
</table>

|| SOURCE OF VARIATION                  | D. F. | Sum of Squares | Mean Squares |
|--------------------------------------|-------|----------------|--------------|
| ICI vs. Dow Chemical Co.             | 1     | .0481          | .0481        |
| Slope                                | 1     | 9.8568         | 9.8568†      |
| Parallelism                          | 1     | .0084          | .0084        |
| Curvature                            | 1     | .0266          | .0266        |
| Opposed curvature                    | 1     | .0368          | .0368        |
| Within tests                         | 6     | .0577          | .0093        |
| Total                                | 11    | 10.0344        | . . .         |

*Log_{10} PFU/ml.
†p > .01.

TABLE IV

COMPARISON OF INACTIVATING POTENCY OF ACETYLETHYLENEIMINE (AEI) STORED AT 23°C AND 4°C

<table>
<thead>
<tr>
<th>STORAGE CONDITIONS</th>
<th>Sample</th>
<th>Time of Inactivation</th>
<th>Log PFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C.</td>
<td>30</td>
<td>5.04</td>
<td>5.18*</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3.83</td>
<td>3.76</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>3.00</td>
<td>3.20</td>
</tr>
<tr>
<td>23°C.</td>
<td>30</td>
<td>6.36</td>
<td>6.40</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5.78</td>
<td>5.73</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>5.58</td>
<td>5.48</td>
</tr>
</tbody>
</table>

*Log_{10} PFU/ml.
4°C Y = 6.74 - 1.32 X.
23°C Y = 6.74 - .42 X.

Potency of room temperature relative to 4°C.

\[ J_1 = \frac{b_2}{b_1} = .31 \text{ or } 31\% \text{ of activity of AEI at 4°C.} \]
FOOT-AND-MOUTH VACCINES

stored at 4°C. No difference between AEI stored at 4°C and -20°C has been observed.

CONCLUSIONS

Acetyleneimine fills the criteria of a perfect vaccine virus inactivant. Infectivity is inactivated rapidly by first order reaction with apparently no damage to the serological or immunizing antigen. Small amounts of inactivant are required. It is a simple compound with the slight disadvantage that storage at temperatures above 4°C will result in slow polymerizations and loss of inactivating potency. Its removal from the vaccine mixture is readily achieved by neutralization with sodium thiosulfate. All considered, AEI is a vast improvement over formaldehyde in the preparation of FMD vaccines.

From the Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Greenport, New York 11944.

REFERENCES

VESICULAR DISEASE COMMITTEE

1967 Report


The vesicular disease committee reports as published in the proceedings of the United States Livestock Sanitary Association provide a continuing summary on the status of vesicular diseases in the United States and in other parts of the world and on vesicular disease research. Since there was no committee report in 1966, the 1967 report will include information for that year.

FOOT AND MOUTH DISEASE

The numerous epizootics and multiplicity of virus types of foot-and-mouth disease (FMD) during 1965 and 1966 created considerable concern throughout the world. Several of the affected countries increased their efforts to control the disease. In several countries massive application of vaccines and strict sanitary measures have helped to reduce the impact and spread of the disease.

The buffer zone established in Thrace to prevent the spread of SAT-1 and Type A_22 from Turkey into Europe continued to be effective. Type SAT_1 has not been reported in the Thrace area since October 1963, and Type A_22 has not been found in the Thrace buffer zone since November 1965. However, the widespread appearance of Type A_22 in the U.S.S.R. posed a new threat of this subtype gaining entry into the livestock population of Central Europe. Romania obtained Type A_22 from the U.S.S.R. and prepared a vaccine which was used to provide a buffer zone of vaccinated animals along her border with the U.S.S.R.

FMD in swine continues to be a major obstacle in attempts to control and eradicate the disease. The control of FMD in swine is more difficult than in cattle because of the high exposure risk from feeding garbage, lack of an effective vaccine, and probably other factors as yet undefined.

Further study of the FMD carrier problem associated with both vaccinated and unvaccinated animals is providing data useful in evaluating the epizootiology of FMD and in developing measures to aid in prevention of the spread of FMD.

The vaccination and systematic slaughter program established in France in 1961 has served to reduce the incidence of FMD in that country. However, between January 1966 and the end of February 1967, two outbreaks were reported. The first involving primarily swine, occurred in early 1966 and was due to virus type C. Nineteen establishments in six departments were involved. The second outbreak occurred in late 1966 and...
extended into 1967. The virus type was O₁ and it affected primarily cattle. Sporadic cases of FMD have been reported in France during 1967; however, information regarding types of virus and extent of disease is not available.

Great Britain, after a period of four years with only one outbreak of FMD, experienced an outbreak of Type O₁ during July 1966, in Northumberland. The initial investigation disclosed necrotic mouth lesions with an offensive smell, a few foot lesions, and no vesicles. Initially, tissue submitted to the laboratory was negative for foot-and-mouth disease virus on the complement fixation (CF) test. Three days later additional animals were observed to be salivating and lame.

Additionally, a similar condition was reported from several other farms. Investigations revealed the presence of FMD. It has been theorized that some other disease condition was present along with FMD. This may account for the initial lesions being inconsistent with those usually produced by FMD. Thirty-two outbreaks were recorded with the last case occurring on September 5, 1966. A total of 5,753 cattle, 38,445 sheep, and 714 pigs were slaughtered.

On September 28, 1966, FMD was confirmed in a herd in Sussex. A second herd 8 miles away was also involved. This outbreak was due to virus type A₂₂. A total of 211 cattle, 159 sheep, 2 goats, and 4 pigs were destroyed. The origin of the infection was not determined. FMD virus type O₁ was confirmed in Hampshire on January 6, 1967. The disease spread to 29 farms, resulting in the slaughter of 7,902 animals.

The most recent outbreak in England occurred in September 1967 at Hampton Lucy Warwick. Details on this outbreak are not available.

The Netherlands experienced an epizootic of FMD Type C in the first half of 1966. A brief quiescent period followed until Type O appeared in November 1966. Over 1,500,000 pigs were vaccinated, and while the immunity was of short duration it was sufficiently long to stem the outbreak. The outbreak continued into April 1967. It was noted during this outbreak that immunization against Type O₁ was apparently not so effective as immunization against Type C.

West Germany, by decree of 12 December 1966, made compulsory the annual trivalent vaccination of all cattle over six weeks of age. It appears that the situation in West Germany has improved. In 1965, 15,952 outbreaks due to types O, A and C were reported in the first half of 1966; however, Type O predominated in the latter half of 1966 and early 1967.

Sweden reported a single outbreak of FMD on March 6, 1966, the first since 1960. The 23 cattle and 43 pigs affected were slaughtered and over 7,000 cattle in the area were vaccinated. No further outbreaks have been reported.

Switzerland experienced a severe epizootic during the winter of 1965-66, followed by sporadic outbreaks until June 1966. In December 1966, four outbreaks due to Type O₁ were reported. As a result of these epizootics it has been decided to make vaccination compulsory for the year 1967.

The incidence of FMD in Italy has steadily declined as the systematic vaccination program expands. This program was started in 1964 in 14
provinces of the Po Valley, extended to 29 northern provinces in 1965 and further expanded in 1966. During this period the recorded outbreaks have dropped from 12,364 in 1964 to 5,842 in 1965 and 1,548 in 1966. Only 169 outbreaks were reported during the first six months of 1967.

In Spain the incidence of FMD increased during 1966 and 1967. Twenty-nine outbreaks were reported in 1966, primarily due to Type O. During the first two months of 1967, 62 outbreaks were reported. Both Type O and Type C were identified.

Portugal reported only five establishments infected with FMD during the first eleven months of 1966. However, in December the disease appeared in twelve establishments and continued to spread. By March 1967, the disease had appeared in nine of the 18 districts in the country. Virus Types C and O have been identified. There is no slaughter or vaccination program in Portugal.

Greece recorded one outbreak of virus Type A_{22} in June of 1966 and outbreaks of virus Type O in March and September, 1967. The type A_{22} outbreak was in a piggery only a few hundred meters from the Greco-Turkish border. The animals in the region had been vaccinated against types A_{22}, SAT 1 and O two months earlier during the buffer zone vaccination program.

During 1966, Turkey had 819 outbreaks of FMD due to virus Type A_{22} and O. This was a 79.3% decrease from 1965. Type O was more prevalent during 1966.

CENTRAL AND SOUTH AMERICA

Foot-and-mouth disease continues to be a serious problem in South America. Types A and O FMD occurred in Colombia, Venezuela, and Ecuador. The other countries are infected with types A, O and C. In some areas vaccine is applied as a control measure. Venezuela and Ecuador are using modified-live virus vaccines and the others are using inactivated products. In recent years, Foot-and-mouth disease has not been reported from the Guianas, Caribbean area, and Central America. Colombia experienced the first outbreak of type C virus in Leticia, in the south of the country. It appears that stamping out action resulted in its eradication.

In early September 1967, an outbreak of Type A FMD in Colombia, within 20 kilometers of the Panamanian border, aroused a great deal of concern in the FMD-free countries to the north. Previously, a barrier zone maintained in Colombia (Department of Choco) along the Panamanian border provided a measure of protection to prevent the spread of FMD into Central America.

The Regional International Organization for Animal and Plant Health (OIRSA) and the Panamanian Government have strengthened their surveillance programs in an effort to prevent the spread of FMD from Colombia into Panama.

In 1966 a new subtype of virus type O appeared in Venezuela. The outbreak which resulted could not be controlled until the new subtype was incorporated into the vaccine. The state of Bolivar was free of the
disease until an outbreak of type A virus occurred near the Brazilian border. This outbreak was controlled by stamping out. FMD in this area is of concern because of the nearness to the Guianas which are reportedly free of the disease.

Several countries were affected with type O virus. A severe outbreak was experienced in Ecuador where the disease reached the cattle breeding area of the Quito Valley. This virus type was also responsible for an extensive outbreak in the Central Zone of Chile.

The situation in Argentina continues to cause considerable concern. An outbreak of type O infection occurred at the Palermo National Cattle Show near Buenos Aires. On Rio Grande Island, National Territory of Tierra del Fuego, Argentina, a ranch populated with approximately 42,000 sheep, 1,000 cattle, 250 horses, and 30 hogs experienced an unusual FMD outbreak in December 1966. In a few days' time, Type C virus caused approximately 300 deaths among the cattle. The sheep and hogs were not affected. The initial investigation attributed the deaths to a "deep disorder of the myocardium." A few animals exhibited erosions on the tongue; however, typical lesions in the mouth or on the feet were not observed. All cattle and swine on the ranch were slaughtered and buried.

The unusual symptomatology of both the English outbreak at Northumberland and the Tierra del Fuego outbreak at the Southern tip of South America emphasized the necessity to consider the possible presence of FMD, even though typical lesions are not seen.

Vesicular stomatitis outbreaks caused by New Jersey and Indiana virus types occurred in various Latin American countries: Mexico, Nicaragua, El Salvador, Costa Rica, Panama, Guatemala, Venezuela, Colombia, Ecuador, Peru, Trinidad, Brazil and Argentina.

**MEXICO**

The last outbreak of foot-and-mouth disease experienced by Mexico was eradicated by a joint Mexico-United States Commission on April 21, 1954. Since that date the joint commission has continued to maintain surveillance of vesicular conditions in the country. Through surveillance it has been possible to determine the existence of New Jersey and Indiana types

<table>
<thead>
<tr>
<th>Total No. of Investigations</th>
<th>Total No. of Samples Processed</th>
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</tr>
<tr>
<td>32</td>
<td>1967*— 87</td>
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*Covers period January 1-October 20, 1967.*
of vesicular stomatitis in certain areas of Mexico. Suspected field samples are tested at the National Livestock Research Institute at Palo Alto, D. F. (Instituto Nacional de Investigaciones Pecuarias) to confirm the diagnosis.

From 1961-66 there was a steady increase each year in the number of confirmed cases of vesicular stomatitis. A sharp drop in incidence took place in 1967. Trends for this period of years are reflected in the following chart. Positive diagnosis is based on CF tests with epithelial material or serum.

New Jersey type stomatitis was found in 8 states during 1967 and Indiana type in two states. Eleven tissue samples processed were positive for VSV and 17 serum samples were positive for VSV.

**VESICULAR STOMATITIS DISTRIBUTION IN THE UNITED STATES DURING 1966**

The 1964 Indiana Vesicular Stomatitis (VS) outbreak involved Texas and eastern Colorado, and the 1965 outbreak involved New Mexico and western Colorado. The 1966 New Jersey VS outbreak covered the same general areas infected in both the 1964 and 1965 outbreaks of Indiana VS.

New Jersey VS was diagnosed in Webb County, Texas during January, 1966 in horses. In mid April, New Jersey VS was diagnosed in Webb and four adjacent counties bordering the Rio Grande River. Subsequently, VS spread northward in Texas to include 409 herds in 72 counties.

Indian type VS was diagnosed by complement fixation with vesicular epithelium from Grant County, New Mexico and Mesa County, Colorado. Complement-fixing Indian type specific equine serums obtained from San Juan County, New Mexico and El Paso County, Colorado also indicated a recent Indiana VS infection.

In some counties only Indiana type serum neutralizing antibodies were detected as indicated in the accompanying chart. Indiana VS had been diagnosed in these counties within the past two years. Complement-fixing antibodies or an increase in serum neutralizing antibodies must be demonstrated before the time of infection can be estimated.

Thirty-two cross-type serum reactions occurred, 25 equine and 7 bovine. Usually, the serums giving cross type CF reactions also neutralize both types of virus in the neutralization test. A high percentage of the cross type reactions occurred in New Mexico and Colorado where both types of virus occurred during 1966 and where Indiana infection had been diagnosed during 1964 and 1965.

Although the Southeast coastal states were the original endemic area for New Jersey VS, very little has been observed in this area since the extensive outbreak in 1963. South Carolina was the only east coast state with a positive case during 1966. Nine cases of New Jersey VS were diagnosed in Mississippi and Arkansas. Oklahoma and Utah had a few cases adjacent to the infected areas of Texas and Colorado.

*Cases confirmed by Animal Health Division, Diagnostic Services at the National Animal Disease Laboratory, Ames, Iowa.*
The outbreak dates and county distribution of confirmed cases of VS during 1966 are tabulated on the accompanying charts.

VESICULAR STOMATITIS DISTRIBUTION IN THE UNITED STATES JAN. 1 TO OCT. 12, 1967

During the first nine months of 1967, 65 investigations of suspected vesicular conditions were conducted in 15 states. Only one case was confirmed as New Jersey VS. Diagnosis was confirmed at the Plum Island Animal Disease Laboratory, Agriculture Research Service, U. S. Department of Agriculture. This case occurred in a swine feeding operation in Catahoula County, Louisiana. Thirty-three 6 month old pigs out of 452 were affected. Lesions were observed on the snout, lips, and coronary band. In some cases the claw had sloughed. It was interesting to note that unruptured vesicles were not observed. Complement fixation tests were conducted on tissue and serum harvested from the affected animals. Positive fixation was obtained using the tissue specimen with New Jersey vesicular stomatitis virus antiserum. Additionally, a serum sample was shown to contain complement fixing antibodies against VSV type New Jersey. Continual surveillance of the herd failed to reveal additional spread.

Serum neutralizing antibodies have been demonstrated on serum specimens submitted from 8 investigations during this period. The incidence of VS in the United States during 1967 is the lowest since 1960. The incidence since 1960 has increased with a peak in 1966. No doubt several factors have influenced this incidence pattern; however, the fact remains that a general increase has occurred followed by a sudden decline. Many theories have been advanced regarding the epizootiology of VS. Factors such as weather, insects, and vegetation are believe to influence the incidence of VS; however, the epizootiology remains unsolved.

RESEARCH VESICULAR STOMATITIS VIRUS

The virus of vesicular stomatitis (VS) has been widely studied by several groups and there is general agreement that the characteristic infective component is bullet shaped about 160 millimicrons long and 60 to 70 millimicrons in diameter with one flat and one round end. Little attention has been given to a variety of other particle forms which have often been interpreted as artifacts. Following further tests it has been concluded that the virus of VS is a system of several distinct forms and that we are unable at present to identify any one of these as the only primary particle or the only infective component. These particle forms are probably released from the cell in distributions determined by cultural conditions and the origin and state of the cell. (Bradish and Kirkham). These studies were continued by Warrington, and his data showed that an infective component with a sedimentation coefficient of 500-S, significantly smaller than that of the main component, 625-S, is present in VS virus (VSV) harvested from chicken embryos and in baby hamster kidney cells (BHK). When VSV of chick embryo origin was x-rayed and passaged in BHK cells, a new
fast sedimenting main component about 750-S was produced. Such velocity sedimentation studies were conducted in sucrose density gradients. The 750-S component which followed after x-ray treatment, was found to be more resistant to heat inactivation at 37°C than either the 625-S or 500-S components. A 625-S component was neutralized more efficiently with specific antiserum than either of the other two components. Further critical analysis of these various fractions when purified by plaque isolation techniques should provide answers to these questions.

VSV has been shown to be a ribonucleic acid (RNA) virus. This information has been developed by chemical analysis of 32-P-labeled virus and by demonstrating unimpaired replication in the presence of desoxyribonucleic acid (DNA) inhibitors.\(^3\)\(^,\)\(^4\)

Brown and Cartwright\(^5\) have studied the immunogenicity of VSV particles. It was not possible to completely separate the antigens thus it has not been possible to decide whether all are immunogenic. A direct comparison in the electron microscope of the antigens has also so far not been possible. At the present time, it is thought that the various antigens are in fact immunogenic. The size of two antigens has been estimated by measuring their rates of sedimentation in sucrose gradients and observing their elution pattern in Sephadex G-200. The larger of the two antigens has an S value intermediate between those of 7-S gamma globulin and bovine plasma albumin. The smaller antigen sediments more slowly than albumin.

Evidence has also been presented by Brown et al.,\(^6\) that controlled disruption of the virion of VSV can lead to an infective viral substructure. Following such treatment it seems unlikely that the infectivity is due to residual traces of virion because the rate of absorption of the infectivity component to BHK cell monolayers is much slower than of the virion. In addition, the infectivity of BHK cells is about 1% as great as it is for mice, whereas the virion is equally as infective for both cells and mice. The substructure is also not neutralized by viral antiserum. The infective substructure also has the same physical characteristic as the complete virus. As the substructure contains most of the RNA of the virion its low infectivity is likely due to the removal of specific attachment sites by the Tween-ether treatment. Electron microscopy shows that the infective component has the same overall bullet like structure as the virion but lacks the outer envelope and fringe structure.

Federer et al.\(^7\) have also studied the serological relationship between some strains of the Indiana sera type. These workers examined four strains of virus identified as VSV some confirming the occurrence for the first time of outbreaks of the disease in Argentina and Brazil. They examined the virus by plaque assay count in BHK cells and by intracerebral inoculation of young mice, titration in cattle, and in pigs. The order of virulence of the four strains for cattle was Indiana classical (C) Brazil, Argentina, and Cocal, and for pigs, Indiana C and Cocal. Complement-fixation tests and virus neutralization tests demonstrated that Argentina and Cocal strains could not be differentiated antigenically one from the other but each differed from the Indiana C strain in the same degree as the Brazil
strain. It was also indicated that the degree of antigenic difference within the VSV Indiana sera type was similar to that found within the groups of FMDV. These authors propose that the three antigenic groups represented by these strains be referred to as Indiana subtypes 1 (Indiana C), 2 (Cocal and Argentina), and 3 (Brazil).

The interest in the virus of VS was stimulated by the recovery from the field of a number of strains related more or less distantly to the classic sera type Indiana but quite distinct from the other sera type New Jersey. One of these strains named Cocal virus was isolated by Jonkers et al. from mites collected from rats trapped in Trinidad and also near Belem, Brazil. In neither case was it associated with clinical stomatitis, however, strain specific antibodies were found in equine animals in Trinidad.

Thormar compared with the Cocal virus with VSV classical types New Jersey and Indiana with respect to the effect of temperature on virus multiplication and plaque formation in cell monolayers. He also studied the rates of inactivation by visible light and showed that Cocal virus was not particularly sensitive to visible light while the classical VS viruses are known to be light sensitive. He also cloned VS viruses at low temperatures and found this to be a useful method for selecting heat sensitive variants which were found to be less infective for their natural host although they retain their immunological characteristics.

The ultrastructure of the new isolate of type Indiana, Cocal, and New Jersey sera types of VSV were studied by Bergold and Munz. No obvious differences in the morphological structure except slight dimensional variations could be found. The electron microscopic investigation revealed that the VSV particles consist of an outer looped helix, an inner beaded helix which probably represents the capsomers, and a solid main central core. This finding together with the relationship reported above by Federer et al. probably indicates that we should retain the two distinct sera types of VSV, New Jersey and Indiana, but recognize among the Indiana sera type, three subsera types which Federer et al. have suggested be called sera types 1, 2, and 3. The ultrastructure findings by Bergold and Munz seem to support the serological findings by Federer et al.

It is also significant to note VSV was isolated from a pool of mosquitoes collected during 1965 in New Mexico. This is the first isolation of VSV to be reported in mosquitoes which were not experimentally infected. This isolation of the virus from the mosquitoes was significant from the standpoint that they may play an important role in the epizootiology of this disease.

In 1965, there was an outbreak of VS in New Mexico and southwest Colorado. The etiology was confirmed as VSV Indiana type. At the same time there were unconfirmed reports of VS infections in humans in late July. These were among individuals who had some degree of contact with infected animals. Results of the study demonstrated that nine of 41 examined had serological evidence of infection of VSV. Seven of these nine persons had experienced clinical illness, the other 2 were asymptomatic.
### DISTRIBUTION OF LABORATORY CONFIRMED VESICULAR STOMATITIS HERDS

**January 1–October 6, 1966**

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*Cross-type reactions obtained with serum.
†Serum Neutralization test.
**TOTALS OF VESICULAR STOMATITIS INFECTED HERDS AND COUNTIES**

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*Cross type VS reactions included with New Jersey VSV as an indication of total incidence.
†In two of these counties there were SN antibodies only.

**VESICULAR EXANTHEMA VIRUS**

The virus of vesicular exanthema of swine (VES) has been characterized by the application of biophysical and biochemical methods. The virus was partially purified by use of differential centrifugation, cesium chloride density gradients, ribonuclease, desoxyribonuclease, and trypsin treatment together with chromatography on Sephadex columns. Virus prepared in this way was shown to contain 20 to 24% ribonucleic acid.

The sedimentation rate of VESV is 207-S compared with a reported sedimentation rate of polio virus of 160-S. The diameter of VESV is estimated to be 35 millimicrons from studies using phosphotungstic acid preparations examined in the electron microscope. VESV does not fit well into any of the previously described virus classifications. The enterovirus group is the most similar one but the size, sedimentation rate, lack of cationic stabilization, and percent RNA of VESV are different from those of the enterovirus group. The hypothesis is advanced that VESV is a member of a previously undescribed group of RNA viruses.

Several types of VESV belonging to the same antigenic group of virus were studied for mutational changes in swine, pig and canine cell cultures. The plaque types differed in virulence and neutralization tests revealed that only animals which showed frank disease developed specific viral antibodies. The inoculated type of virus was generally mixed with other types after recovery from the host. The contaminating new types were not the same for the different plaque type inoculations. Similar changes were observed in cell systems. Since all inocula consisted of virus from single cell yields which were clonal in origin, these authors concluded that mutational changes occurred in vivo and in vitro. They also warned that mutations together with the requisite selective courses could lead to an erroneous classification of a variant in regard to virulence.
VESV is known to produce frank disease only in swine. The virus has been shown to replicate only in cells of porcine or canine origin. Jenkins, Hackett, Madin and Schaffer\textsuperscript{15} from the University of California, Berkeley, have shown that the viruses of VES will propagate in an established line of African green monkey kidney cells. This is the first indication that this virus will propagate in primate cells. These authors did not report on the infectivity spectrum of the virus propagated in this system. This would, however, be of considerable interest.

FOOT-AND-MOUTH DISEASE

Since the time of the last Vesicular Disease Committee report, there have been instances where foot-and-mouth disease (FMD) in man have been reported. Probably the best described and illustrated of these is by Pilz and Garb in Germany.\textsuperscript{16}

The disease has also been recently reported in man in England\textsuperscript{17} and in Denmark.\textsuperscript{18} The disease which occurred in England is also being reported at this meeting.

Since the time of the last Committee report, two articles on the transmission of air-borne nature of FMD virus (FMDV) have been published. The virus transmits frequently by direct or indirect contact but there is some information to suggest an animal may be infected occasionally by air-borne virus. Danish investigators\textsuperscript{19} have attributed outbreaks on the islands of Denmark during periods of strong winds to air-borne spreads from Germany. In these instances, however, it has not been possible to exclude other sources of spread of the infection. In work conducted in England and reported by Hyslop,\textsuperscript{20} air in animal boxes housing experimentally infected cattle was sampled. The virus collected from the air was concentrated by absorption and detected by intraperitoneal inoculation into unweaned mice. From the studies it was concluded that airborne spread of virus may possibly occur under natural conditions in the field.

In studies at the Plum Island Animal Disease Laboratory, McKercher \textit{et al.}\textsuperscript{21} conducted experiments with susceptible cattle which were exposed to air from an area where experimentally infected cattle had been held. In both of the studies the susceptible controlled cattle developed signs of FMD 4-7 days following the time they were exposed to the air from the contaminated areas. These two experiments, the one in Britain and the one in the U.S., add to the evidence that under certain conditions air may serve as a vehicle for transmission of FMD at least under experimental conditions.

During recent years, much experimental evidence has been advanced concerning the existence of a carrier state in cattle following exposure to FMDV. As early as 1931, Waldmann\textsuperscript{22} showed that cattle could become biological carriers of the virus for an extended period of time following recovery. In this work he demonstrated that urine collected from cattle which had recovered from FMD infection and which appeared to be normal, contained virus for as long as 246 days post-inoculation. van Bekkum \textit{et al.}\textsuperscript{23} reported that virus could be detected regularly in saliva
from a large proportion of cattle over a period of 7 months after recovery from clinical disease. These findings were confirmed by Sutmoller et al.\textsuperscript{24} in 1965. Both van Bekkum and Sutmoller used similar techniques for recovery of virus from field outbreaks. When vesicular material was no longer available they used a sampling instrument to collect fluid specimens from the upper part of the esophagus and pharynx. These studies have been continued and confirmed at Pirbright by Burrows.\textsuperscript{25,26} He found that a carrier state persisted in a majority of animals following infection. The sites of virus persistence have been identified as being in the dorsal surface of the soft palate and the pharynx. The group at the Pirbright laboratory recently reported that the virus may persist as long as 18 months following infection. Studies on this subject are continuing in various laboratories and the information which may be developed during the next few years should contribute substantially to the knowledge relative to the epizootiology of FMD. A paper "Carriers of Foot-and-Mouth Disease" by P. Sutmoller et al. will appear elsewhere in these proceedings.

The subject of immunization of pigs against FMD has been a perplexing problem for many years. During recent years, type C infection in swine has occurred rather frequently in some places in Europe especially The Netherlands and Belgium. The 1965 epizootics in swine in The Netherlands were particularly serious and resulted in pigs being vaccinated. In some instances, there was not sufficient time to use the vaccine especially compounded for swine. In these instances vaccine prepared for cattle was used. Even though the vaccine had been compounded for use in cattle, it, along with sanitary measures stopped the spread of infection. The serious epizootic of the disease in pigs has stimulated many researchers to investigate the problem of a more suitable vaccine for this purpose. Nathans\textsuperscript{27} reported on such a vaccine in 1965. He concluded that the resistance produced in the pig as the result of this vaccine was of limited duration. About 4 years ago, Cunliffe and Graves\textsuperscript{28} at the Plum Island Animal Disease Laboratory, reported on studies of an FMD vaccine in cattle which appeared to be a better immunizing agent than those heretofore reported. This vaccine was different in that the adjuvant was an oil or incomplete Freund adjuvant. Pigs vaccinated with this type of product appeared to be immune for a longer period than those receiving a conventional vaccine. These studies have been continued and recently McKercher\textsuperscript{29,30} reported on tests in larger numbers of animals. His findings have corroborated and extended those previously reported by Cunliffe and Graves.

Several years ago, British investigators from the Pirbright laboratory\textsuperscript{31} reported on the susceptibility of BHK cells to FMDV. During recent years, this work has been extended and these same investigators now report on the growth of cells in deep suspension cultures. BHK cells are being used in a number of other laboratories for the production of FMDV for research purposes as well as for the production of FMDV for compounding into vaccine.

The complexity of the typing problem with reference to FMD may be exemplified by information released by the Typing Laboratory at
They revised their list of sub-type reference strains of FMDV and in this show that at the present time there are 10 subtypes of type O virus, 22 of A, 2 of C, 7 of SAT-1, 3 of SAT-2, 4 of SAT-3, and 2 of type Asia-1. Thus of the 7 immunologic types of FMDV, 50 subtypes have been identified.

Breese and Graves\textsuperscript{33} at the Plum Island Animal Disease Laboratory have studied the size and shape of the FMDV particle by using special staining techniques coupled with electron microscopy. On the basis of a rotation technique and comparison of virus images and models, FMDV has been classified as a 32 unit virus.

Since the last USLSA Vesicular Diseases Committee report, the USDA amended the Federal regulations\textsuperscript{34} to permit importation of animal semen and to make it possible for additional germ plasm to be imported into the U. S.

Basic research on the virus, viral components, and antibodies have continued at the PIADL. In studies by Graves et al.,\textsuperscript{35} two types of neutralizing antibodies with different chemical, physical, and complement fixing properties, are found after inoculation of guinea pigs with an inactivated FMDV. One antibody is present during the first and second week after inoculation and is of the 19-S class sedimentation. The other appears during the second week after inoculation and continues in excess of 30 days and is of the 7-S class sedimentation. These antibodies have markedly different properties because of which it has been concluded that quantitative studies of neutralizing antibodies to assay antigens, should not be done during the first few weeks after inoculation. This points out that the time of collecting the serum samples for assay may conceivably influence the interpretation of potency determinations of vaccines. Cowan et al.\textsuperscript{36} and Polatnick\textsuperscript{37} have also studied the apparent non-viral but infection-associated antigen in FMD. This has been referred to as a third antigenic material produced upon infection with FMDV. These studies indicate that the antigen is less dense than the 23 mu particle and it is antigenically distinct from both the 23 and 7 mu particles but is not type specific. This work has concluded that it is not a virus component. Basic studies on these various antibody components are continuing.

Bachrach and Polatnick\textsuperscript{38} have studied the amino acid composition by analysis of three immunological types of FMDV which were produced in tissue cultures. They found significant differences in several of the 18 amino acids of FMDV. They studied the immunologic types A, O, and C which had been passaged only a few times in tissue cultures. In addition, low passage A-119 virus differed from high passage A-119 virus in the three amino acids glycine, tryptophan, and tyrosine. The differences were correlated with previous findings concerning virulence, electrophoretic mobility, and degradation temperatures. These basic studies on further differentiation and identification of the amino acids in the various types and sub-types of FMDV should provide significant basic information about the virus.

Evidence of the existence of different subtypes of virus within the seven principal types of FMDV has been known for many years. Fagg and Hyslop\textsuperscript{39} (1966) recently reported on the isolation of an immunologic-
ally distinct variant strain of virus which evolved when the strain was passed serially through 34 partially FMD resistant cattle. The variant which evolved was different from the other subtype strains of this serological type of the virus against which it was tested. These observations provide experimental support for theories on the mechanism by which new subtypes arise during outbreaks of FMD in the field.

For the first time in many years, FMD occurred on the Argentine side of the Island of Tierra del Fuego. The virus responsible for the outbreak was identified as type C. Approximately \( \frac{3}{4} \) of the cattle on the one ranch where the disease occurred were infected and \( \frac{1}{2} \) of the infected cattle died as the result of coronary lesions attributed to the virus of FMD. The high mortality in this instance in cattle which are fully susceptible serves as a reminder of what FMDV can do when it is introduced into a fully susceptible population.\(^{40}\)

**Recommendations:**

I. The committee recommends that the U.S. Department of Agriculture ask the Congress for funds to study the epidemiology of vesicular stomatitis. This recommendation is based on the following facts:

1. Vesicular stomatitis is recognized as a public health problem. Human cases occur during epizootics in livestock.
2. The reservoir or reservoirs of VS are not known.
3. The method or methods of spread of VS are not known.
4. In individual herds, particularly dairy cattle, loss from the disease may be devastating.
5. Several countries will not accept United States cattle because VS is known to occur in this country.
6. It is the judgement of the committee that full time studies extending over a period of at least three years must be conducted if losses from vesicular stomatitis are to be reduced.
7. Vesicular stomatitis usually occurs in a cyclic pattern and no state suffers annual losses therefore individual states are reluctant to utilize their research facilities to study the disease.
8. Vesicular stomatitis is clinically indistinguishable from foot-and-mouth disease. Whenever vesicular lesions are found they must be considered suspicious of foot-and-mouth disease and a differential diagnosis secured.

II. The committee recommends that the U.S. Department of Agriculture adopt a specific policy concerning the use of the probang test.

**Background:**

The "probang" test is one of many procedures which may be used to help determine the foot-and-mouth disease status of animals. Conduct of the test involves collection of throat and esophageal fluid preferably containing mucous, cellular debris and a minimum of saliva with the objective of isolation of virus from the collected material. Actual
isolation of virus from such material may be accomplished in a number of ways such as inoculation of test animals or inoculation of tissue culture.

Demonstration of the virus in such a sample is positive evidence that the virus was present in the body of the tested animal when the test material was collected. Failure to demonstrate virus in a particular specimen does not assure that the animal had not been exposed to FMD virus, virus was not present in the tested animals body, and further does not assure that subsequent tests would also be negative. Therefore, the committee recommends that the USDA adopt the following policy:

1. A positive probang test is conclusive evidence that the tested animal has been exposed to, and is harboring the virus and is sufficient cause for the animal to be classed as an FMD virus carrier for purposes of regulatory action.

2. A negative probang test does not assure that the tested animal had never been exposed to FMD virus or is free of FMD virus. For that reason, such negative tests must be supported by other evidence if the animal involved is to be considered free from FMD.

REFERENCES


34. Graves, J.H., Cowan, K.M., Trautman, R.: Characterization of Antibodies Produced


REPORT OF DELEGATES TO THE NATIONAL ASSOCIATION OF STATE DEPARTMENTS OF AGRICULTURE


This report covers the NASDA meetings held in Honolulu in November, 1966 and in Atlanta earlier this month.

In addition, the Board of Directors of NASDA, in April of 1967, published revised procedural guide lines designed to coordinate the work of a number of affiliated or related groups, including the U.S.L.S.A., with that of NASDA. Of particular concern was the need and desirability of a unified approach to federal agencies and the Congress.

Copies of the guide lines have been distributed to members of the executive committee. Your NASDA delegates are of the opinion that a close working relationship between NASDA and the U.S.L.S.A. is in the best interests of effective handling of national animal disease problems. It is recommended that the Board of Directors of the United States Livestock Sanitary Association give early consideration to this matter.

Dr. John G. Milligan and Dr. Ernest H. Willers attended the 1966 NASDA meeting at which the following resolutions pertaining to animal health were adopted.

1966 RESOLUTION NO. 1

Animal Disease Reporting System

WHEREAS, the livestock industry’s need for a meaningful and feasible animal disease reporting system has been discussed for many years; and

WHEREAS, little has been accomplished as to realization of such a system due to two factors: (1) An economically compelling reason; and (2) we have not had the machine capability to store and retrieve the kind of data involved; and

WHEREAS, some world markets are closing their doors to our livestock producers because we do not have an acceptable method for certifying that animals or animal food products originate from areas not having certain specific animal disease; and

WHEREAS, an inefficient livestock industry means increased cost to the consumer; and

WHEREAS, an effective reporting system is necessary for (1) The development of a disease eradication program and the measurement of the progress of such program; (2) sound epidemiology, which in turn, is the basis for the effective management of animal health programs; (3) provid-
ing a surveillance mechanism to alert the industry of new disease threats before they become catastrophic problems; (4) a guiding stimulus and source of epidemiological information to animal disease research agencies; (5) maintaining and/or increasing potential foreign markets; and

WHEREAS, the lack of machine capability to store and retrieve massive data has been effectively resolved with the advent and perfection of the computer system; therefore

BE IT RESOLVED, That the National Association of State Departments of Agriculture in convention assembled in Honolulu, Hawaii, November 13-18, 1966 endorses the need for a meaningful and feasible animal disease reporting system; and

BE IT FURTHER RESOLVED, That these efforts be organized by the Animal Health Division, Agricultural Research Service, United States Department of Agriculture, in cooperation with the various states to collect, compile, and analyze the data.

1966 RESOLUTION NO. 2

Biological Product Safety

WHEREAS, disease outbreaks have been attributed to contaminated or virulant vaccines; and

WHEREAS, complete eradication of many disease will be impossible without more stringent biological safety regulations; and

WHEREAS, product labels do not adequately warn the user of the potential dangers; and

WHEREAS, the Secretary of Agriculture under the Virus Serum Toxin Act has responsibility to assure the purity, safety and potency of all licensed animal biologics, and not only a portion thereof, for the diagnosis, prevention, and treatment of animal diseases; and

WHEREAS, more complete and detailed testing is possible; therefore

BE IT RESOLVED by The National Association of State Departments of Agriculture in convention assembled in Honolulu, Hawaii, November 13-18, 1966, that the United States Secretary of Agriculture be urged to support, and the Congress of the United States to provide more funds for facilities and personnel so that testing controls of animal biologics can be expanded to prevent the marketing of impure, unsafe, or impotent products.

1966 RESOLUTION NO. 3

Calfhood Vaccination

WHEREAS, the federal government has notified 22 states that plans leading to the phasing out of calfhood vaccination should be initiated and
that federal funds would be reduced or withdrawn in fiscal year 1967 for vaccine purchases and for fee-basis vaccination; and

WHEREAS, vaccination has proved to be of significant value in contributing to the control and eradication of bovine brucellosis; and

WHEREAS, epizootics of bovine abortions from brucellosis are more likely to occur in non-vaccinated populations; and

WHEREAS, it has been shown that vaccination of only a portion of the cattle population is significantly less efficient in contributing to the control and eradication of brucellosis than vaccination of 100% of the cattle; therefore

BE IT RESOLVED by The National Association of State Departments of Agriculture in convention assembled in Honolulu, Hawaii, November 13-18, 1966, that in states where the federal government recommends phasing out of calfhood vaccination, it presents a written evaluation of why these states no longer need vaccination; and

BE IT FURTHER RESOLVED, That they propose additional regulations which would be helpful in controlling potential epizootics of bovine brucellosis; and

BE IT FURTHER RESOLVED, That vaccination be supported until such time as it can be discontinued completely, unless special geographic areas or types of herds can be specified.

1966 RESOLUTION NO. 4

Movement of Livestock

WHEREAS, losses from animal disease are of great economic importance to all segments of the livestock industry; and

WHEREAS, the movement of livestock is the principal means of dissemination and perpetuation of infectious disease, such as tuberculosis, brucellosis, scabies, hog cholera, etc.; and

WHEREAS, sources of animal disease have been impossible to locate because of lack of identification; and

WHEREAS, several states have successfully established compulsory or voluntary market cattle identification programs involving intrastate movement; and

WHEREAS, the control and eradication of infectious diseases is a cooperative state-federal undertaking; therefore

BE IT RESOLVED by The National Association of State Departments of Agriculture in convention assembled in Honolulu, Hawaii, November 13-18, 1966, that the federal government develop proposed regulations for the individual identification of all livestock moving in interstate traffic for any purpose, including slaughter; and
BE IT FURTHER RESOLVED, That similar state regulations be developed by those states which do not have an adequate system of livestock identification to cover intrastate movements of all livestock.

1966 RESOLUTION NO. 5

*Market Cattle Testing Program*

WHEREAS, in the past, losses from animal disease such as bovine brucellosis and tuberculosis have been of great economic importance to the Livestock Industry; and

WHEREAS, the location of herds infected with bovine brucellosis and tuberculosis have required total dependence upon down-the-road testing of individual animals which was done at considerable expense to Governmental agencies and was burdensome to cattle owners; and

WHEREAS, the development of the Animal Identification and Market Cattle Testing Program will reduce the testing of individual animals to those herds where infection is known to exist, reducing dollar costs of disease eradication and relieving cattle owners of the burden of repeatedly assembling herds for test purposes; and

WHEREAS, it is apparent from reports gathered from all parts of the country that the present system of maintaining identity of animals until completion of post-mortem inspection is not operating effectively in all states; therefore

BE IT RESOLVED, That the National Association of State Departments of Agriculture in convention assembled in Honolulu, Hawaii, November 13-18, 1966:

1. Request that the Agricultural Research Service and Consumer and Marketing Services and representative of the United States Livestock Sanitary Association develop the necessary procedures to assure carcass identification throughout the post-mortem inspections operations.

2. Urge the United States Department of Agriculture to take the lead in cooperation with The National Association of State Departments of Agriculture in initiating the action needed to accomplish the objective of this resolution.

3. That The National Association of State Departments of Agriculture Board of Directors engage in conference with all appropriate United States Department of Agriculture officials concerning this matter at the earliest possible date.

1966 RESOLUTION NO. 6

*Animal Health Division Budget*

WHEREAS, there are fine, well organized cooperative agreements covering most all animal disease eradication work between Animal Health...
Division of the Agricultural Research Service and State Departments of Agriculture; and

WHEREAS, there has been close working relationship in the establishment of budget and appropriations covering the various disease eradication programs; and

WHEREAS, it is very apparent that the present budget system is obsolete and grossly unworkable because the appropriation allocations are made for specific disease under direct line budget; and

WHEREAS, this type of budgeting does not provide for flexibility or new emphasis as disease conditions change from state to state and between state and federal; therefore

BE IT RESOLVED by The National Association of State Departments of Agriculture in convention assembled in Honolulu, Hawaii, November 13-18, 1966, that the United States Department of Agriculture and the Congress be requested to set up the Animal Health Division budget to provide for flexibility between diseases (such as most state budgets are) thus enabling immediate action for changed emphasis as problems arise either in the existing disease programs or as new areas for immediate attention come to light. We further request that the Executive Committee pursue this in depth with the United States Department of Agriculture officials and with Congress where deemed necessary.

NOTE: This resolution did not receive the required two-thirds vote of the Resolutions Committee as specified in "Guidelines for Resolutions."

1966 RESOLUTION NO. 7

Bovine Mastitis

WHEREAS, bovine mastitis is of great economic importance to dairy farmers and is of concern to all segments of the dairy industry; and

WHEREAS, estimates based on figures from the United States Department of Agriculture Statistical Reporting Service indicate an annual loss of more than four hundred million dollars for the period 1951-60, representing loss of animals and milk, not including the cost of treatments; and

WHEREAS, there has been a general lack of progress in mastitis control; and

WHEREAS, it has been demonstrated that an effective control program based on education, herd management, sanitation, proper use of equipment, and treatment of affected animals is beneficial to the dairy industry; and

WHEREAS, successful mastitis control programs have been established in several states; therefore
BE IT RESOLVED by The National Association of State Departments of Agriculture in convention assembled in Honolulu, Hawaii, November 13-18, 1966, that the United States Department of Agriculture be requested to immediately study mastitis programs in the several states, contact research workers, develop several proposed programs on an area basis, evaluate these programs, and then develop a proposed national mastitis program suitable for use in the various geographic regions of the country.

1966 RESOLUTION NO. 8

Tuberculin Sensitivity in T.B.

WHEREAS, heterospecific tuberculin sensitivity has widespread distribution in both man and cattle; and

WHEREAS, it confuses interpretation of the standard caudal tuberculin test; and

WHEREAS, in cattle it is caused by acid-fast bacteria other than Mycobacterium bovis, which include M. avium, M. paratuberculosis, M. tuberculosis, and Runyon’s Anonymous Mycobacteria; and

WHEREAS, many of these organisms have high infectivity, but do not cause disease without predisposing factors; and

WHEREAS, there are means available for improving specificity of the tuberculin test, some of which are: Use of tuberculin of reduced concentration; use of PPD’s; and comparative test procedures; and

WHEREAS, there was an expert advisory committee to the United States Department of Agriculture in matters of tuberculosis eradication; which in the past has furnished valuable counsel in matters of tuberculosis eradication; and

WHEREAS, this committee has not recently functioned; therefore

BE IT RESOLVED by The National Association of State Departments of Agriculture in convention assembled in Honolulu, Hawaii, November 13-18, 1966, that this committee be reactivated with new personnel assigned to study and recommend the most effective means to reduce effect of non-specific tuberculin sensitivity in the bovine tuberculosis eradication program; and

BE IT FURTHER RESOLVED, That these methods be evaluated by the United States Department of Agriculture in controlled field trials on a nationwide basis.

I was joined at the Atlanta meeting of NASDA by Dr. James Andrews, Dr. George Stiles, Francis Buzzell and Edward Dwyer of the Executive Committee. Two resolutions were adopted.
MEAT INSPECTION

WHEREAS, the National Association of State Departments of Agriculture has supported the strengthening and modernization of State and Federal meat inspection laws, recognizing that there exists a need for cooperation between the State and Federal programs to safeguard the public interest. The Association urges implementation of such laws consistent with good government; by directing its efforts toward cooperation with the U.S. Department of Agriculture in developing proposals and by reaffirming their position in communications to the Congress; and

WHEREAS, dynamic technological and economic changes in the livestock and meat industry necessitate changes in the traditional Federal Meat Inspection Program; and

WHEREAS, many states have established new and modern state and/or cooperative State-Federal Meat Inspection Programs providing additional service and protection to the consuming public; and

WHEREAS, the National Association of State Departments of Agriculture Board of Directors named a special meat inspection committee this past year to work closely with the U. S. Department of Agriculture toward the development of a more effective Federal Meat Inspection Act, and for improved working relations with the U. S. Department of Agriculture; and

WHEREAS, in recent weeks mutual understanding and cooperation has been actively sought by both NASDA and the USDA administration; and

WHEREAS, NASDA firmly believes the provisions of HR-12144 will afford consumer protection and at the same time be administratively feasible; and

NOW, THEREFORE, BE IT RESOLVED, that the National Association of State Departments of Agriculture in convention assembled in Atlanta, Georgia, October 1-5, 1967, reaffirms its position as favoring HR-12144 thereby strengthening State responsibility in intrastate commerce and Federal responsibility in interstate commerce and the authority to enter into State-Federal cooperative agreements; and

BE IT FURTHER RESOLVED, that NASDA pledges and seeks united effort with USDA, the Congress, the meat industry and with all consumers toward establishment of the best effective State and Federal meat inspection program possible; and

BE IT FURTHER RESOLVED, that the NASDA Board of Directors be instructed to name a Meat Inspection Committee to work and consult with the USDA officials in interpreting and implementing the Meat Inspection Act in such areas as:
1. In the development of a uniform procedure of evaluating acceptability of facilities and inspection procedures for use by both Federal and State programs.

2. To develop a uniform classification system for types of meat packing plants and measurement of inspection needs.

3. In identifying and establishing priorities for assistance to the states in upgrading and developing their meat inspection programs both now and in the future.

4. In developing procedures for equitable allocation of Federal assistance to the states, should such legislation providing for this be passed.

5. To participate in assembling information and evaluating the need for new standards for products, ingredients and labeling.

6. In planning for staffing and training including employee interchange and employee development for both Federal and State Inspection Programs.

7. In developing a concentrated approach to encourage and assist those states in need of a stronger and more effective mandatory meat inspection program, providing information, data, and specific personal assistance.

BE IT FURTHER RESOLVED, that the National Association of State Departments of Agriculture recognizes the need for effective enforcement programs by the states; and

BE IT FURTHER RESOLVED, that copies of this resolution be forwarded to the Secretary of Agriculture, members of his staff and the Congressional Agricultural Committees.

1967 RESOLUTION NO. 2

Reaffirmation of 1966 Resolutions

WHEREAS, the National Association of State Departments of Agriculture has in conventions past resolved for action and follow-up by its Board of Directors on several Animal Health matters; and

NOW, THEREFORE, BE IT RESOLVED, that the National Association of State Departments of Agriculture in convention assembled in Atlanta, Georgia, October 1-5, 1967, reaffirms and keeps active resolutions one (1) through eight (8) of the 1966 convention in Hawaii, asking the Board of Directors to follow up and report back to the National Association of State Departments of Agriculture.
# INDEX OF THE C. V. L. D.

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CONSTITUTION AND BYLAWS
of the
CONFERENCE OF VETERINARY LABORATORY
DIAGNOSTICIANS

CONSTITUTION

ARTICLE I—NAME

The name of the Conference shall be "Conference of Veterinary Laboratory Diagnosticians."

ARTICLE II—

UNITED STATES LIVESTOCK SANITARY ASSOCIATION AFFILIATION

The Conference of Veterinary Laboratory Diagnosticians shall be an affiliate of the United States Livestock Sanitary Association in accordance with a memorandum of agreement mutually agreed upon between the two parties and approved by a two-thirds majority vote of those voting and present at any annual business meeting.

ARTICLE III—PURPOSE

The purpose of this Conference 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 Livestock Sanitary 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 Conference shall be annual and special.

ARTICLE VI—OFFICERS

The officers of this Conference shall be: Chairman, Chairman-Elect, and Secretary-Treasurer.

ARTICLE VII—EXECUTIVE COMMITTEE

The Executive Committee shall be composed of the Chairman, Chairman-Elect, and Secretary-Treasurer.
The Executive Committee shall constitute the administrative body of this Conference and shall determine its activities and policies.

The Chairman of the Conference shall be the Chairman of the Executive Committee.

ARTICLE VIII—PROGRAM COMMITTEE

The Program Committee shall consist of the Chairman-Elect and four other members, one each, respectively, from the four districts of the United States, appointed by the Conference Chairman. 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 Southem, 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. Chairman: It shall be the duty of the Chairman to preside at all meetings of this Conference and to appoint all committees. The Chairman shall be an ex-officio member of all committees and Chairman of the Executive Committee. (Also see Article II).

2. Chairman-Elect: In the absence of the Chairman, the Chairman-Elect shall preside at the meetings of the Conference. In the event of the absence, disability or resignation of the Chairman, he shall perform all duties of the Chairman. He shall be a member of the Executive Committee and Chairman of the Program Committee.

3. Secretary-Treasurer: The Secretary-Treasurer shall keep an accurate record of the meetings of the Conference. Whenever authorized by the Executive Committee, he shall publish newsletters and distribute them to the members of the Conference. The Secretary-Treasurer shall also keep an accurate record of the meetings of the Executive Committee and shall furnish a copy to each member of said Executive Committee.

He shall keep an accurate account of all Conference moneys received and disbursed. He shall also present to the Chairman 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 Executive Committee. He shall be the Secretary of the Executive Committee, also an ex-officio member of the Program Committee.

ARTICLE X—AMENDMENTS

The Constitution of this Conference may be amended by a two-thirds
vote of the members of the Conference present and voting at an annual meeting, provided that the specific amendment to be acted upon shall have been presented in writing at a previous annual meeting.

BYLAWS

ARTICLE I—ORDER OF BUSINESS

Registration.
Call to order.
Report of Secretary-Treasurer.
Chairman's Address.
Committee Reports.
Discussion.
Unfinished Business.
New Business.
Nomination and Election of Officers.
Adjournment.

A suspension of the Bylaws may be made by a two-thirds majority vote of those present and voting 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 recommendation of the Executive Committee and a majority vote of a regularly scheduled meeting of the membership.

ARTICLE III—MEETINGS

The annual meeting of the Conference will be held the two days preceding the dates of the annual meeting of the United States Livestock Sanitary Association and at the same location.

ARTICLE IV—QUORUM

Twenty members of the Conference shall constitute a quorum.
Two members of the Executive Committee shall constitute a quorum.

ARTICLE V—PROCEEDINGS


(The articles for publication shall be assembled by the Chairman of the Program Committee and forwarded by him to the Secretary of the United States Livestock Sanitary Association immediately following each annual meeting.
All articles for publication in the United States Livestock 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 meeting shall be assembled by the Chairman of the Program Committee and submitted to the Secretary of the United States Livestock Sanitary Association prior to thirty days in advance of the scheduled annual meeting dates.

ARTICLE VI—Amendments

The Bylaws may be amended by a majority vote of the members of the Conference present and voting at an annual meeting.

ARTICLE VII—Election of Officers

All elective officers (Chairman-Elect and Secretary-Treasurer) shall be nominated by a nominating committee appointed by the Chairman, and by nominations from the floor of the annual business meeting. The terms of office for the above shall be for one year following their election. Interim vacancies shall be filled by appointment by the Executive Committee and such appointees will serve until the next regularly scheduled business meeting.

ARTICLE VIII—Dues

The amount of registration fees and/or dues shall be determined by a two-thirds majority vote at any regularly scheduled annual business meeting.

ARTICLE IX—Standing Committees

The Executive Advisory Council shall be composed of five members, one member from each of the four districts and the immediate past Chairman. The four district members shall have staggered terms of office not to exceed four years each. Vacancies are to be filled through appointment by the Chairman. The immediate past Chairman’s term of office shall be for one year. The duties of the council is to act as an advisory administrative body to the Chairman and actively implement the C.V.L.D. goal to achieve the establishment of a National Reference Assistance Laboratory for State-level veterinary diagnostic laboratories.

ARTICLE X

Minimum Standards for Veterinary Diagnostic Laboratories

The Association shall develop minimum standards for the certification of Veterinary Diagnostic Laboratories.
The report of the panel on minimum standards for veterinary diagnostic laboratories is an outgrowth of the work of the committee that reported last year on diagnostic laboratory facilities in the United States. The objective of the work of this committee is to provide a standard for comparison for various laboratories in the country. Diagnostic laboratories have made tremendous progress in the last twenty years in physical facilities, staff qualifications and services offered. This progress has been the result of the effort of individual and groups of veterinarians in various states. This progress could not have been made without the help and support of administrators. One benefit of the panel's report will be to aid veterinarians in soliciting the help of administrators in providing assistance in areas where a given laboratory may be weak.

The committee has purposely aimed high in the setting of "minimum standards." No one wants to be "average," and we have attempted to delineate criteria for a complete veterinary diagnostic laboratory service. The requirements of different states and locations will vary and, therefore, the emphasis placed on them in different locations will differ.

It is not intended that each laboratory have all of the recommended services and equipment under one roof. This is desirable but not necessary as long as the service is available to the laboratory through some other agency.

A possible natural outgrowth of the activities of this committee is a laboratory certification program. Such an activity is in the minds of the officers of this association and may become a reality.

Dr. Robert Schroeder, current president of the American Veterinary Medical Association, has set as one of his goals the certification of veterinary diagnostic laboratories. We welcome this support from the AVMA and feel that this organization should be in the forefront of any such activity.

Minimum Standards
For Veterinary Diagnostic Laboratories

Report and Recommendations
Section One:

LABORATORY SERVICE AREAS

by K. D. Weide, D.V.M., Ph.D.
Brookings, South Dakota

I. MICROBIOLOGY

A. Bacteriology
   1. Aerobic, CO₂, anaerobic
   2. Sensitivity testing
   3. Bacterial characterization

B. Virology
   1. Tissue culture
   2. Chick embryo

C. Mycology

D. Mycoplasma

E. Serology
   1. Serotyping

F. Fluorescent Antibody

G. Laboratory Animals

II. PATHOLOGY

A. Gross

B. Microscopic
   1. Histochemistry
   2. Frozen sections

C. Clinical Pathology
   1. Body fluids
   2. Function tests

C. Parasitology

III. TOXICOLOGY

A. Analytical and Biological Chemistry

B. Laboratory Animals

IV. MISCELLANEOUS

A. Field Investigations

B. Consultation and Continuing Education

C. Prompt and Accurate Reporting

D. Data Storage and Retrieval; and Epidemiology

E. Personnel Training Programs

F. Developmental Programs
Section Two:

PHYSICAL FACILITIES AND EQUIPMENT

by E. P. Pope, D.V.M.

Ames, Iowa

I. MICROBIOLOGY

A. Virolology

1. Refrigerators
   a. Walk-in
   b. Small home type

2. Freezers
   a. Chest type, $-80^\circ$ C
   b. Upright Revco, $-10^\circ$ C

3. Incubators
   a. Tissue culture $37^\circ$ C
   b. Egg incubators $37, 35^\circ$ C
   c. CO$_2$ incubator

4. Water baths (agitator type)
   a. $56^\circ$ C
   b. $37^\circ$ C

5. Autoclave (self-contained steam, anti-scale compound free) Best with its own steam supply. Building steam may contain anti-scale compounds which will kill tissue cultures.

6. Large drying oven (dry heat sterilizer)

7. Mixers
   a. Blenders
   b. Homogenizer
   c. Grinders

8. Centrifuges
   a. Heavy duty, refrigerated (International PR-2 type)
   b. Ultra centrifuge (desirable)

9. Microscopes
   a. Fluorescent
   b. Binocular microscope for tissue culture observation
   c. Dissecting microscope

10. Analytical balance

11. Lyophilization apparatus

12. Distillation apparatus, glass-lined

13. Separate glassware washing facilities

B. General Bacteriology

1. Refrigerators

2. Freezers

3. Incubators
   a. $37^\circ$ C
   b. $25^\circ$ C

4. Water baths
   a. $37^\circ$ C
   b. $56^\circ$ C
   c. $42^\circ$ C

5. Autoclave

6. Dry-heat sterilizer

7. Centrifuges

8. Microscopes
   a. Binocular
   b. Fluorescent
   c. Dissecting
   d. Darkfield

9. Balances
   a. Torsion
   b. Analytical

10. pH meter

11. Spectrophotometer
12. Automatic pipetting machine
13. Lyophilization apparatus
14. Safety hood

II. SEROLOGY
1. Refrigerator
2. Incubator
3. Water baths
   a. 56°C
   b. 37°C
4. Autoclave
5. Centrifuge
6. Freezer, storage
7. Spectrophotometer
8. pH meter (for tube test)
9. Torsion balance
10. Rotator
11. Automatic pipetting apparatus

III. PARASITOLOGY
1. Incubator
2. Centrifuge
3. Microscopes
   a. Binocular
   b. Wide-field dissection microscope

IV. GROSS PATHOLOGY
1. Cooler—walk-in type
2. Autopsy table
3. Band saw
4. Rail and mechanical hoist
5. Animal holding area
6. High pressure cleaner and/or steam
7. Necropsy room
8. Scales, weighing
9. Humane euthanasia equipment

V. HISTOPATHOLOGY
1. Automated tissue processor
2. Microtome
3. Cryostat
4. Microscopes
   a. Binocular
   b. Fluorescent
5. Microtome knife sharpener
6. Vacuum infiltration apparatus
7. Paraffin oven

VI. CLINICAL PATHOLOGY
1. Microscope
2. Freezer
3. Refrigerator
4. Centrifuge/s
5. Spectrophotometer
6. Microhematocrit centrifuge
7. Electrophoresis and integrator
8. Blood counting apparatus
9. Fluorometer
10. pH meter
11. Water bath
12. Incubator
13. Refractometer

VII. TOXICOLOGY
1. Atomic Absorption
   Spectrophotometry
2. Gas Chromatography
3. Chromatography Equipment
4. Microscope, Binocular
5. pH Meter
6. Spectrophotometer
   a. Infra Red
   b. U.V.
7. Distilling Apparatus, Glass-lined
8. Analytical Balance
9. Fumehood (Perchloric)
10. Wylie Mill
11. Refrigerator
12. Freezer

VIII. OFFICE
1. Medical record storage and data retrieval room
2. Duplicating apparatus
3. Adding machine
4. Calculator
5. Dictating/Transcribing equipment
6. Intralaboratory communication equipment

IX. MISCELLANEOUS
1. Alcohol storage area
2. Volatile chemical storage area
3. Instrument room
4. Dark room and equipment
5. Cameras and Photomicrograph equipment
6. Library and/or conference room
7. Media preparation area
8. Glass washing and general service area
9. Maintenance shop and tools
10. Protective clothing
11. Transportation motor vehicle for personnel and supplies

X. GENERAL REQUIREMENTS FOR LABORATORY
1. Air circulation and temperature control
   Air conditioning without recirculation of air. Air from the outside to be filtered to remove dust. This requirement is for all laboratory areas.
2. Wall, ceiling, and floor construction
   Must be air tight, smooth, impervious to liquids and disinfectant solutions.

XI. LABORATORY ANIMAL AND ISOLATION FACILITIES
1. These must meet the requirements of the Laboratory Animal Welfare Act.
2. Cage washing apparatus.

XII. DISPOSAL SYSTEMS
1. Carcass disposal and burnable refuse
   a. By incineration
2. Sewage disposal
   a. sewage must go to approved sewage disposal plant for treatment.

XIII. UTILITIES
1. Gas
2. Electricity (110, 220 V)
3. Water (tap and distilled)
4. Steam
5. Negative and Positive pressure
6. Telephone
**Section Three:**

**PERSONNEL QUALIFICATIONS**

by V. A. Seaton, D.V.M., Ph.D.

Ames, Iowa

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<tr>
<th>POSITION</th>
<th>MINIMUM</th>
<th>ADDITIONAL DESIRABLE QUALIFICATIONS</th>
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<tbody>
<tr>
<td>Director</td>
<td>1) D.V.M.; 2) 5 years experience in diagnostic veterinary medicine;</td>
<td>1) Graduate training; 2) Administrative and supervisory experience</td>
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<tr>
<td></td>
<td>3) Management training; 4) Broad knowledge of laboratory disciplines</td>
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<tr>
<td>Pathologist/s</td>
<td>1) D.V.M. and M.S. or equivalent</td>
<td>1) Board of the ACVP and/or PhD in pathology</td>
</tr>
<tr>
<td>Diagnostician/s</td>
<td>1) D.V.M. with 2 years experience in pathology</td>
<td>1) 2 years practice experience; graduate work; or specialized training</td>
</tr>
<tr>
<td>Bacteriologist/s</td>
<td>1) M.S. degree in microbiology + 2 years experience in pathogenic bacteriology</td>
<td>1) D.V.M. and/or PhD in microbiology</td>
</tr>
<tr>
<td>Virologist/s</td>
<td>1) M.S. in microbiology and 2 years experience in virology</td>
<td>1) D.V.M. and/or PhD in veterinary virology; Board diplomat</td>
</tr>
<tr>
<td>Biochemist</td>
<td>1) M.S. in Biochemistry</td>
<td>1) PhD in Biochemistry</td>
</tr>
<tr>
<td>Technicians—Histo.</td>
<td>1) High school + 2 years experience</td>
<td>1) Med. Tech. (ASCP certification) or B.S. + 4 years experience</td>
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<tr>
<td>Technicians—Bact.</td>
<td>1) High school + 2 years experience</td>
<td>1) Med. Tech. (ASCP certification); B.S. + 4 years experience</td>
</tr>
<tr>
<td>Technicians—Chem.</td>
<td>1) High school + 2 years experience</td>
<td>1) Med. Tech. (ASCP certification); B.S. + 4 years experience</td>
</tr>
<tr>
<td>Technicians—Serol.</td>
<td>1) High school + 2 years experience</td>
<td>1) Med. Tech. (ASCP certification); B.S. + 4 years experience</td>
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<tr>
<td>Laboratory Aids</td>
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<td>1) High School</td>
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Personnel Qualifications, cont.

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<th>POSITION</th>
<th>MINIMUM</th>
<th>ADDITIONAL DESIRABLE QUALIFICATIONS</th>
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</thead>
<tbody>
<tr>
<td>Animal Caretakers</td>
<td>1) High School</td>
<td>1) Certificate by American Association of Laboratory Animal Science</td>
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<tr>
<td>Secretary/s</td>
<td>1) Business School graduate</td>
<td>1) Medical secretary classification or B.S. in Business Education</td>
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<tr>
<td>Maintenance Engineer</td>
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**ADDITIONAL DESIRABLE CATEGORIES:**

<table>
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<tr>
<th>Clinical Toxicologist</th>
<th>1) D.V.M.</th>
<th>1) D.V.M.; Board in Toxicology</th>
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<tbody>
<tr>
<td>Epidemiologist</td>
<td>1) D.V.M.</td>
<td>1) D.V.M.; MPH in Epidemiology</td>
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**Section Four:**

A Method for Determination of Budgets for Veterinary Diagnostic Laboratories

KENNETH K. KEAHEY, D.V.M., PH.D.*
East Lansing, Michigan

A review has been made of the Conference of Veterinary Laboratory Diagnosticians (CVLD) Laboratory Facilities Questionnaire results, in an effort to ascertain helpful information as it relates to budgets and salaries. Factors which determine an annual operational budget for a diagnostic laboratory are many and varied. It is thought, however, that quality, quantity and types of services rendered are common to most, if not all, veterinary diagnostic laboratories. Quality of services may be used as a common denominator in determining guidelines for an operational budget, if data extrapolated from the CVLD questionnaire are valid.

The budget categories requested on the CVLD questionnaire were: operating funds, maintenance funds, inventory value, salaries, travel, and library. The categories of salaries were broken down into professional salaries and supportive salaries. Professional salaries included individuals with the D.V.M., Ph.D., or D.V.M.-Ph.D. and, in some instances, the M.S. degree. Supportive salaries included medical technologists, technicians, clerical, and supportive personnel. A total of professional salaries and supportive salaries represented approximately 65% of the total operating budget.

*Department of Pathology, Michigan State University.
budgets of reporting laboratories. It was believed that the expenditures in
the category of salaries represented the most accurate gauge of budget
information.\(^1\) The category of inventory value included major items, such
as microscopes, spectrophotometers, etc., that are of a permanent inventory
nature. These items, therefore, would not be in their entirety an annual
expense. This inventory category represented only 5.8\% of total budgets.
The other categories requested in budget information were considered
annual recurring expenditures.

During the 8th Annual Meeting of the Conference of Veterinary
Laboratory Diagnosticians it was reported,\(^2\) "a level of 2.5 to 3.0 supportive
personnel to each professional staff member is desirable."

The ratio of supportive to professional personnel was computed for the
diagnostic laboratory at Michigan State University with the following
results: There were 2.68 supportive to each professional staff member. The
figure of 2.68, however, did not include individuals that often service phys-
ical plant equipment. If these individuals were considered, the figure would
be slightly higher.

Depending on the anticipated or actual volume and types of services
rendered, the number of professional employees can usually be determined.
The salaries of professional employees will vary considerably because of
qualifications, experience, geographical location, and cost of living differen-
tial. An inquiry by an administrator from another establishment of the
region will provide information on going salaries and needed increases to
lure desired professional employees, so that professional salaries may be
budgeted. Multiply the number of professional employees times 2.75, an
average figure, and this will give an estimate of numbers of needed suppor-
tive personnel. The same criteria as stated for professional employees' salaries
apply for salaries of supportive personnel. A total of professional and
supportive personnel salaries should represent approximately 65\% of the
annual operating budget.

The above information has many applications and provides the best
guide of establishing budgets for veterinary diagnostic laboratories that
is presently available.

REFERENCES

2. Sipple, W. L.: Veterinary Diagnostic Laboratory Personnel Qualifications and
SAFETY MEASURES

I. Health Program for Laboratory Workers

A. Pre-employment examination—TB skin test and chest X-ray.
B. People working with tuberculosis should have TB skin test every six months and chest X-ray (14" x 17") once a year.
C. People that have a positive skin test should not be skin tested but required to have a chest X-ray at the discretion of his physician.
D. People associated with laboratory, i.e., clerical workers, should have skin test and chest X-ray once a year.

II. Procedures in Contaminated Areas

A. Biological safety cabinets (Hoods) with a filtered exhaust air system should be used. A negative pressure of 0.75 inches of water is maintained in the cabinet. Material to be removed from the hood is either decontaminated as a batch or individual articles are placed in an airlock at end of the hood and decontaminated with a suitable disinfectant. A filtered air inlet is desirable to keep contamination to a minimum.

B. Pipettes are used only with a safety pipette bulb. (Materials are never pipetted by mouth.) All discarded pipettes are immersed in a suitable disinfectant. Contaminated pipettes should be sterilized by pressure steam sterilization 121 C for one hour.

C. Blenders should be used only in a safety cabinet. This device can produce considerable aerosol and is very hazardous to use in a tuberculosis laboratory.
D. All cultural tubes and petri dishes should be opened only in a safety cabinet.

E. Animal rooms—Before entering animal rooms where animals are infected with mycobacteria, clothes should be changed (everything). Rubber gloves should be worn. A surgical mask is worn over mouth and nose. An eye shield is used over eyes if animals are to be inoculated with live mycobacteria. Upon leaving the animal room, the individual should completely disrobe and take a complete shower including hair unless a head protector is used. Clothes used in the animal room are autoclaved at 121°C for 45 minutes.

F. Syringes—Only syringes of the luer-loc type are used.

III. Disinfectants

A. Disinfectants used in the laboratory are 2 percent Amphy* or 5 percent phenol.

B. Quaternary ammonium compounds should not be used in a tuberculosis laboratory.

**ISOLATION AND TYPING OF MYCOBACTERIA**

I. Isolation of Mycobacteria

A. Identification—The establishment of an identification system for specimens throughout the processing is essential.

B. Preliminary Treatment of Specimens—Tissue specimens are rinsed in a dilute solution of sodium hypochlorite (76.0 ml of commercial bleach, 5.25% NaOCl per 4000 ml of tap water) as soon as possible after receipt. All fat is removed from tissues using separate sterile scissors and forceps for each specimen.

   The treated tissues are submerged in fresh, dilute sodium hypochlorite solution and allowed to stand undisturbed over night at 4°C. The following day the tissues are washed twice in fresh dilute sodium hypochlorite solution.

C. Grinding and digesting2—The washed tissues are placed in sterile petri dishes and transferred to a biological safety cabinet where they are dissected using sterile scissors and forceps. The cut surfaces are examined for lesions.

   The central area from each tissue is transferred to a blender jar. Lesions are included with adjacent tissue. Fifty ml of nutrient broth containing 0.4% phenol red indicator is added to the blender jar. The mixture is exposed for two minutes to the action of the rotating blender knives. A teflon magnetic stirring bar and 100 ml of papain are added to the blended tissues. Enough 4.0% NaOH is added to change the phenol red indicator from yellow to red.

   The blender jar is disinfected with 2.0% Amphy solution and removed from the safety cabinet.

   Digestion of the tissues is enhanced by placing the blender jar

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*Lehn & Fink Products Corporation, 4934 Lewis Avenue, Toledo, Ohio 43612.
on a magnetic mixer for one hour. The temperature of the digestion mixture should be maintained at 37 C in a waterbath.

D. Concentration—The jar is then returned to the biological safety cabinet. Ten ml of pentane is added to the contents. The contents are mixed by two or three vigorous shakes and then allowed to stand undisturbed for 30 minutes.

The mycobacteria which were released from the tissue during digestion are oriented in the pentane-water interface.

The interface between the pentane layer and the aqueous layer is removed with a sterile 10 ml pipette and filtered through a double layer of sterile unbleached muslin. The muslin is conveniently supported by a glass funnel. The filtrate is collected in a sterile 20 x 125 mm test tube.

The tube and contents are centrifuged at 1636 RCF* for 20 minutes inside a screw cap safety centrifuge capsule. The sediment is deposited in the bottom of the tube and a pellicle is formed on top of the liquid during centrifugation. The liquid separating the sediment and pellicle is removed with a pipette and autoclaved.

One ml of nutrient broth is added to the centrifuge tube which is shaken vigorously, producing a homogenous mixture of sediment, pellicle and broth. This mixture is used to inoculate media and animals.

E. Culture Media—Each of eight tubes of culture media is inoculated with four drops of inoculum delivered from a pasteur pipette.

<table>
<thead>
<tr>
<th>Media Inoculated for Each Case</th>
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<tbody>
<tr>
<td><strong>No. Tubes</strong></td>
</tr>
<tr>
<td>2</td>
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</tbody>
</table>

The tubes containing inoculated slants are disinfected and removed from the biological safety cabinet. Each tube is held so that the inoculum flows evenly over the surface of the medium. The tubes are placed in a rack so that the horizontal position is maintained during an overnight incubation period at 37 C. This step

\*RCF = \text{Relative Centrifugal Force} = \frac{4 \times (3.14)^2 \times r \times n^2}{32 \times 2}

\begin{align*}
r &= \text{radius in feet} \\
n &= \text{revolutions per second}
\end{align*}

\textbf{Example:} A centrifuge head which accommodates screw cap capsules is used at NADL. The extended capsules provide an effective radius of 9\frac{1}{2} inches. The RCF is 1636 when this equipment is used.
insures uniform contact of the inoculum over the entire surface of each slant. The slants are returned to the vertical position for further incubation at 37 C.

The excess inoculum is mixed with an equal volume of 0.2% benzalkonium chloride to suppress the growth of contaminating microorganisms. If the inoculated medium is overgrown or hydrolyzed by contaminating microorganisms, a second set of mediums is inoculated with the treated inoculum.

F. Incubation Period—The incubating culture mediums are examined each week for a period of eight weeks for the presence of mycobacterial colonies. If bacterial colonies resembling those of mycobacteria are found, a smear is made from each. The smear is stained by the Ziehl-Neelsen technique and observed under oil immersion for the presence of acid-fast bacilli. Each colony of acid-fast microorganisms is used to inoculate a single tube of Dubos broth containing Tween 80* and albumin. The broth is used at a later time for biochemical tests and to study cultural characteristics.

II. Mycobacterial Typing Procedures

A. Observation of Primary Colonies—An understanding of the variations in colonial morphology resulting from changes in the medium or by subculturing is essential for an effective mycobacterial typing scheme. Isolated colonies are observed for morphology and pigmentation with the aid of a hand lens or other optical device.

1. *Mycobacterium bovis*—Primary colonies of this species on Middlebrook's 7H-10 medium usually appear colorless, flat, irregular, rough and very dull by transmitted light. They appear flat and dry or resemble the convex, white, moist colony of *M. avium* and the anonymous mycobacteria on Herrold's medium. *M. bovis* isolants subcultured on Lowenstein-Jensen medium produce mammilate, colorless colonies. (Figure 1) *M. bovis* colonies growing on Stonebrink medium usually appear as white, moist, convex colonies resembling those of *M. avium*.

2. *Mycobacterium avium*—Primary colonies of this species usually appear moist, white and convex on all four types of media.

3. Runyon Group III—Same as *M. avium*.

4. *M. tuberculosis*—Primary colonies are very dry, rough, flat to raised, and white to buff with irregular edges when grown on Lowenstein-Jensen medium.

Cellular and colonial characteristics of several mycobacteria and their respective biochemical reactions are presented in Table I.

B. Biochemical and Cultural Procedures—Smears are made of all colonies resembling those of mycobacteria. The smears are stained with the Ziehl-Neelsen procedure described in the appendix.

*Difco Laboratories, Detroit, Michigan 48201.*
Figure 1. Diagrammatic sketch of a typical *M. bovis* colony.

### TABLE I—CELLULAR AND COLONIAL CHARACTERISTICS OF SEVERAL MYCOBACTERIA AND THEIR RESPECTIVE BIOCHEMICAL REACTIONS

<table>
<thead>
<tr>
<th>MYCOBACTERIAL TYPE</th>
<th>APPEARANCE TIME (days)</th>
<th>PIGMENT</th>
<th>CELL LENGTH</th>
<th>COLONIAL MORPH.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Light</td>
<td>Dark</td>
<td></td>
</tr>
<tr>
<td>M. bovis</td>
<td>13-20</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M. tuberculosis</td>
<td>13-20</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M. avium</td>
<td>13-20</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Runyon Group I Photochromogens</td>
<td>13-20</td>
<td>+</td>
<td>+</td>
<td>U</td>
</tr>
<tr>
<td>Runyon Group II Scotochromogens</td>
<td>13-20</td>
<td>+</td>
<td>+</td>
<td>U</td>
</tr>
<tr>
<td>Runyon Group III Non-Photochromogens</td>
<td>13-20</td>
<td>6-13</td>
<td>-</td>
<td>U</td>
</tr>
<tr>
<td>Runyon Group IV Rapid growers</td>
<td>6</td>
<td>growth</td>
<td>±</td>
<td>+</td>
</tr>
</tbody>
</table>

### P & B and Cellular Morph. Code

- **G** = Granular growth
- **U** = Uniform homogenous growth
- **S** = Short 0.5 - 1.0 μ
- **P** = Plump
- **C** = Coccoidal
- **M** = Medium length 1.1 - 2.0 μ
- **L** = Long 2.0 μ+

### Colonial Morphology Code

- **M** = Mamillate (See Figure 1)
- **F** = Flat
- **C** = Convex
- **S** = Smooth
- **R** = Rough
- **Mst** = Moist
- **E** = Entire
- **I** = Irregular
- **D** = Dry
The remaining portion of each colony is transferred to Dubos broth (containing albumin and Tween 80) with a sterile wire loop. The broth is incubated for one week at 37°C. The purity of new growth is confirmed by smear and Ziehl-Neelsen staining. The incubation period is extended for another week if no growth appears.

Both fluid and solid media are inoculated with the pure Dubos broth culture for use in subsequent typing procedures.

Proskauer and Beck Medium (Karlson) is used to study four cultural characteristics in fluid medium:

Observations and Tests
in
Proskauer and Beck Medium

1. Granular-uniform growth characteristics

Cells of *M. tuberculosis* and *M. bovis* cultures have a granular (clumped) appearance in transmitted light. All other mycobacteria usually have a uniform (homogenous dispersion of cells) appearance in transmitted light. Rough strains of *M. avium* and Runyon's anonymous mycobacteria often produce clumps of cells in a background of uniform cells. (Figure 2)

![Figure 2. Clumping of cells in Proskauer and Beck Medium (left) and background uniform growth with clumps of cells interspersed.](image)

2. Cording

A smear of the bacterial sediment in a Proskauer and Beck culture is stained by Ziehl-Neelsen technique and examined by oil immersion microscopy for the presence of cords and for cellular morphology. (Figure 3)

Cording is a term used to describe a parallel alignment of bacterial cells that results in a long serpentine or string-like formation. *M. tuberculosis* and *M. Bovis* produce cords consistently. The phenomenon is also reported to be an indicator of virulence.
3. Cellular morphology may be studied on the same smear and the observations used to aid in determining the bacterial type. The morphology of several mycobacteria is presented in Table I.

4. Niacin

The presence of niacin in a culture growing in Proskauer and Beck medium may be used to differentiate \textit{M. tuberculosis} from all other mycobacteria.\textsuperscript{1} \textit{M. tuberculosis} is a vigorous producer of niacin. \textit{M. fortuitum} has also been reported as a niacin producer.

One ml of 4.0\% aniline in ethyl alcohol is added to the culture growing in Proskauer and Beck medium. This is followed by the addition of 1.0 ml of aqueous cyanogen bromide (deadly poison). (See appendix) The appearance of a yellow color within five minutes is indicative of the presence of niacin.

Antibiotic sensitivity tests are conducted in the basal medium, Dubos broth with albumin and Tween 80, so nicotinic acid hydrazide (INH) (See appendix) is prepared in final concentration of 10 \textmu gm per ml of basal medium. Thiophen-2-carboxylic acid hydrazide (TCH) (see appendix) is prepared in a final dilution of one part neotetrazolium to 40,000 parts basal medium.

One tube of each inhibitory agent medium and one tube of basal medium (control) is used to type each isolant. Each tube is inoculated with 0.4 ml of a Dubos broth culture of the isolant to be typed. Inhibition is indicated when no growth is present in the medium containing the inhibitory agent. The control medium must have luxurient growth.

Bacterial growth is purple in color in the neotetrazolium medium because neotetrazolium salts are converted to colored compounds called formazans.

\textit{M. bovis} strains are are inhibited by all three agents but growth is luxurient in the control medium. (Figure 4)

\textit{M. tuberculosis} strains are inhibited by INH and neotetrazolium but grow well in TCH medium and control medium.
M. avium and Runyon's anonymous groups are not inhibited by any of the three agents. Therefore, luxurient growth occurs in all inhibitory media and in the control medium.

M. bovis and M. tuberculosis cultures are identified on the basis of growth characteristics in Proskauer and Beck medium, niacin test, drug resistance and cellular morphology.

M. avium and Runyon's anonymous groups are differentiated by additional observations of culture growth on Lowenstein-Jensen medium. Appearance time, pigmentation and colonial morphology are observed and recorded.

Colonies of M. avium and Runyon Group III strains appear in 13 to 20 days at 37 C and in 6 to 13 days at 45 C. Pigmentation is usually absent. The colonies are moist, white, entire and convex to raised. Final differentiation between these two types is made by animal inoculation (chickens). M. avium causes generalized lesions in the liver, spleen and kidneys. Runyon Group III cultures do not produce lesions in the chicken.

Both Runyon Group I and II produce orange to yellow pigment under certain light conditions. Group I colonies are pigmented when grown in light but colorless when grown in darkness. Group II colonies are pigmented when grown in light or in darkness.

The light requirement for the differentiation of Group I and II colonies can be satisfied if the culture tubes occupy a position near an incubator door which is opened several times a day. Satisfactory conditions of darkness can be produced by covering the appropriate culture tubes with aluminum foil.

Runyon Group IV microorganisms are characterized by bacterial growth appearing in six days or less at 37 C. Temperature requirements for growth are not restrictive with growth occurring from room temperature to 45 C for many strains. Pigment is not usually present in colony growth but may occur occasionally.
The mycobacterial typing scheme described here is illustrated in Figure 5.

![Mycobacterial Typing Diagram](image)

C. Animal Inoculation—The inoculation of laboratory animals may be used as an adjunct to the cultural and biochemical typing techniques presented in this manual. *M. tuberculosis*, *M. bovis*, *M. avium* and Runyon Group III isolants are commonly differentiated by this method. Other Runyon anonymous microorganisms are not typed by animal inoculation because they are not normally virulent for laboratory animals.

The virulence of several mycobacteria for selected laboratory animals is presented in Table II.

Guinea pigs and chickens are each inoculated intraperitoneally with 1.0 ml of a saline suspension containing 0.1 mg of bacterial cells per ml of saline. Rabbits are inoculated intraperitoneally with
1.0 ml of saline suspension containing 0.01 mg of bacterial cells per ml of saline.

Inoculated animals are kept for two months. Any that die within that period of time are necropsied for evidence of tuberculosis. All surviving animals are necropsied for evidence of tuberculosis at the end of the two-month period.

The concentration of bacterial suspensions for animal inoculation is determined with the Fitch-Hopkins tube and centrifuged at 2357 RCF for 20 minutes. The bacterial cells are packed in the bottom of the tube constriction by centrifugal force. The tube calibration adjacent to the top of the packed cell column is used to determine the net weight of cells in the Dubos broth culture. The translation from Fitch-Hopkins tube calibrations to wet weight of bacterial cells is illustrated in Figure 6.

The method is explained in the following example:

**Needed:** 50.0 ml of a 0.1 mg per ml suspension

**Available:** 10.0 ml of visibly turbid Dubos broth culture

**Procedure:**

1. Transfer 2.0 ml of the Dubos broth culture to a clear Fitch-Hopkins tube.
2. Centrifuge at 2357 RCF.
3. Translate the 0.01 reading on tube constriction to 5.0 mg cells/ml.
4. Add 49.0 ml of sterile physiologic saline solution to 1.0 ml of the original Dubos broth.

---

*3000 rpm with a 9.25 inch effective radius is sufficient for 2357 RCF.*

### TABLE II—PATHOGENICITY PATTERN FOR LABORATORY ANIMALS

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>GUINEA PIG</th>
<th>RABBIT</th>
<th>CHICKEN</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. bovis</em></td>
<td>++++</td>
<td>++++</td>
<td>—</td>
</tr>
<tr>
<td><em>M. tuberculosis</em></td>
<td>++++</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>M. avium</em></td>
<td>—</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Runyon Group III</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Figure 6. Fitch-Hopkins Tube Readings.

(5) Proof.

Needed: 50 ml of 0.1 mg/ml = 5.0 mg total wet weight of cells

Add: 49.0 ml saline solution
1.0 ml Dubos broth

50.0 ml total volume of inoculum

Result: 5.0 mg in 50.0 ml
Same as: 1.0 mg in 10.0 ml
Same as: 0.1 mg in 1.0 ml

APPENDIX

ZIEHL-NEELSEN ACID-FAST STAIN

Stock Saturated Alcoholic Basic Fuchsin* Solution

95% Ethyl Alcohol 100 ml
Basic Fuchsin 3 gm

*Both basic fuchsin and brilliant green are available from: National Aniline Division, Biological Stains Department, 40 Rector Street, New York 6, New York.
Working Solution Basic Fuchsin
- Saturated Alcoholic Basic Fuchsin: 10 ml
- 5% Ag Phenol: 90 ml

Acid Alcohol
- Hydrochloric Acid (conc.): 3.2 ml
- 95% Ethyl Alcohol: 97.0 ml

Brilliant Green Counterstain
- Brilliant Green: 1 gm
- 0.01% NaOH: 100 ml

ZIEHL-NEELSEN-ACID-FAST STAIN
1. Stain the smear for three minutes in steam Ziehl's carbol fuchsin.
2. Rinse in distilled water.
3. Decolorize in 95% ethyl alcohol, containing 3% by volume of concentrated hydrochloric acid, until only a suggestion of pink color remains. 2-3 minutes.
4. Wash in distilled water.
5. Counterstain for three minutes with alkaline brilliant green.
6. Wash in distilled water and dry—read with oil-immersion lens of microscope.

NIACIN TEST REAGENTS
Cyanogen Bromide Solution
- Cyanogen Bromide: 10 gm
- Distilled Water: 90 ml

Ethyl Aniline Solution
- Aniline: 4 ml
- 95% Ethyl Alcohol: 96 ml

CAUTION: Cyanogen bromide solution should be prepared and used in fume hood. Both solutions should be stored in brown bottles. They should be kept no longer than four weeks before replacement with fresh solutions.

MIDDLEBROOK'S 7H10 AGAR WITH MIDDLEBROOK OADC ENRICHMENT

Ingredients
- Middlebrook's 7H10 Agar Base (Difco 0627): 20 gms
- Distilled Water containing 5 mls Glycerol: 900 mls
- Middlebrook OADC Enrichment (Difco 0722): 100 mls
**Method of Preparation**

Mix and heat Middlebrook's 7H10 Agar Base and water containing Glycerol until in complete solution. Pour into aspirator bottle with attachment for aseptic dispensing of flask for pouring plates. Sterilize in autoclave at 121 degrees C for 15 to 20 minutes. Cool to 56 degrees C and add Middlebrook OADC Enrichment. Mix well. Dispense in 20 x 125 Screw Cap tubes in 9 ml amounts and allow to solidify in slant position. For plates, pour medium into petri dishes in 25 ml amounts. Incubate at 37 degrees C to check sterility.

**HERROLD'S EGG YOLK MEDIUM**

**Ingredients**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone (Difco 0118)</td>
<td>9.0 gms</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>4.5 gms</td>
</tr>
<tr>
<td>Agar (Special Noble-Difco 0142)</td>
<td>15.3 gms</td>
</tr>
<tr>
<td>Beef Extract (Difco 0126)</td>
<td>2.7 gms</td>
</tr>
<tr>
<td>Glycerol</td>
<td>27.0 mls</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>870.0 mls</td>
</tr>
<tr>
<td>Egg Yolks</td>
<td></td>
</tr>
</tbody>
</table>

Aseptically prepared from strictly fresh eggs—if source is known—eggs should be from hens that have had no antibiotics in feed or medicine.

2% Malachite Green dye solution

**Equipment:** Prepare and sterilize the following:

- One 2 Liter Aspirator bottle with cotton gauze plug, small bell filling attachment for dispensing and preferably a magnetic stirring bar for mixing. Rat tooth forceps for each egg.
- Test tubes—20 x 125 S. C. tubes in sufficient quantity.

**Method of Preparation**

Mix and heat all ingredients (except egg yolks and dye solution) until agar is melted. Cool to 60 degrees C and adjust pH to 7.5 with 1 N. Sodium Hydroxide. Pour into asp. bottle and sterilize in autoclave 25 minutes at 121 degrees C. Cool to 56 degrees C and add six sterile egg yolks prepared as follows: Scrub eggs with a brush in warm detergent water and rinse with clear water. Let air dry on a towel. Soak in 75% isopropyl alcohol for 30 minutes. Dry between layers of sterile towels. Crack shell at one end with sterile forceps, make 10 mm hole and remove egg white forceps and gravity. Make hole larger and break yolk. Twirl forceps to mix yolk, pick out yolk sac with forceps and pour mixed yolk into sterile media in asp. bottle. Repeat

**This medium is also prepared without Glycerol and Malachite Green—usually called Herrold's Egg Yolk Medium—without—without.**
process for each egg. Mix media gently on magnetic stirrer. Add Malachite Green dye solution with sterile pipette. Blend again on stirrer. Dispense aseptically into 20 x 125 S. C. tubes in 9 ml amounts. Dispense on bell side of tube only. Do not shake tube after filling to avoid film on more than bell side. Allow medium to harden in slant position. Check sterility by incubation for 48 hours at 37 degrees C.

LOWENSTEIN-JENSEN MEDIUM*

**Ingredients**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>For 1612 mls Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowenstein Medium Base (Difco 0444)</td>
<td>37.2 gms</td>
</tr>
<tr>
<td>Glycerol</td>
<td>12 mls</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>600 mls</td>
</tr>
<tr>
<td><strong>Whole Eggs—asceptically prepared</strong></td>
<td>1000 mls (24 large eggs)</td>
</tr>
</tbody>
</table>

**Equipment needed: Prepare and sterilize:**

- One 2 Liter aspirator bottle with small bell filling attachment for dispensing and preferably a magnetic stirring bar for mixing.
- Large funnel—lined with two thicknesses of unbleached muslin. Tape edge to hold in place. Huck towel folded over muslin lined funnel—tape in place.
- Funnel wrapped in paper for sterilizing.
- Two quart Bell jars with Osterizer blender tops—autoclave in inverted position.
- Test tubes—20 x 125 S. C. tubes in sufficient quantity.
- Pipette or glass rod for stirring eggs.

**Method of Preparation**

Add Lowenstein Medium Base to water containing glycerol. Mix on Waring Blender to obtain a smooth solution. Heat in flask until dissolved. Pour into a 2 L. asp. bottle. Sterilize in an autoclave for 20 minutes at 121 degrees C. Cool to room temperature.

Egg Preparation: Scrub eggs in warm detergent water, rinse in clear water and air dry on towel. Soak in 75% isopropyl alcohol for 30 minutes. Dry eggs between layers of sterile towels. Dip top of beaker in alcohol and flame dry to sterilize. Break eggs on sterile beaker edge and drop in sterile Ball blender jars. Mix on Osterizer just enough to homogenize but not to cause excessive air bubbles. Filter through sterile muslin lined funnel into sterile media in asp. bottle. (Put gauze plug from bottle inside towel layer to keep sterile while eggs are filtering.) Stir eggs with sterile pipette or rod if necessary to help filtering process but keep eggs in funnel covered with towel gauze plug in asp. bottle. Mix gently but thoroughly on mag-

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*This media is also made without Glycerol when specified.

**Eggs should be strictly fresh. If source is known, eggs should be from hens that have had no antibiotics in feed or medicine.
netic stirrer until media is evenly blended. Dispense into tubes aseptically. Dispense on bell side of tube only. Do not shake tube after filling to avoid film on more than bell side. Dispense in 9 ml amounts in 20 x 125 S. C. tubes. Inspissate for 40 minutes at 80 degrees C with tubes in slant position. Cool slightly. Incubate 24 to 48 hours in slant position so excess moisture will be reabsorbed by media.

**STONEBRINKS MEDIUM**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>For 1200 ml Total Media</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salt Mixture</strong></td>
<td></td>
</tr>
<tr>
<td>Sodium Pyruvate (Sodium Salt-Pyruvic Acid)</td>
<td>5 gms</td>
</tr>
<tr>
<td>Potassium Phosphate Monobasic KH$_2$PO$_4$</td>
<td>2 gms</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>300 mls</td>
</tr>
<tr>
<td>Sodium Phosphate Dibasic (Na$_2$HPO$_4$) to obtain pH of 6.5</td>
<td></td>
</tr>
</tbody>
</table>

| **Dye Mixture** | |
| Crystal Violet | 100 mg |
| Malachite Green | 800 mg |
| Distilled Water | 100 mls |

| **Egg Mixture** | |
| **Whole egg (Aseptically prepared)** | 800 mls (20 eggs) |

This makes an average of 140 slants of media.

**Equipment** to Prepare and Sterilize

1—250 ml Screw Cap Flask
1—2 Liter Aspirator Bottle with magnetic stirring bar within a small bell filling attachment on rubber hose to dispense aseptically into tubes.
1—large funnel lined with 2 layers of unbleached muslin and covered with huck towel (muslin taped in place) then wrapped in paper and taped to sterile.
2—quart jars with Osterizer blender tops—sterilized in inverted position.
1—pipette or glass rod to stir eggs in funnel.
Tubes—20 x 125 Screw Cap in sufficient quantity.

**Method of Preparation**

Mix salt solution until completely dissolved. Adjust pH to 6.5 with

**Eggs should be strictly fresh. If source is known—eggs should be from hens that have had no antibiotics in feed or medicine.**

Prepare eggs as follows: Wash with brush in warm detergent water, rinse in clear water and lay out on towel to dry. Soak in 75% isopropyl alcohol solution for 30 minutes. Dry by inserting between layers of sterile towels. Dip top of a beaker in alcohol. Burn off alcohol with bunsen burner to sterilize. Break eggs on sterile edge of beaker, drop into sterile blender jars. Mix gently on Osterizer just enough to homogenize egg mixture but not enough to cause air bubbles. Add dye mixture to salt mixture in the aspirator bottle. Filter eggs through sterile muslin lined funnel into solutions in the aspirator bottle. Put cotton gauze plug from bottle between layers of the huck towel while eggs are filtering through. Stir eggs with sterile pipette or rod if necessary to help filtering process and keep eggs in funnel covered with towel as much as possible. When egg mixture has completed filtering, then replace cotton gauze plug in aspirator bottle and mix gently but thoroughly on magnetic stirrer, until the media is evenly blended. Dispense into tubes aseptically. Dispense on bell side of tube only and do not shake tube after filling to avoid film on more than bell side. Dispense in 9 ml amounts in 20 x 125 S. C. tubes. Inspissate for 40 minutes at 80 degrees C with tubes in slant position. Cool slightly. Incubate 24 to 48 hours in slant position so excess moisture will be reabsorbed by media.

MODIFIED P & B MEDIUM WITH 5% HORSE SERUM

Ingredients

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Asparagine</td>
<td>5 gms</td>
</tr>
<tr>
<td>Potassium Phosphate Monobasic</td>
<td>5 gms</td>
</tr>
<tr>
<td>Potassium Sulfate</td>
<td>5 gms</td>
</tr>
<tr>
<td>Glycerol</td>
<td>20 mls</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>930 mls</td>
</tr>
<tr>
<td>Magnesium Citrate</td>
<td>1.5 gms</td>
</tr>
<tr>
<td>Horse Serum (sterile)</td>
<td>50 mls</td>
</tr>
</tbody>
</table>

Method of Preparation

Dissolve L-Asparagine by heating in part of the water until clear. Dissolve each of the next two chemicals in small amounts of water separately. Add to asparagine mixture, add glycerol and rest of water. Mix thoroughly. Adjust pH to 7.0 with 10 Normal Sodium Hydroxide. Now add the 1.5 gms of Magnesium Citrate. Mix until in solution. Pour into aspirator bottle with attachment for aseptic dispensing. Sterilize in autoclave at 121 degrees C for 20 minutes. Cool to 50 degrees C and add aseptically the sterile Horse Serum. Dispense aseptically into 20 x 125 Screw Cap test tubes in 4 ml amounts. Incubate at 37 degrees C to check sterility.
### DUBOS BROTH WITH TWEEN 80 WITH DUBOS OLEIC ALBUMIN COMPLEX WITH INH*

**Ingredients**

<table>
<thead>
<tr>
<th></th>
<th>Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dubos Broth Base (Difco 0385)</td>
<td>6.5 gms</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>890 mls</td>
</tr>
<tr>
<td>0.1% INH solution (Isoniazid) (We use the Isoniazid from Squibb &amp; Sons called Nydrazid 6375)</td>
<td>10 mls</td>
</tr>
<tr>
<td>Dubos Oleic Albumin Complex (Difco 0375)</td>
<td>100 mls</td>
</tr>
</tbody>
</table>

**Method of Preparation**

Mix Dubos Broth Base and water until in complete solution. Add the INH solution and mix thoroughly. Pour into aspirator bottle with attachment for aseptic dispensing. Sterilize in autoclave at 121 degrees C for 20 minutes. Cool to below 56 degrees C. Add aseptically the Dubos Oleic Albumin Complex and mix well. Dispense aseptically into 20 x 125 Screw Cap tubes in 8 ml amounts. Incubate at 37 degrees C to check sterility.

### DUBOS BROTH WITH TWEEN 80 WITH DUBOS OLEIC ALBUMIN COMPLEX WITH TCH†

**Ingredients**

<table>
<thead>
<tr>
<th></th>
<th>Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dubos Broth Base (Difco 0385)</td>
<td>6.5 gms</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>900 mls</td>
</tr>
<tr>
<td>Dubos Oleic Albumin Complex (Difco 0375)</td>
<td>100 mls</td>
</tr>
<tr>
<td>1% solution of Thiophencarbonsaure-Hydrazid** (filter sterilized)</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

**Method of Preparation**

Mix Dubos Broth Base and water until in complete solution. Pour into aspirator bottle with attachment for aseptic dispensing. Sterilize in autoclave at 121 degrees C for 20 minutes. Cool to below 56 degrees C. Add aseptically the Dubos Oleic Albumin Complex and the filter sterilized TCH solution. Mix well. Dispense aseptically into 20 x 125 Screw Cap tubes in 8 ml amounts. Incubate at 37 degrees C to check sterility.

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*INH is abbreviation for Isoniazid—also called by other trade names. INH concentration to be 10 micrograms per ml of media.

†TCH is an abbreviation of Thiophencarbonsaure-Hydrazid—used at the rate of 10 micrograms per ml of media.

**Thiophencarbonsaure-Hydrazid can be obtained from the following sources:
Karl Roth, Herrenstrasse 26-28, Karlsruhe, Deutschland (Germany)
Aldrich Chemical Co., Inc., 2371 N. 30th St., Milwaukee, Wisconsin
(Thiophen-2-Carboxylic Acid Hydrazide) $200.00 per 100 grams.
DUBOS TWEEN ALBUMIN BROTH WITH NEOTETRAZOLIUM CHLORIDE.*

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dubos Broth Base without Tween 80 (Difco 0435)</td>
<td>6.5 gms</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>890 mls</td>
</tr>
<tr>
<td>Tween 80</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>50% glucose solution (filtered sterilized)</td>
<td>10 mls</td>
</tr>
<tr>
<td>Dubos Oleic Albumin Complex (Difco 0375)</td>
<td>100 mls</td>
</tr>
<tr>
<td>1% Neotetrazolium Chloride solution**</td>
<td>2.5 mls</td>
</tr>
</tbody>
</table>

Method of Preparation

Mix and warm Dubos Broth Base and water until in complete solution. Add Tween 80 to the hot medium and mix thoroughly. Pour into aspirator bottle with attachment for aseptic dispensing. Sterilize in autoclave at 121 degrees C for 20 minutes. Cool to below 56 degrees C. Add aseptically the sterile Glucose solution, Dubos Oleic Albumin Complex and the Neotetrazolium Chloride solution. Mix thoroughly. Dispense aseptically into 20 x 125 Screw Cap test tubes in 8 ml amounts. Incubate at 37 degrees C to check sterility.

GLASSWARE INSTRUCTIONS:

Test tubes for this medium should be new or absolutely free from scratches so as not to hinder vision of media.

REFERENCES


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*Neotetrazolium Chloride concentration to be 1 to 40,000.

**Autoclaved 15 minutes at 121 degrees C to sterilize.
REPORT OF C.V.L.D. COMMITTEE ON STANDARD PROCEDURES FOR THE ISOLATION OF SALMONELLA FROM ANIMAL FEEDS AND MEAT BY-PRODUCTS

L. C. Grumbles, Chairman, College Station, Texas; T. Bartram, Washington, D. C.; E. M. Ellis, Ames, Iowa; M. M. Galton, Atlanta, Georgia; W. Henderson, Lafayette, Indiana; B. S. Pomeroy, St. Paul, Minnesota; J. E. Williams, Athens, Georgia.

The Committee on Standard Procedures for the Isolation of Salmonella was requested to consider revision of ARS 91-36, Recommended Procedures for the Isolation of Salmonella Organisms from Animal Feeds and Meat By-products.

The various suggestions for revision proposed by the Technical Service of the Animal Health Division were circulated to the members of the committee and each member was requested to review ARS 91-36 and forward suggestions to the chairman of the committee. Response to this request was good and most members of the committee responded with definite suggestions. Unfortunately the committee did not have an opportunity to meet as a group to resolve areas of disagreement. There was good agreement on some points and the suggested revisions in ARS 91-36 at this time are:

1. Include lysine iron agar along with triple sugar iron agar for presumptive screening of suspected colonies of Salmonella (page 2, paragraph 5).

2. Add as last sentence—page 3, paragraph 5: "lysine iron agar is especially useful to differentiate between Salmonella and Citrobacter. Most Salmonella produce lysine decarboxylase indicated by an alkaline reaction in the medium (blue or purple). The typical reaction of Citrobacter is an alkaline slant with an acid (yellow) butt. H₂S production can be detected in lysine iron agar, but formation is often irregular or suppressed except in the Citrobacter group."

3. Add the following sentence as a last sentence on page 6, paragraph 3: "Sodium sulfadiazine is available in sterile solution from the American Cyanamid Company."

4. Add the following statement about preparation and storage of brilliant green agar as last sentence on page 5, paragraph 3: "Do not expose brilliant green agar to direct sunlight. If the medium is not used the day the plates are prepared, they should be stored in the dark at 4° C."

5. Revise Table I, page 6 as follows: Title—Change to Read: Reactions of Several Groups of Organisms on Triple Sugar Iron Agar. Footnote 2—Eliminate and change reaction of Salmonella spp. under H₂S column to read: "+ or -".

Suggestions were made which are not included in the recommended change. For example, a majority of the committee does not believe there is good reason to change the size of sample from 30 grams to 25 grams. Pre-enrichment in a non-selective broth may be a useful procedure, however,
the committee would like to see additional comparative studies on this in several laboratories before recommending it as a universal procedure.

This committee believes that the procedures outlined in ARS 91-36 are still sound and are a good guide for laboratories conducting cultural examinations of animal feeds and meat by-products. Improvements and revisions should be made to keep the publication current and make it most useful. It will be necessary for the committee to meet in a work session to resolve many of the questions and prepare a more thorough revision. Hopefully this can be done at some future date.
STANDARD PROCEDURES FOR THE ISOLATION AND IDENTIFICATION OF PARATUBERCULOSIS ORGANISMS

C. S. Roberts, D.V.M., M.S., Chairman* A. B. Larsen, D.V.M., M.S.**

Specimens

The specimen submitted to the laboratory should consist of about 6 inches of the terminal portion of the ileum, including the ileo-cecal valve. In preparing this material for shipment, the intestinal content should be flushed out carefully with water without splitting the intestine. The specimen should be immediately frozen and sent to the laboratory.

Diagnostic Procedure

Three methods are used for the diagnosis, they are:

I. Demonstrating the bacillus in histological sections;
II. Demonstrating the bacillus in smears prepared from digested intestinal tissue.
III. Culturing the bacillus.

Method I. Select a piece of tissue about ¾” square from the terminal portion of the small intestine including a very small piece of the ileocecal valve. Place the tissue in 10% formalin, dehydrate with ethyl alcohol, clear, embed, section and stain using Ziehl-Neelsen acid-fast technique as modified for staining tissues. Most of the acid-fast bacteria will be found in macrophages or giant cells. The bacillus is a short thick red rod about 1.5 μ x 0.5 μ in size. The advantage of using this method is that only M. paratuberculosis will be found in the tissues and they will not be confused with other acid-fast bacteria.

Method II. Obtain 1-2 gm. of intestinal mucosa ½” anterior to the ileocecal valve. Macerate this with a Tyndal glass grinder or blender and completely disperse in about 40 ml. of 1% trypsin solution. Pour into a 50 ml capped centrifuge tube, adjust to pH 9 and shake for 40 minutes. Centrifuge at 2500 r.p.m. for 45 minutes, decant the supernatant and make a smear from the sediment. Stain, using the Ziehl-Neelsen acid-fast technique. This method is the most convenient to use, but has the disadvantage that it is possible to confuse other acid-fast bacteria with M. paratuberculosis. However, this is not likely to happen if an experienced observer is examining the smears.

Method III. To culture the bacillus, add 40 ml of 0.1% Benzalkonium Chloride to the sediment remaining in the centrifuge tube after the smear has been prepared as described in Method II. Shake well and allow to remain

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**National Animal Disease Laboratory, Animal Disease and Parasite Research Division, P. O. Box 70, Ames, Iowa.
at room temperature for 1 day. Then seed, .5 ml of the sediment on 4 slants of modified Herrold's medium. Incubate in a slanted position at 38°C with caps loose for 1 week to allow excess fluid to evaporate. Tighten caps and continue incubating for 12 weeks. Small white, acid-fast colonies are verified by subinoculation onto a fresh tube of modified Herrold's medium and on another tube that does not contain M. phlei or and extract of M. phlei.* If growth occurs only in the tube containing M. phlei, the culture is considered to be M. paratuberculosis. This method has the advantage that if carefully carried out, it is possible to cultivate the bacillus when it is present in such small numbers that it cannot be demonstrated by the other methods.

Comments

Most specimens received at a diagnostic laboratory will be from suspected clinical cases and generally these are far enough advanced that the bacillus will be present in large enough numbers to be detected by Methods I or II.

Slides from positive cases of paratuberculosis should be kept on hand and the morphology of the bacillus should be compared with the morphology of the bacilli observed on the slides prepared from suspected cases.

A stock solution of 5% trypsin in 5 ml vials is kept in the deep freeze and the quantity desired is thawed just before use. Trypsin solution must be frozen as soon as prepared, and used as soon as it is thawed. This is very important.

REFERENCES FOR ADDITIONAL READING


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*A crude mycobactin, prepared by acetone extraction of M. phlei, now replaces the M. phlei suspension.
INTRODUCTION

Histopathologic and mycobacteriologic examinations should be conducted by the same laboratory to enable continuing evaluation of results obtained from each examination. For reference of mycobacteriology see "A Manual for the Isolation and Typing of Mycobacteria," by Diagnostic Services, NADL, Ames, Iowa 1967. All diagnoses of tuberculosis should be confirmed by histopathologic examination. The hazards of basing a diagnosis of tuberculosis on gross lesions alone has been repeatedly emphasized. Davis and Anderson reported 41 of 77 (53.3 per cent) lesions suspected of being tuberculous were granulomas due to other causes.¹ These same workers in another series of 418 cases demonstrated that 45.7 percent of the lesions were not tuberculous.² In a study of 1608 bovine specimens Morehouse et al. confirmed the diagnosis of tuberculosis in 49.8 per cent of the cases.³

1. Standard procedures should include the microscopic examination of tissue sections stained to demonstrate cellular components of the lesions as well as acid-fast bacilli and other likely etiologic agents.⁴ Combination stains are not recommended.

Acid-fast bacilli have been cultured from lesions caused by a variety of other etiologic agents.⁵ ⁶ ⁷ Isolations of M. bovis,⁵ ⁶ M. avium,⁴ ⁷ ⁸ and the anonymous mycobacteria⁹ ¹⁰ have also been made from apparently normal animal tissues.

2. Adequate controls on the processing of tissues for histopathologic examination should be required. Control tissue sections of known tuberculous and normal tissue should be processed by the routine procedures and examined by the pathologist or laboratory supervisor. This should be done at weekly intervals, or if tuberculous tissues are not routinely processed, controls should be run on each group of tissues.

Staining of sections of known tuberculous tissues will test the reliability of the stain components and operational techniques in demonstrating the acid-fast bacilli. Similar processing of normal tissues will reveal the presence of contaminating acid-fast bacilli. These ubiquitous organisms have...
produced erroneous diagnoses on three occasions in a hospital laboratory.\textsuperscript{10} The organisms have been isolated from gelatin, a water aerator and water sediment\textsuperscript{10} as well as paraffin.\textsuperscript{11}

3. Where prompt results are desired, the use of rapid decalcification and frozen sectioning is recommended.\textsuperscript{12,13} In such cases adjacent blocks of lesions should be prepared by conventional methods for later examination.

4. In those lesions where tissue changes are suggestive of tuberculosis, and where no acid-fast bacilli have been demonstrated by routine staining procedures; fluorescent stains should be employed. The following auramine 0 staining procedure is recommended:

A. Deparaffinize for five minutes each in three changes of xylene.
B. Place the slides in each of the following solutions successively for approximately 5 minutes: three changes of absolute alcohol; one change of 95\% alcohol; 80\% alcohol; and 70\% alcohol.
C. Stain for 10 minutes in Auramine 0 staining solution prepared as follows:

\begin{verbatim}
Auramine 0 (C.I. 41000, Matheson Coleman & Bell) 3 gm
Liquefied phenol 32 ml
Glycerin 70 ml
Distilled water 900 ml
\end{verbatim}

The auramine 0 should be dissolved in the phenol, and the glycerin and distilled water added to the phenol-auramine 0 mixture. The final solution should be filtered through gauze and cotton. The staining solution should age for at least 24 hours before use. The auramine 0 stains in the staining dish should be changed at least every two weeks and the stain should not be used for more than 8 weeks after preparation.

D. Decolorize with one quick dip in 20\% sulfuric acid.
E. Dip quickly in 80\% alcohol.
F. Place in 10\% ferric chloride for 5 minutes.
G. Place in 95\% alcohol for 30 seconds. (This solution and the 80\% alcohol in the next step should be changed frequently.)
H. Place in 80\% alcohol for 30 seconds and allow the slides to air dry.
I. Coverslip with mounting media for fluorescent microscopy and coverslips of no. 1\frac{1}{2} thickness.

The control procedures described in Section 3 should also be followed with the auramine 0 technique.

The slides should be examined with a microscope suitably equipped for fluorescent microscopy. Bacteria demonstrated to be acid-fast by the auramine 0 technique exhibit a yellow-gold fluorescence. Acid-fast bacilli detection was increased (approx. 28\%) in cattle granulomatous lesions by auramine 0 staining (unpublished data, N.A.D.L.).

5. Specimen description data should be recorded on the ANH form 1-15 Mycobacteriology and Pathology Data for machine records processing and submitted to the Chief Staff Officer for bovine tuberculosis, Federal Center Building, Hyattsville, Maryland 20782.
6. The pathology section of Diagnostic Services, NADL, should serve as the reference center for Histopathologic examination of specimens suspected of being tuberculous. Approval of laboratories to conduct histopathological examination of suspected tuberculous specimens as part of the national T.B. eradication program should be contingent upon the results of a site visit by a designated representative from the National Conference of Veterinary Laboratory Diagnosticians and a representative of Diagnostic Service, NADL, and continual approval would be based on findings during periodic visits by these representatives.

7. Reporting will be carried out in accordance to directive from Chief Staff Veterinarian, Tuberculosis Eradication.

8. Lesions typical of tuberculosis in which acid-fast bacilli are demonstrated should be considered as tuberculous. Reports should indicate one of the following categories:

A. Compatible—mycobacteria and tuberculous lesion present.
B. Suggestive—granulomatous lesions present, but neither mycobacteria nor other etiologic agents were found.
C. Not suggestive—neither mycobacteria nor granulomatous lesions were found.

REFERENCES
A questionnaire concerning the charging of fees for animal diagnostic laboratory services was made in September and October of 1967. Questionnaires were submitted to all laboratories listed in the Manual and Directory of Animal Diagnostic Laboratories in the United States prepared by Technical Services, Animal Health Division, ARS, USDA, the January 1967 edition. The purpose of this survey was to determine the numbers of laboratories making a change for a portion or for all of their services offered.

Eighty-nine laboratories responded. The information is summarized as follows:

No. making no charges—60
No. making charges for a portion of their services—20
No. making charges for all services offered—9

Governmental Supported Laboratories—51 reporting
No charges—38
Partial charges—8
Total charges—5

University Associated Laboratories—32 reporting
No charges 18
Partial charges—11
Total charges—3

Commercial Laboratories—6 reporting
No charges—4
Partial charges—1
Total charges—1

The items listed where charges were made on only a portion of the services offered were:

1. Poultry serology
2. Serology—all species
3. Fecal flotations
4. Milk bacteriology
5. Histopathology
6. Out of state cases
7. Poultry cases
8. Pet animals
9. Antibiotic sensitivities
10. Blood counts
11. Pregnancy tests—mares
12. Trichomoniasis exams
13. Vibrio fetus exams
14. Toxicology

Of the nine laboratories reporting a charge for all services offered, three offer milk bacteriology only, two do poultry diagnosis only, and four offer general diagnostic service on all species.

The most commonly listed advantages of charging a fee were:

1. Eliminates nuisance or curiosity cases.
2. Provides additional funds for equipment and personnel in otherwise inadequate budgets.
3. Places the major cost on the benefactors of laboratory diagnosis.
4. Selective charges can be made for exams which often are not essential to diagnosis.
5. Charging fees encourage the submission of better selected specimens with adequate histories, with better utilization of the results.

The most commonly listed disadvantages of charging a fee were:

1. Fees would reduce the volume of specimens, thus reducing the surveillance and observation functions of a diagnostic laboratory to aid in the control of infectious diseases.
2. No fees encourage more specimens for teaching purposes.
3. In most government supported laboratories, any fees collected return to the general fund and no direct monetary support by the laboratory is realized.
4. The extra bookkeeping, billing, and auditing procedures required are prohibitive.
5. Our fees can be “token fees” only. Fees adequate to cover all costs of the service are prohibitive.
6. Most laboratories that do charge do so to eliminate nuisance cases—if this is a problem, other methods should be employed to eliminate this.
7. It costs us about $5,000.00 a year to collect $8,500.00 in fees.
8. Actual costs would be prohibitive. Our cost would be $30.00 per accession. The actual cost for an autopsy and routine tests for a typical case would be about $240.00.
9. Fees would eliminate services to the small farmer and tend to limit service to the larger commercial groups.
10. It is my opinion that a tax supported veterinary diagnostic laboratory cannot effectively provide a complete service to the livestock industry on a fee basis.
THE KOH METHOD FOR DETECTING THE GRAM-STAINING POTENTIAL OF BACTERIA WITHOUT STAINING

D. M. FLUHARTY, D.V.M., M.S.*
Pullman, Washington

In teaching veterinary students to effectively utilize laboratory diagnostic aids, we have always been interested in finding screening methods that are simple to run, yet accurate. Two of these methods in the area of diagnostic bacteriology will be described in this paper and the one that follows.1 These methods may be new to many diagnosticians and should prove useful in their laboratories.

In 1940, a Japanese investigator2 at the Kitasato Institute, Tokyo, described three simple methods for detecting potential Gram-staining characteristics of bacteria without staining. Of the three methods, the caustic potash (KOH) test was most practical and dependable. With this method, a pure culture of bacterial growth from solid medium was mixed with a drop of 3% aqueous KOH on a glass slide. Gram-negative bacteria formed a viscous mass and Gram-positive bacteria did not. The second method involved mixing bacteria with half a drop of concentrated sulfuric acid. Cultural masses of Gram-negative organisms became transparent, while Gram-positive organisms showed no change. In the third method, a fresh growth of organisms from solid medium was smeared over a slide rather thickly, then dried and fixed with a flame. Several drops of water or 5% aqueous carbolic acid were then placed on the smear. The slide was then passed through a flame and shaken lightly. With preparations of Gram-negative organisms treated in this fashion, most of the organisms remained fixed to the slide. This latter method was called the carbolic acid method.

The investigator, Eihyo Ryu,3 then utilized these three methods to check fresh pure cultures from solid media of a number of various bacteria and compared results with those from the Gram-stain. The following Gram-negative organisms were tested: Actinobacillus mallei (3)**; Aerobacter aerogenes (1); Alcaligenes faecalis (1); Brucella sp. (6); Eberthella typhosa (7); Escherichia coli (13); Hemophilus sp. (2); Klebsiella pneumoniae (2); Meningococcus intracellularis (1); Neisseria sp. (12); Pasteurella sp. (9); Proteus vulgaris (8); Pseudomonas aeruginosa (1); Salmonella sp. (28); Shigella dysenteriae (3); and Vibrio cholera (4); The Gram-positive organisms tested included: Actinomyces bovis (2); Bacillus sp. (4); Clostridium chauwei (1); Cl. tetani (20); Cl. welchi (4); Corynebacterium sp. (32); Diplococcus pneumoniae (4); Erysipelothrix rhusiopathiae (1); Mycobacterium tuberculosis (4); Staphylococcus sp. (7); Streptococcus pyogenes (20); and Str. viridans (10).

Ryu determined that the KOH method agreed with the Gram's differential stain without exception in the various genera, species, and 210

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*Associate Professor, Department of Veterinary Pathology, College of Veterinary Medicine, Washington State University.
**Numbers in parentheses represent the number of strains tested.
stains tested. Although Clostridium chauvei and Cl. tetani are ordinarily considered Gram-positive organisms, he found that all stains tested became Gram-negative after culturing 18-24 hours on artificial media and likewise produced a viscous mass with the KOH reagent. The concentrated sulfuric acid reagent also produced results that were in agreement with the Gram-stain, whereas the carbolic acid method showed several discrepancies.

MATERIALS AND METHODS

For the last 4 or 5 years, the KOH method has been used successfully along with the Gram-stain on routine cultures made in the Veterinary Clinical Diagnostic Laboratory at Washington State University (WSU). The KOH method has proven particularly time-saving and useful in screening various colonies picked from blood-agar plates. The KOH method was selected in preference to others suggested by Ryu because the reagent involved was less hazardous than concentrated sulfuric acid, and the method seemed more accurate than the carbolic acid method.

To confirm the validity of the KOH method on a wider spectrum of bacterial genera than might be encountered in routine diagnostic bacteriology, the stock culture collection maintained by the Department of Veterinary Microbiology at WSU was tested. First, bacterial growth was removed directly from the surface of blood or brain-heart-infusion agar upon which the stock cultures had been maintained at 4°C for 2 to 10 months. The routine Hucker's modification of the Gram-stain and the KOH test were conducted on each culture. In a second trial, stock cultures were first transferred to blood, brain-heart-infusion, or nutrient agar slants and incubated for 16 to 20 hours, and then the young cultures were tested as before. The details of the KOH test as conducted as follows:

1.) One or two loopsful (4 mm inside diameter) of 3% KOH were transferred to a microscopic slide.
2.) A second loop (2 mm inside diameter) was used to transfer the pure culture or isolated bacterial colonies from the surface of the solid medium into the KOH solution on the slide.
3.) The two materials were mixed on the slide. If the mixture became markedly viscid or gelled within 5 to 60 seconds, the isolate was considered Gram-negative. It was also noted that such isolates often produced a string-like mass that adhered to both the loop and the slide when separated. If no such viscosity or gel was produced, the isolate was considered Gram-positive.

Three preliminary experiments were conducted also in an effort to define the mechanism by which KOH produces viscosity in Gram-negative organisms. In one trial, several Gram-negative and Gram-positive isolates were mixed with California Mastitis Test reagent on the premise that the viscosity might be related to DNA or RNA, as is the case with the CMT reaction in milk. In another trial, 3% and 10% NaOH was substituted for 3% KOH and tested with several Gram-negative and Gram-positive isolates. In a third trial, 2 ml masses of coliform and staphylococcic organisms
were subjected to ether and ether-alcohol extractions in an attempt to remove lipid fractions from bacterial cell walls. The washed cells were spun-down in a refrigerated centrifuge. The bacterial cell masses at the bottom

### TABLE I
**Comparison of Results Obtained with the KOH Method and the Gram-Strain on Pure Cultures of Bacteria in the WSU Stock Culture Collection, Department of Veterinary Microbiology**

<table>
<thead>
<tr>
<th>Gram-Negative Bacteria Tested</th>
<th>Number of Strains</th>
<th>KOH</th>
<th>Gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobacter sp.</td>
<td>1</td>
<td>+. *</td>
<td>- *</td>
</tr>
<tr>
<td>Bordetella sp.</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Brucella suis</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>9</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia freundii</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Moraxella bovis</td>
<td>1</td>
<td>-</td>
<td>var.*</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Past. pseudotuberculosis</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>7</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Shigella dysentariae</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Vibrio sp.</td>
<td>3</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>31</strong></td>
<td><strong>30</strong></td>
<td><strong>30</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gram-Positive Bacteria Tested</th>
<th>Number of Strains</th>
<th>KOH</th>
<th>Gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Corynebacterium equi</td>
<td>2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Coryn. pyogenes</td>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Coryn. pseudotuberculosis</td>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Gaffkya sp.</td>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Listeria sp.</td>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sarcina lutea</td>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>14</strong></td>
<td><strong>14</strong></td>
<td><strong>14</strong></td>
</tr>
</tbody>
</table>

*(+ or -) signifies positive or negative to all strains tested.

*Var. = Gram-variable.

†Total strains negative to the Gram-stain.
of the tubes were then agitated with 10 ml of ether for 30 minutes at room temperature. After re-centrifugation and separation, the supernatant ether extracts were evaporated and the residues tested with 3% KOH. Also the bacterial cell masses that had been exposed to the extraction were tested with 3% KOH. This trial was repeated using a mixture of 50% ether and 50% ethyl alcohol as the solvent.

**RESULTS**

The older stock cultures in the WSU collection tested with KOH produced results essentially similar to those obtained with younger cultures from the same source. The only difference was that Gram-negative isolates among the younger cultures required 5 to 15 seconds to form a gel with KOH. Similar Gram-negative isolates from some of the older stock cultures required up to 60 seconds. A summary of the results obtained with the KOH test and the Gram-stain on the younger stock cultures is given (Table I). As the table shows, 30 of 31 cultures of bacteria currently accepted as Gram-negative formed a gel or viscous mass with the KOH test. The one exception, *Moraxella bovis*, proved to be Gram-variable with the Gram-stain used in the trial. Also, 14 of 14 bacterial cultures currently considered to be Gram-positive did not form a viscous mass with KOH reagent, i.e., they were negative to the KOH test.

In a trial in which CMT reagent was mixed with several Gram-negative and Gram-positive cultures, the reagent failed to cause a viscous mass. In a trial, mixing 3% or 10% NaOH with Gram-negative cultures, results were somewhat variable. The 3% NaOH caused clumping or agglutination of organisms without a consistent, grossly visible gel formation. The 10% NaOH did cause a grossly recognizable gel formation with most Gram-negative cultures tested. Gram-positive cultures failed to show any gel formation with either 3% or 10% NaOH. It was noticed also that when gelled, Gram-negative bacteria were washed from slides with about 10 volumes of distilled water, then placed into a test tube and shaken, a surface foam resulted on the column which persisted for 10 to 30 minutes. Gram-positive bacteria treated in the same fashion failed to elicit this phenomena. In the solvent extraction trials, the gelling agent which reacts with 3% KOH apparently was unaffected. Residues from solvent extracts made with ether or ether-alcohol on coliform and staphylococcic organisms did not gel with 3% KOH. The coliform organisms, after being subjected to the solvents, still produced a gel with 3% KOH and the staphylococcic organisms did not.

A further investigation using known mixtures of Gram-negative rods and Gram-positive rods or cocci, grown for 16 to 20 hours on solid media, demonstrated that all cultures containing mixed growth of Gram-positive and Gram-negative bacteria resulted in gel formation when tested with KOH. This suggests that well isolated colonies and cultures of bacteria must be used in order for this test to be reliable.
DISCUSSION

Results reported here essentially confirm those of the original investigator, namely, that many medically important bacteria from a number of genera can be separated as to Gram-staining potential simply by mixing pure growth from solid media with 3% KOH. The few discrepancies found in these trials and those of Ryu have concerned bacteria which changed their Gram-staining characteristics on artificial media or which were indefinite as to their Gram-staining characteristics. Moraxella bovis has been variously described by one author as Gram-negative and by another as Gram-variable.

The nature of chemical or morphologic changes occurring in Gram-negative bacteria when they are mixed with KOH is unknown. With present knowledge, the KOH test must be considered empirical. Whether or not the KOH reaction on bacteria is chemically related to the changes occurring during the Gram-reaction is open to speculation. Since Christian Gram of Denmark first introduced the "Gram-technic" 83 years ago, several theories to explain the phenomenon have been proposed but remain unproven. (Fig. 1) The theory favored by Oginsky and Umbriet (Theory 1, Fig. 1) is that in Gram-positive bacteria a magnesium, ribonucleic acid, sulfur-containing protein, crystal-violet, iodine complex is formed at the outer surface of cells which is insoluble in alcohol. Such a complex does not form in Gram-negative bacteria. Another theory is that the cell membrane or the cell wall of Gram-positive bacteria are not permeable to the

![Diagram of bacterial cell with labels: CAPSULE, CELL WALL, G(-) = 20% Fats, G(+) = 2% Fats, G(+) = No a.a. with S-groups, *1. Mg-RNA-S-Prot.-CV-1, Complex at outer surface, CELL MEMBRANE, *2. Membrane in G(+) Not permeable to CV-1, CYTOPLASM, *3. Isoelectric: G(+) have low IP and hold basic dyes, * = Theories of Gram Stain. Figure 1. KOH Method.
crystal violet-iodine complex which forms within the cell, whereas Gram-negative organisms are permeable. It is difficult to relate the KOH reaction to either of these theories. It is known that the CMT reagent, alkyl-aryl-sulfonate, reacts with leukocyte DNA to produce a gel-like complex.7 Also, the reagent in the Whiteside test, 4% NaOH, reacts with inflammatory material in milk samples, presumably leukocytes, to produce a gell-like mass.8 On the premise that the viscosity produced in Gram-negative bacteria by KOH might be related to more exposed nucleic acids (RNA), the CMT reagent was tested against both Gram-negative and Gram-positive organisms. No viscosity resulted with either type of organism. The third theory for the Gram-stain (Theory 3, Fig. 1) is that the cytoplasm of Gram-positive bacteria has a lower isoelectric point and hence, holds basic dyes, such as crystal violet, better than the cytoplasm of Gram-negative bacteria. Since both a strong base (KOH) and a strong acid (H2SO4) in Ryu's4 methods will cause grossly visible differences between masses of Gram-negative and positive bacteria, one might conceive a relationship between a theory involving pH factors in the Gram-stain and the findings of Ryu.

Some other morphologic and chemical features of bacterial outer surfaces are summarized (Fig. 1). The capsule or outer slime layer varies in composition from species to species and even from strain to strain.6 These substances are thought to function variously as protective materials, nutrient substrates, or by-product reservoirs and have been variously identified as polysaccharides, polypeptides, lipo-proteins, or combinations of these. Due to their variability, it would seem unlikely that there would be a chemical relationship between the capsular substances and the Gram-stain or KOH reaction.

Bacterial cell walls are thought to have little selective permeability, but give bacteria their semi-rigid borders. Orginsky and Umbriet6 have indicated that there seems to be considerable difference in chemical composition between Gram-negative and Gram-positive bacterial cell walls, with one difference being the content of lipid. Gram-negative bacteria contain about 20% lipid in cell walls and Gram-positive about 2%. From the above characteristics, one might assume that fatty-acid soaps could be formed when strong alkali solutions are mixed with Gram-negative organisms and not with Gram-positive. Possible support for this explanation is the finding reported in these trials that 10% NaOH as well as 3% KOH will produce a gel with certain Gram-negative bacteria. Also, aqueous solutions of such gels, when shaken, show a detergent-like foam on their surface. On the other hand, attempts made in these trials to extract the gelling agent from E. coli organisms with ether and ether-alcohol solvents were unsuccessful. Possibly the lipids are so intimately incorporated in bacterial cell walls that their solvent extraction is inhibited, whereas hydrolytic release of their fatty-acid fractions may occur readily with strong alkali solutions.

Recently, studies by Bartholomew, Cromwell, and Gan9 indicate that the phenomenon in the Gram-stain mainly concerns the permeation rate of dye, iodine, and solvent molecules through the interstitial spaces in cell-wall
material. Their results suggest that the thicker cell walls and the smaller "pore" size in Gram-positive bacteria may be involved in greater retention of dye. How the KOH reaction might relate to such a Gram-stain hypothesis is purely speculative. Perhaps, if lipids are involved in both reactions (KOH and Gram's), the higher lipid content in cell walls of Gram-negative cells forms a more miscible base through which alcohol solvents can carry the dye and iodine from such cells. In conclusion, the KOH reaction and others described by Ryu, are useful adjuncts to both applied bacteriology (as useful screening tests to supplement the Gram-stain) and to basic research (as additional reactions to help explain the nature of bacterial surfaces).

REFERENCES

THE DIRECT COAGULASE-TEST ON INCUBATED MILK FOR DETECTING PATHOGENIC STAPHYLOCOCCI

D. M. FLUHARTY, D.V.M., M.S.*
Pullman, Washington

Diagnostic laboratory personnel and veterinarians involved in mastitis control programs often need to know if a given herd, udder, or lactating quarter contains pathogenic staphylococci. Many mastitis tests (the catalase test,¹ the Whiteside test,² the California mastitis test,³ the Hotis test,⁴ and stained milk smears⁵) are not specific for diagnosing mastitis caused by pathogenic staphylococci. Other methods require special media or several time-consuming manipulations in the laboratory (the various tellurite, glycine, polymyxin, or egg-yolk agars,⁶ or rabbit-plasma agar⁷).

The ability to produce coagulase and to clot plasma has been accepted generally as the most reliable in vitro criterion for determining pathogenicity of staphylococci.⁸ The coagulase enzyme has been detected by three methods: by mixing a colony or fluid culture of staphylococci with decalcified animal plasma in a test tube (the tube test); by observing for a halo around colonies when pathological material is cultured on nutrient agar containing plasma (the agar-plate test); or by observing for clumping of organisms when a heavy suspension of staphylococci is mixed with plasma (the slide test). The tube test has been considered the most reliable of the three methods.⁸

The coagulase enzyme is not only found within bacterial cells but much is released into surrounding media.⁸ This occurs when pathogenic staphylococci are cultured in or on a variety of nutrient media. These facts led to the idea that a suspected milk sample itself might serve as an adequate medium for coagulase production. In other words, with a carefully collected sample from a cow with staphylococcal mastitis, the diagnostician should have per se the biological system necessary for coagulase production. Both the inoculum and a nutrient substrate should be present in the sample as collected and the diagnostician merely would need to incubate the sample, then check for coagulase production by the tube test. The following trials were conducted to determine the potential value of such a diagnostic test.

MATERIALS AND METHODS

Eighty-five milk samples as routinely received by the Washington State University Veterinary Diagnostic Laboratory between July 8 and October 28, 1963, were tested. These were collected into sterile Hotis tubes,⁴ then iced or delivered directly to the laboratory. Various types of samples were included among those tested. Some were from herds tested by

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DETECTING PATHOGENIC STAPHYLOCOCCI

a composite sampling from the four quarters of each cow. Others were separate quarter samples. A few were from cows with acute or gangrenous mastitis, others from cows with subacute, chronic or latent mastitis, and others from cows free of mastitis.

The flow chart (Fig. 1) shows methods employed in these trials to compare the efficiency of several culture procedures for detecting presence of coagulase-positive staphylococci in milk samples. In method No. 1, after the Hotis tube was thoroughly shaken, several loopsful of fresh milk were streaked directly onto a sheep blood-agar plate which was then incubated at 37°F. for 24 to 48 hours. Typical staphylococcal colonies were then removed from the plate and introduced into 0.5 ml. of rabbit plasma. In method No. 2, 0.5 ml. of fresh milk first was introduced and incubated in thioglycolate enrichment medium. Any surface or deep anaerobic bacterial growth was then removed with a sterile Pasteur pipette and streaked on a sheep blood-agar plate which was then processed as in method No. 1. In method No. 3, the milk was first incubated in the Hotis tube for 18 to 24 hours after which the Hotis reading was taken. The tube

*Medium (B-432), Difco Laboratories, Detroit, Michigan.
TABLE I
SUMMARY OF RESULTS OBTAINED BY VARIOUS MASTITIS TESTS ON 85 MILK SAMPLES FROM JULY 8 TO OCTOBER 28, 1963

<table>
<thead>
<tr>
<th>Tests</th>
<th>% Positive*</th>
<th>% Negative*</th>
<th>Total Samples Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. CMT Scores: (1 or greater)</td>
<td>72.5</td>
<td>27.5</td>
<td>80</td>
</tr>
<tr>
<td>B. Fresh Milk Smears:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Leukocytes (5 \times 10^5 or more)</td>
<td>67.1</td>
<td>32.9</td>
<td>85</td>
</tr>
<tr>
<td>C. Bacteria Smears:</td>
<td>Sta. †</td>
<td>Str. †</td>
<td>Oth. †</td>
</tr>
<tr>
<td>1. Fresh milk</td>
<td>8.2</td>
<td>4.7</td>
<td>3.5</td>
</tr>
<tr>
<td>2. Incubated milk</td>
<td>57.6</td>
<td>38.8</td>
<td>27.6</td>
</tr>
<tr>
<td>D. Hotis Test Readings</td>
<td>24.7</td>
<td>10.6</td>
<td>64.7</td>
</tr>
<tr>
<td>E. Bacterial Colonies:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meth. No. 1. (FM \rightarrow SBA) †</td>
<td>78.3</td>
<td>27.7</td>
<td>13.0</td>
</tr>
<tr>
<td>Meth. No. 2. (FM \rightarrow Thio. \rightarrow SBA) †</td>
<td>61.2</td>
<td>24.7</td>
<td>15.4</td>
</tr>
<tr>
<td>Meth. No. 3. (IM \rightarrow SBA) †</td>
<td>68.3</td>
<td>34.1</td>
<td>31.7</td>
</tr>
<tr>
<td>F. Camp Results</td>
<td>52.0</td>
<td>48.0</td>
<td>25</td>
</tr>
<tr>
<td>G. Coagulase Results:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meth. No. 1. (FM \rightarrow SBA \rightarrow RP) †</td>
<td>41.0</td>
<td>59.0</td>
<td>83</td>
</tr>
<tr>
<td>Meth. No. 2. (FM \rightarrow Thio. \rightarrow SBA \rightarrow RP) †</td>
<td>38.5</td>
<td>61.5</td>
<td>52</td>
</tr>
<tr>
<td>Meth. No. 3. (IM \rightarrow SBA \rightarrow RP) †</td>
<td>47.6</td>
<td>52.4</td>
<td>82</td>
</tr>
<tr>
<td>Meth. No. 4. (IM \rightarrow RP) †</td>
<td>5.0</td>
<td>95.0</td>
<td>60</td>
</tr>
<tr>
<td>Meth. No. 5. (IM \rightarrow RP) †</td>
<td>48.2</td>
<td>51.8</td>
<td>85</td>
</tr>
</tbody>
</table>

*Percentage of total samples run by each procedure that were positive or negative.

†Code: Sta. = staphylococci-like clusters in smears, staphylococci Hotis readings or staphylococci-like colonies on agar plates; Str. = streptococci chains in smears, streptococci Hotis readings, or streptococci-like colonies on agar plates; Oth. = other bacteria; FM = fresh milk; IM = incubated milk; SBA = sheep blood-agar; Thio. = thioglycolate medium; and RP = rabbit plasma.
was then shaken thoroughly and several loopsful of milk were streaked on sheep blood-agar and processed as in method No. 1. In method No. 4, two loopsful of milk from the fresh Hotis tube were introduced directly into a tube containing 0.5 ml. of rabbit plasma. This tube was then incubated at 37 F and checked at 4, 8, and 24 hour intervals for coagulation. In method No. 5 (the direct coagulase test on incubated milk), the Hotis tube was first incubated 18 to 24 hours at 37 F and then several loopsful of milk were inoculated directly into rabbit plasma.

The plasma employed in these tests was obtained from healthy rabbits and 2 mgm of sodium oxalate per ml. of blood collected was used as an anticoagulant. After centrifugation of blood to recover plasma, the latter was diluted 1:5 with physiological saline containing 2 mgm of sodium oxalate per ml. The diluted plasma was then dispensed in 0.5 ml. amounts into 10 x 75 ml. test tubes which were covered with Parafilm-M.* Such tubes were then held at -20 F until needed. Each batch of plasma employed was checked for accuracy against known coagulase-negative and coagulase-positive staphylococci and compared with results obtained using BactoR dessicated coagulase-plasma** as a control.

The following additional tests were conducted on most milk samples: A California mastitis test (CMT); a microscopic leukocyte count on a smear of the fresh milk using the method of Breed and Prescott; a microscopic examination of incubated milk for bacteria; a Gram-stain and KOH test on bacterial colonies; and a CAMP test on streptococcic colonies to detect Streptococcus agalactiae or Str. uberis.

RESULTS

General results of the study are summarized (Table I). Of 80 milk samples scored by the CMT, 72.5% were 1 or greater. This percentage of samples positive for the CMT compared closely with the percentage showing a leukocyte count of 500,000 or more per ml. (67.1%). When examined for bacteria, stained fresh milk smears were negative in 83.5% of the samples checked. However, when the same samples were incubated, bacteria were seen in all but 5.9% of the smears. Part of the samples contained more than one type of bacteria. Staphylococcic-like clusters were seen in 57.6% and streptococcic chains in 38.8% of the smears from the incubated samples. Hotis test results were indefinite or negative in 64.7% of the samples checked.

Types of bacterial colonies observed when samples were cultured on sheep blood-agar plates after three different methods of pre-treatment before streaking are compared (Part E, Table I). Considering the three methods over-all, approximately 60 to 80% of the samples produced staphylococcic-like colonies and 25 to 35% produce streptococcic-like colonies. Other bacteria were recovered from part of the samples and included: coliform bacilli, corynebacteria, Proteus sp, Pasteurella hemolyticum, Bacillus sp., Sarcina sp., and several unclassified organisms. Streptococcic colonies from

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*Marathon Division of American Can Co., Neenah, Wisconsin.
**Difco Laboratories, Detroit, Michigan.
25 samples were checked by the CAMP procedure and 52% were positive. Considering the total 85 milk samples, only 15.3% were found to contain *Str. agalactiae* or *Str. uberis*.

The percentage of samples found to contain coagulase-positive staphylococci, as detected by different methods of handling samples prior to introduction of milk or colonies into rabbit plasma, are compared (Part G, Table I). Coagulase-positive staphylococci were detected in 48.2% of samples when incubated milk was introduced directly into rabbit plasma (method No. 5). Other methods did not detect as high a percentage of pathogenic staphylococci in milk samples, but with method No. 3 (incubated milk to sheep blood-agar, then to rabbit plasma) there was only a slight difference. With method No. 4, in which fresh milk was introduced directly into rabbit plasma, only 3 of 60 samples (5%) caused coagulation of rabbit plasma.

Individual samples showing coagulase-positive or -negative results with method No. 5 were totaled and compared with results obtained on the same samples when three other methods of coagulase testing were used. (Table II) Analysis of the agreement between method No. 5 and the

### TABLE II

AN ANALYSIS OF AGREEMENT OF THE DIRECT COAGULASE TEST ON INCUBATED MILK WITH THREE OTHER METHODS FOR DETECTING COAGULASE-POSITIVE STAPHYLOCOCCI IN MILK SAMPLES

<table>
<thead>
<tr>
<th>Method No. 5 (IM—→ RP)*</th>
<th>Positive</th>
<th>Negative</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method No. 1 .............</td>
<td>Pos. 28</td>
<td>Neg. 13</td>
<td>78.3</td>
</tr>
<tr>
<td>(FM—→ SBA—→ RP)* ..........</td>
<td>Pos. 16</td>
<td>Neg. 11</td>
<td>71.2</td>
</tr>
<tr>
<td>Method No. 2 .............</td>
<td>Pos. 34</td>
<td>Neg. 5</td>
<td>89.0</td>
</tr>
<tr>
<td>(IM—→ SBA—→ RP)* ..........</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*See Table I for the code giving meanings of letter abbreviations.*

three other methods is given also. It can be seen that the best agreement (89.0%) occurred between methods No. 5 and No. 3, i.e., 73 of 82 samples tested by both methods agreed and nine disagreed. False negatives and false positives (5 and 4) were about the same. Method No. 5 detected appreciably more coagulase positive samples than did methods No. 1 and No. 2, accounting for the lesser degrees of agreement, 78.3% and 71.2% respectively, with these methods.
DISCUSSION

Results indicate that the direct coagulase test on incubated milk (method No. 5) was as effective or more effective than other more complicated methods tested in these trials for detecting coagulase-positive staphylococci in milk samples.

Although the tube-method for coagulase testing has been described as the most reliable in vitro method for determining pathogenicity of staphylococci, it has not been used consistently in mastitis testing programs. Milk samples generally are streaked on blood-agar plates and hemolysin production has been used more widely as a criterion for pathogenicity than has coagulase production. The extra time and labor involved in previously described coagulase tube-testing methods, in which individual staphylococcal colonies must be picked and transferred to coagulase plasmas, perhaps has discouraged more extensive use of these tests. Using hemolysin production as a sole criterion for staphylococcal pathogenicity is fraught with certain problems or possible inaccuracies. In a study by Elek and Levy, of 59 coagulase-positive animal staphylococci, 26% did not produce the alpha-hemolysin and 12% did not produce the beta-hemolysin. Types of blood agar employed for staphylococcal hemolysin detection also are critical. Blood-agars containing horse or human erythrocytes will not detect the alpha-hemolysin, and agars containing horse, human or rabbit erythrocytes fail to detect the beta-hemolysin. In addition, staphylococcal anti-hemolysins should be removed from blood agars employed for hemolysin detection.

Over-all evaluation of milk samples tested in these trials shows that about 70% contained products of udder inflammation as judged by CMT and leukocyte scores. Staphylococcus aureus, i.e., coagulase-positive staphylococci (in about 50% of samples) and Stt. agalactiae or Str. uberis (in about 15%) accounted for most pathogens present.

It is interesting to note that when fresh milk samples were streaked on blood-agar, 78.3% of samples produced staphylococcal-like colonies, but when these were introduced into rabbit plasma, only 41% of samples were found to contain coagulase-positive staphylococci. Apparently milk samples, as routinely received at diagnostic laboratories, may contain a number of micrococcic forms which are not necessarily pathogens and these may represent either so-called "normal udder micrococci" or contaminants derived from the streak-canal, the udder exterior, or the environment during collection. A good diagnostic screening method in addition to hemolysin production is needed to help distinguish Staph. aureus from those other micrococci.

Fresh milk produced fewer coagulase-positive staphylococci (41%) than incubated milk (47.6%) when cultured on blood-agar. Incubation of milk thus may reveal latent staphylococcal infection where very few organisms are shed and these can be missed when only a few loops of fresh milk are streaked on agar plates. A few staphylococci present can also multiply and produce coagulase when the milk is incubated. Several cases of latent staphylococcal infection were detected during these trials in which the blood-agar streak from fresh milk produced only Stt. agalactiae or
Str. uberis colonies. Incubated milk revealed dual infection with both streptococcic and Staph. aureus colonies on blood-agar plates; and in addition, direct coagulate tests on these incubated samples were positive.

Several precautions about use of the direct coagulate test on incubated milk should be mentioned. Coagulate plasmas with citrate salts as anticoagulants are unsuitable, because certain bacteria can metabolize citrate, release calcium, and give false positive results. Milk samples containing antibiotics or highly alkaline udder secretions composed mostly of serum and leukocytes may fail to support proper growth or produce coagulate on incubation. Such samples likewise often fail to give definitive results with other cultural methods for mastitis testing. Milk samples from nonlactating glands or very abnormal secretions can be mixed with several parts of sterile milk to produce better growth of staphylococci during enrichment.

Veterinarians in practice could easily add the direct coagulate test to office-laboratory procedures, since coagulate plasma is the only reagent needed after the milk samples have been incubated. Quality controlled and lyophilized rabbit plasma is available from biological supply companies.* The cost of such plasmas average about 15 cents per test or less if larger batches are ordered. The lyophilized product can be kept indefinitely at 2 to 10 C. The direct coagulate test on incubated milk is recommended as a valuable adjunct to mastitis testing programs because of its simplicity and apparent accuracy in detecting Staph. aureus in milk samples.

REFERENCES

2. Bacto-Coagulate Plasma (0286-33) from Difco Laboratories, Detroit, Mich. 48201.
CHARACTERIZATION OF HOG CHOLERA VIRUS BY ELECTRON MICROSCOPY

B. E. SHEFFY*, P. A. BACHMANN**, and G. SIEGL**

Earlier studies of hog cholera virus described it as being spherical, probably containing RNA, 22-50 μ in size, lipid solvent labile with a density of 1.13-1.14 grams per milliliter. Subsequent reports confirmed data on chemical characteristics but considerable disparity in size was found.

Recent studies have fixed the particle size to be 39-40 μ, consisting of a lipid containing envelope of approximately 6 μ in thickness with an inner core of 28-29 μ in diameter. As measured in cesium chloride gradients, the mean buoyant density of these particles was 1.15-1.16 g/ml. In addition to hog cholera virus particles, however, tissue cultures of hog cholera virus prepared in kidneys from pigs from slaughter houses were found to contain adenovirus and picornavirus particles.

This report is an extension of the above studies to include a comparison of electron optical evaluations of 2 noncytopathogenic hog cholera viruses, strain A and Ames with those reported for 2 cytopathogenic hog cholera virus strains, München 1 and PAV-1.

MATERIALS AND METHODS

**Virus:** München 1 strain had been cultured for 35 passages in primary pig kidney (PK) cell cultures and PAV-1 had been cultured similarly for 205 passages but in cell cultures prepared from specific pathogen free (SPF) pigs. Strains A and Ames hog cholera virus were cultured from spleen and defibrinated blood of infected SPF pigs.

All virus strains were grown in monolayer cell cultures of a pig kidney cell line (PK-15). Cell cultures were prepared in Roux flask by standard methods, and when monolayers were confluent, were overlayed for 2 hours with fluids containing suspensions of the various strains of hog cholera virus. Medium consisting of bovine amniotic fluid with 15.0 mg phenol red, 100,000 units penicillin and 100 mg streptomycin per liter was added to all of virus infected cultures.

Concentration, Purification and Determination of Buoyant Density: Virus was harvested by alternate freezing and thawing of cell cultures 4-5 days after infection. Cellular debris was removed by centrifugation at 2,200 g for 30 minutes. The supernatant (1-2 liters) was concentrated to 10% of original volume by vacuum evaporation (Rotavapor, Fa. Büchi, Flawil, Switzerland) at 25°C. The virus was then pelleted by ultracentrifugation (Spinco model L, Beckman Instruments) at 100,000 g for 2 hours.

Further purification of the virus and bouyant density determinations were

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**Institut für Mikrobiologie und Infektionskrankheiten der Tiere, Ludwig Maximilians Universität München, Prof. A. Mayr, Director.
†The American Type Culture Collection, Rockville, Maryland.
made in the following manner: Distilled water was mixed with a saturated solution of cesium chloride (20°C) in the proportions 8:9; 8:7; 8:5; 8:3; 8:1. An aliquot (0.8 ml) of each dilution was carefully layered in cellulose nitrate tubes, beginning with the highest concentration of cesium chloride. This gradient was overlaid with 0.8 ml of concentrated virus suspensions. The gradients were centrifuged in a swinging-bucket SW 39 rotor at 100,000 g for 14-16 hours.

In other buoyant density determinations, 8 parts of virus suspension were mixed with 2 parts of a saturated cesium chloride solution. This solution had a density of 1.18 g/ml. After centrifugation in a swinging-bucket SW 39 rotor at 100,000 g for 24 hours, gradients with densities between 1.25 and 1.11 g/ml were recorded.

Consecutive fractions of 0.3 ml were collected. Density of each fraction was computed by measurements of their respective refractive indices by an Abbe 3L refractometer (Carl Zeiss, Oberkochen, Germany), and by subsequent extrapolation from standard curves established by measurements of weighed samples. Virus was separated from the cesium chloride by centrifugation. The pelleted virus was suspended in 0.15 M ammonium acetate and held at +4°C prior to the electron microscopic studies.

Uninfected culture fluid of PK-15 cells were similarly concentrated and prepared for electron optical studies.

**Electron Microscopy:** Negatively stained preparations of virus particles were prepared by mixing equal parts of purified virus suspensions with a 2% solution of either phosphotungstic acid (PTA) at pH 4.9 and 6.7 or silicotungstic acid (STA) at pH 7.8. A drop of 0.01% aqueous solution of bovine serum albumin was added to each staining solution. After mixing, drops of the above suspensions were transferred to Formvar-coated grids. Excess solute was withdrawn by touching the edge of the grid with filter paper. After drying, the grid preparations were examined in a Siemens Elmiskop I under double condensor illumination at an instrumental magnification of 60,000.

For demonstration of envelope presence, drops of virus suspension were allowed to dry on Formvar-coated grids. After short washing in distilled water, the grids were placed upside-down on the surface of a 2% osmium tetroxide solution for 30 minutes. For contrast enhancement, the preparations were stained with PTA (pH 6.7).

**RESULTS**

Examination of preparations from München 1 strain showed the majority of particles had mean diameters of 39-40 µ (Table I). A significant number of particles 14-16 µ in diameter, however, were found in these fractions. The 39-40 µ particles consisted of a lipid-containing envelope (Fig. 1) of approximately 6 µ in thickness and an inner core of 29-30 µ in diameter. The 14-16 µ particles did not appear to be enveloped and were hexagonal in shape. Peak concentration of these particles were found in gradient fractions with a density of 1.15-1.16 g/ml (Fig. 2). No particles
**TABLE I**

**Size of Particles Found in Fractions of Different Buoyant Density**

(München 1)

<table>
<thead>
<tr>
<th>Buoyant Density—g/ml</th>
<th>Peak</th>
<th>Particle Size—μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.14–1.20</td>
<td>1.16</td>
<td>38–40</td>
</tr>
<tr>
<td>1.21–1.36</td>
<td></td>
<td>14–16</td>
</tr>
<tr>
<td>1.37–1.44</td>
<td>1.38</td>
<td>None</td>
</tr>
</tbody>
</table>

(Fig. 1)

(Fig. 2)
were seen in electron optical studies of fractions with densities between 1.21 and 1.36 g/ml, but these fractions contained small amounts of hog cholera virus. Numerous particles were found at densities of 1.37 to 1.44 g/ml, with peak density at 1.38 g/ml. The particles measured 20-22 μm in diameter and were hexagonal in shape (Fig. 3). These particles were detected in strain München 1 (Fig. 3) but were not present in cultures of PAV-1.

Electron optical studies of hog cholera virus strains A and Ames (Table II) revealed 39-40 μm particles at the same density as in virus strains München 1 and PAV-1. No 14-16 μm particles or 20-22 μm particles were found.

Uninfected control PK-15 cell culture fluids contained no virus particles at any of the buoyant densities studied.

**DISCUSSION**

This study confirms previous work of Mayr et al., Horzinek et al. regarding the density and size of hog cholera virus. Polymorphic enveloped
particles, mostly round to oval, measuring 39-40 μm were found in highest concentration at buoyant densities of 1.15-1.16 g/ml. In spite of variations in staining technique employed, clear resolution of inner structure of intact particles was not obtained. However, close study of negatively contrasted disrupted particles revealed twisted threadlike structures, suggesting a helical symmetry for hog cholera virus particles. Additional studies employing further modification of staining methods to demonstrate clearly this structure are indicated.

The fact that cultures of HCV strains A and Ames (noncytopathogenic) contained only hog cholera virus particles while strains München 1 and PAV-1 (cytopathogenic) consisted of several distinctly different particles may be of significance with reference to the cytopathogenicity of these viruses. Adenovirous has been shown not to be required for expression of cytopathogenicity of PAV-1 strain HCV but additional studies are needed to establish the role of the 14-16 μm particles in this phenomenon.

Several possible explanations for the presence or absence of the various particles in different strains can be advanced. First it cannot be said with absolute certainty that because particles other than hog cholera were not seen in cultures of strain A and Ames, they were not present, but rather that they were not present in sufficient concentrations to be seen. It is possible that these particles did not grow to sufficiently high concentrations to be observable after only 2 subcultures in PK-15 cells. Additional subculture in axenic cultures and subsequent electron optical studies may be necessary.

The second and more likely explanation is that the original tissue source of these strains contained only HCV and contaminations was introduced from the primary pig kidney cell cultures used. The limitations of such cultures for continuous propagation of virus has been recognized. Adenovirus infections were recognized in control kidney cultures of pigs from slaughter houses,9,10 porcine enterovirus have been isolated from normal kidney cultures of pigs 1-6 weeks of age11 and a picodna virus was found in kidney cell cultures of healthy 3 week old piglets.12

SUMMARY

Round to oval virus particles 39-40 μm in diameter with an envelope containing lipids of approximately 6 μm in thickness were found in cultures of hog cholera virus strains A and Ames. Other particles as reported in cultures of München 1 and PAV-1 were not seen. The need for purification of stock viruses and subsequent culture in axenic cell systems was emphasized.

REFERENCES


INTRODUCTION

Fragmentary evidence incriminating the virus of bovine virus diarrheca (BVD) as an abortifacient agent has been available since the disease was first reported in 1946. This evidence comes from clinical observations, serological data, examination of gross and microscopic fetal lesions, experimental induction of abortion with BVD virus and from isolation of BVD virus strains from aborted fetuses submitted to a diagnostic laboratory. These observations all suggest that BVD can produce bovine abortions. The extent to which BVD abortion actually occurs, the conditions under which it occurs, and the best methods of diagnosing and preventing it are presently unknown. We would like to discuss current thinking about BVD abortion and tentatively outline procedures useful in attempting to diagnose this condition.

HISTORY AND LITERATURE REVIEW

The first description of BVD reported 20 abortions occurring ten days to three months after clinical disease was observed in five herds containing 127 cattle (Olafson 1946). Some cattle never observed to be clinically ill aborted. This clinical and pathological description of the disease in adult cattle did not include description of the fetuses nor designation of the specific etiologic agent of the abortions and because techniques for identification of viruses were limited at the time, the possibility of concomitant infections with other agents was not excluded.

Abortions have been reported occurring concurrently with outbreaks of BVD or mucosal disease among neonatal calves (Schipper and Eveleth 1957, and Romvary 1965). In these outbreaks, most adult cattle including the dams of affected calves, had no clinical disease. Some calves in these episodes appeared normal at birth, but within one to four days developed fatal disease with erosive lesions of the mucous membranes of the gastrointestinal tract. Other calves had signs and lesions of virus diarrheca at birth and succumbed shortly thereafter. These observations suggested prenatal infection, but this hypothesis could not be adequately tested. Although virological studies implicated BVD virus in the neonatal fatalities, the specific etiologic agent of the accompanying abortions was not ascertained. Further, one of these reports (Schipper and Eveleth 1957) is complicated by later discovery that the virus implicated either was IBR virus or later became contaminated with IBR virus (Fernelius and Ritchie, 1966). In this outbreak, an 8½ month fetus with erosive lesions characteristic of BVD was observed in utero during the necropsy of a mature cow with mucosal disease (Noice and Schipper 1959). Similar lesions have been found in an
aborred fetus (Jubb and Kennedy 1963). Neither of these reports describes isolation of virus from the fetus.

Abortion was reported in two bovine virus diarrhea transmission experiments with pregnant cattle. One abortion occurred about six weeks post BVD inoculation (Baker 1954) and another 60 days post inoculation (Huck 1957). Neither of these authors described pathological examination of the fetus or placenta nor included the serological status of the dam at the time of abortion.

Non-cytopathogenic BVD virus strains were recovered from two aborted fetuses (Gillespie, et al. 1967). One of the isolates was obtained from lung and intestine of a twin fetus removed from a five-year-old cow which had a dystocia when 260 days pregnant. The cow had diarrhea and an undiagnosed chronic respiratory disease at the time of the dystocia. The second isolate was obtained from liver and spleen of a fetus aborted by a six-year old cow which was 225 days pregnant. The herd experienced respiratory infection of undetermined etiology one month earlier. Specimens for serological testing of these cows were not available.

Attempts to incriminate BVD virus on the basis of BVD virus neutralization tests on serums taken from cattle at the time of abortion or both at the time of abortion and again 20-30 days later have been attempted with unconvincing results (Robinson 1961, and Kahrs 1965).

Olafson (1946) first described BVD as a disease characterized by a high clinical attack rate (most animals in herds showed clinical signs) with few fatalities. Later reports indicate clinical disease observed in a small proportion of cattle in herds (low clinical attack rate) with many of the sick cattle dying (high case fatality rate). Our experiences investigating BVD episodes in dairy herds with less than 100 cattle usually have presented a picture of one, two or three fatal cases in cattle 6 months to two years of age without clinical signs in older cattle. Virus neutralization tests on serums collected from entire herds, as soon as possible after BVD appeared on farms, indicated BVD antibody present in greater than 90% of the cattle in the herds. Cattle without evidence of antibody frequently developed clinical BVD and died. Inquiry regarding abortions sometimes revealed history of one or two abortions two to six weeks preceding the diagnosis of BVD in the herd. The aborting cattle, if still present at time of the investigation, had BVD serum antibody. Usually no attempt was made to determine the etiology of the abortion. Retrospective diagnosis of BVD as the cause of such an abortion is speculative. Frequently immune pregnant cattle in these herds had normal parturitions.

Clinical observation of abortion and birth of calves with cerebellar hypoplasia following undiagnosed infections of pregnant cattle have been recorded. Retrospective serological studies incriminating BVD as the possible etiologic agent have led to further speculation of the effects of this virus on prenatal calves (Sharp 1965).

A recent attempt to clarify the role of BVD is bovine abortions by intravenous inoculation of non-cytopathogenic BVD virus strain isolated from a fetus into eleven susceptible cattle during their third trimester of pregnancy resulted in birth of one calf at 261 days of pregnancy. The
remaining calves were born at term. The premature calf had a large erosive
lesion on the oral mucous membranes. When necropsied following eutha-
nasia at 24 hours of age extensive petechial hemorrhages in the abomasum
were the only additional gross lesions observed. Histopathological examina-
tion of the oral lesions revealed a series of ulcers in various stages of develop-
ment. To this date, virus has not been isolated from the tissues of this calf.
Serum samples from this calf and four full-term calves from dams inocu-
lated with this BVD isolate during pregnancy were collected before the
calves suckled by a veterinarian present at birth. These serums were found
to contain BVD antibody.

ASSUMPTIONS NEEDED FOR DISCUSSION OF BVD ABORTION

The following assumptions, subject to verification by later studies,
are used to describe BVD.

1. BVD is a very common parasite of the cattle population of the USA.
2. Exposure of cattle to BVD virus usually results in an inapparent
   or undiagnosed infection with minimal clinical signs.
3. Overt clinical disease manifested by anorexia, salivation, diarrhea and
   erosive lesions of the mucous membranes of the gastro-intestinal
   tract, dehydration, and death are infrequent sequellae to BVD
   infection.
4. Active immunity following BVD infection protects cattle from sub-
   sequent reinfection and is usually lifelong.
5. Pregnant cattle, exposed to BVD virus, will not abort if they are
   immune from previous exposure.
6. If abortion is to occur, primary infection of susceptible cattle must
   occur during pregnancy and possibly during a certain (as yet
   unknown) stage of pregnancy.
7. All susceptible cattle infected with BVD virus during pregnancy
   do not abort, but the conditions determining whether or not abor-
   tion occurs are unknown.

Under conditions outlined above, the occurrence of BVD abortion
requires that cattle which have escaped effective exposure to a very common
virus for their entire non-pregnant life be exposed during a vulnerable
stage of pregnancy.

Under these conditions BVD abortion should occur principally as a
sporadic event with little concurrent evidence suggesting BVD etiology. However, under rare circumstances abortion storms accompanied by overt
clinical disease may occur.

DIAGNOSIS OF BVD ABORTION

When the herd history is suggestive of BVD, a concerted effort should
be made to identify the etiologic agent. The absence of clinical signs in
the herd of origin does not exclude BVD as the etiologic agent. If the
entire herd is tested for BVD serum antibody, and ninety percent or more
of the cattle possess BVD serum antibody, you have presumptive evidence
that recent BVD infection has occurred. BVD immunity in individuals is virtually lifelong, but in the absence of infection, herd immunity wanes due to immigration of susceptible animals. An assumption of recent infection would be erroneous in the situation where immigrants were mostly immunized cattle. If clinical BVD has been observed recently in a herd, the herd should contain at least 90% immune animals and the cattle lacking antibody have a poor prognosis for life (particularly those in the 6-24 month age group). It has been observed that these cattle without BVD serum antibody in BVD infected herds frequently develop fatal clinical BVD, sometimes lasting for several weeks, and die. Immunological tolerance is postulated as playing a role in this phenomena (Lambert 1966).

Data on the cow which aborted should include history of any recent disease, recent vaccination, previous breeding problems. Specimens of placenta should be obtained and examined. However, we presently have no evidence that BVD abortion is accompanied by characteristic lesions of the placenta.

Serum specimens collected from the cow at the time of abortion and again 4 weeks later can be examined for BVD antibody. We have arbitrarily chosen a four-fold rise in titer as significant. We have not been able to demonstrate this rise in BVD titer in the 3-4 week time interval following abortion. (We have demonstrated such a titer increment associated with some IBR abortions). We speculate that the time interval between exposure of pregnant cattle to BVD virus and abortion is sufficient for the cow to develop circulating antibody, so that any further rise is not readily demonstrable. The 50-80% prevalence of BVD serum antibody in bovine populations renders a single positive serum worthless for diagnostic purposes. A single negative serum collected at time of abortion has diagnostic significance if accompanied by a positive serum collected from the same cow at a later date. A negative serum accompanied by a second negative serum collected 3-4 weeks after abortion indicates that the cow was not infected with BVD during the period immediately preceding abortion and should eliminate BVD as the etiologic agent unless clinical or pathological evidence to the contrary is present.

Serums from stillborn calves and aborted fetuses which never ingested colostrum contained BVD antibody. This observation suggested in utero infection. However, the absence of this antibody cannot be used to exclude BVD from the diagnosis since the mechanism by which the fetus gets this antibody and the stage of gestation at which this fetal antibody can be present are still unknown.

The mucous membranes of gums, tongue, hard and soft palate of fetus and dam alike should be examined for ulcerative or erosive lesions suggestive of BVD. Again, the absence of these lesions does not rule out BVD. At necropsy of the fetus, the pharynx, larynx, trachea, esophagus, and the mucous membranes of the gastro-intestinal tract should be examined for erosions or hemorrhages suggestive of BVD.

Tissues for virus isolation attempts collected aseptically from the fetus should include lung, liver, spleen, kidney, brain and placenta, plus any other organ or tissue with lesions suggestive of BVD. These specimens
can be stored at \(-70^\circ\) C. until results of the bacteriological cultures and/or histological exams suggest that virus isolation attempts are indicated. The specimens are then ground in tissue culture media containing antibiotics and fungistats and inoculated into primary embryonic bovine kidney tissue cultures and examined daily for evidence of cytopathic changes. Cultures lacking cytopathic changes after 6-8 days should be harvested and reinoculated into fresh cultures as these changes may be evident only on 2nd or 3rd passage. After three passages without evidence of cytopathic changes it is desirable to assay for the presence of non-cytopathogenic agents by means of the interference test (Gillespie, Madin and Darby 1962). This technique is based on fact that some non-cytopathogenic strains of BVD will grow in tissue cultures and inhibit the growth of known cytopathogenic BVD strains. If the known cytopathogenic strain produces characteristic changes in control cultures but fails to do so in the test cultures, inoculated three days earlier with materials from the fetus, the presence of non-cytopathogenic virus is suspected. This suspicion must be confirmed by calf inoculation and subsequent demonstration of infection by clinical observations and by demonstration of BVD antibody production by the calf. Thus the interference test serves to indicate which materials justify the time and expense of calf inoculation procedures.

Isolation of non-cytopathogenic BVD virus from fetuses without characteristic BVD lesions has been accomplished by this technique (Gillespie et al 1967).

Both cytopathogenic and non-cytopathogenic strains of BVD virus can be identified in tissue culture systems and probably in pathological specimens by use of fluorescent antibodies (Fernelius 1964). This procedure has potential for the diagnosis of BVD abortion.

Attempts to isolate BVD virus from fetuses are handicapped by the need for relatively fresh fetuses from which tissues can be harvested with minimal bacterial contamination. In addition, experimental evidence indicates that cattle infected with BVD during pregnancy can give birth to calves with circulating BVD antibody and presumably the same could happen with aborted fetuses. We speculate that the presence of non-colostral antibody in tissues of calves from dams experimentally infected during pregnancy contributed to the fact that BVD virus was not isolated from the tissues of one calf born prematurely or from tissues of ten calves born at full term.

**CONTROL OF BVD ABORTION**

Until details of the role of BVD in bovine abortion are more clearly elaborated, only tentative recommendations for its control can be stated. BVD virus eventually infects most cattle, usually producing a lifelong immunity. Present reasoning suggests that abortion can be avoided if this infection occurs prior to breeding age. Since colostrally-transferred maternal antibody may interfere with vaccination, it is recommended that healthy unstressed calves be vaccinated with modified live virus after 8 months of age. To be of value, such a program must be carried out continuously.
SUMMARY

Bovine virus diarrhea joins the list of systemic infectious diseases to be considered in the differential diagnosis of bovine abortions. BVD abortion usually appears sporadically, but may occur in abortion storms if the virus infects a highly susceptible herd containing many pregnant cattle. All pregnant cattle exposed to BVD do not abort. It is speculated that some calves prenatally exposed to BVD have neurological defects.

It is difficult to make a specific etiological diagnosis of BVD abortion because:

1. BVD infection in pregnant cattle is frequently inapparent, lacking characteristic clinical signs, so that when abortion occurs, BVD is no more strongly suspected than numerous other abortifacient agents, and a detailed specific search for evidence of BVD is omitted.

2. Interpretation of tests on cattle serums collected following abortion is confused by the high prevalence of BVD antibody in the normal cattle population and by the fact that the interval between BVD infection of pregnant cattle and fetal expulsion is usually long enough for the dam’s BVD antibody titer to be stabilized at a high level by the time of abortion.

3. Although BVD infected fetuses can have grossly visible characteristic erosive lesions of the mucous membranes, these lesions may be absent.

4. Isolation of BVD virus from fetuses is frequently difficult. Fresh sterile fetal tissues are required. Some BVD virus strains, being non-cytopathogenic are demonstrable only by indirect procedures such as the induction of cellular resistance to cytopathogenic BVD virus strains or the inoculation of susceptible calves. Also some fetuses exposed in utero possess antibody.

Despite these difficulties, BVD abortion can be presumptively diagnosed if the dam exhibited clinical signs and lesions of BVD prior to abortion or if the fetus has lesions of BVD. BVD abortion can be confirmed by isolation of the virus from fetal tissues.

When a laboratory diagnosis of BVD abortion is made, the veterinarian or cattle owner should be told that remaining pregnant animals in the herd are probably already exposed and that although a vaccine is available, it is unadvisable to vaccinate pregnant cattle. He should also be told that future occurrences can best be minimized by a control program based on vaccination of healthy unstressed calves over eight months of age.

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REFERENCES

FLUORESCENT STAINING OF MYCOBACTERIA IN BOVINE TISSUES WITH AURAMINE O DYE—A COMPARATIVE EVALUATION OF A MODIFIED STAINING PROCEDURE

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INTRODUCTION

One of the most tedious tasks confronting veterinary pathologists is the search for mycobacteria in tissue sections suspected of containing tuberculous lesions. Whenever mycobacteria are not demonstrated, the diagnosis of tuberculosis is presumptive. The problem is compounded because tissue changes found in tuberculosis may be produced by a number of other etiologic agents.

Each case of laboratory confirmed bovine tuberculosis encountered in slaughtering establishments and during routine tuberculosis eradication work must be traced to the herd of origin. Epidemiologic trace-back procedures are initiated upon the histopathologic demonstration of mycobacteria within tissue changes typical of tuberculosis. It is imperative to find the mycobacteria if they are present.

Ideally, mycobacteria should stain equally well in all instances. However, experience has shown that staining characteristics of mycobacteria in tissue sections are variable. Mycobacteria are not always demonstrated by conventional methods even though cultures may be obtained from adjacent areas in the same lesion.

Fluorescent staining offers another means of demonstrating mycobacteria. It is accepted for staining sputum smears7,8 and is used on tissue section13,14 from human patients. Fluorescent staining is effective in rapidly demonstrating mycobacteria and is effectual in certain cases when conventional methods have failed.5,9,15

Application of fluorescence microscopy for the detection of mycobacteria in bovine tissue sections has not been reported in the available literature at this time.

The purpose of this investigation was to evaluate the fluorescent staining of mycobacteria in bovine tissues stained with auramine O dye by a modified staining procedure.

PROCEDURE

Material Specifications

The material for this investigation consisted of formalin-fixed specimens of bovine tissues taken from the tissue repository of the Pathology

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Unit, Diagnostic Services at the National Animal Disease Laboratory. The tissue specimens were restricted to those that were received during the calendar years of 1964 and 1965.

The bovine tissue specimens originated from either of two sources: (1) from cattle that were reactors or members of quarantined herds in the cooperative state-federal bovine tuberculosis eradication program, or (2) from bovine carcasses that contained lesions suspected of being tuberculous upon examination by state or federal meat inspectors. The bulk of the submitted bovine tissue specimens were lymph node tissue; however, liver, spleen, lung, kidney, heart, mammary gland, skeletal muscle, adrenal gland, and salivary gland tissues were received in some instances. Skin tissue lesions were not considered in this investigation.

The design of this investigation was constructed so that one could classify the tissues into either granulomatous disease conditions, nongranulomatous disease conditions, or no microscopic lesions on the basis of a hematoxylin and eosin stain. Harris's alum hematoxylin and ethylenesin combination stain was used for demonstrating cytological detail in this study. All tissue sections were stained by the new-fuscin technique to detect acid-fast bacilli. This was done to further classify the respective tissues into either tuberculous or non-tuberculous categories by the presence or absence of acid-fast bacilli in conjunction with the hematoxylin-eosin staining results.

Tissues in Group I were all granulomatous disease conditions. Tissues in this group were observed to contain at least two acid-fast bacilli per granulomatous lesion. This group was composed of 1,111 submissions of positive tuberculous specimens of bovine tissue origin.

Tissues included in Group II had no microscopic lesions. These submissions were noted to have no acid-fast bacilli anywhere in the apparently normal tissue. This group consisted of 327 submissions of negative specimens of bovine tissue origin.

All tissues in Group III were of granulomatous and non-granulomatous disease conditions. No acid-fast bacilli found in any of the tissues. This group was composed of 296 submissions of granulomatous disease conditions other than tuberculosis as well as non-granulomatous disease conditions. Among the granulomatous disease condition were actinomycosis, actinobacillosis, chronic bacterial abscesses, parasitic lesions, coccidioidomycosis, botryomycosis, phycomycosis, lipogranulomatosis, and foreign body granulomatous cases. The non-granulomatous disease conditions consisted of a multiplicity of neoplasms, septicemias, eosinophilic myositis, bronchopneumonitis, and acute bacterial abscesses.

The control group consisted of three cattle as follows: two adult cattle were experimentally infected with Mycobacterium bovis and held in isolation approximately two years. At necropsy, specimens containing tuberculous lesions were collected from these animals. Negative specimens were collected from one animal procured from a herd known to be free of bovine tuberculosis. This animal was negative on at least two tests using mammalian tuberculin injected intradermally while in detention for at least 15 months.
at the National Animal Disease Laboratory. A thorough post-mortem examination was conducted and no lesions were found.

**Methods**

*Histopathologic preparation*

The tissue specimens were fixed in ten percent buffered formalin. Tissue specimens were adequately trimmed and blocked into sizes sufficient to be processed through routine histologic procedures. The specimens were demineralized, if necessary, in five percent nitric acid solution for a minimum of two and one-half hours under negative atmospheric pressure in a modified household pressure cooker. More time was allowed if the tissue specimens were excessively mineralized. The tissue specimens were neutralized in five percent ammonium hydroxide for one-half hour under negative atmospheric pressure.

The tissues were prepared for histologic processing by conventional methods.

*Fluorescent Staining Procedure*

The procedure involved staining the tissue sections with auramine O as modified by Howell through the following specific steps:

1. Deparaffinized through three changes of xylene for two minutes each, three changes of absolute ethanol for two minutes each, 95 percent ethanol for two minutes, and 80 percent ethanol for one minute.
2. Stained in auramine O solution for ten minutes.
3. Decolorized in aqueous 20 percent sulphuric acid for approximately 10 seconds.
4. Dipped in 80 percent ethanol for one minute.
5. Immersed in 10 percent ferric chloride solution for five minutes.
6. Immersed in 80 percent ethanol for one minute.
7. Dipped in 95 percent ethanol for one minute.
8. Dried in slide dryer for 15 minutes.
9. Mounted in non-fluorescent medium under special coverslip, Corning cover glass, No. 1.5 thickness.1

The auramine O stain was prepared by the following formula:5

\[
\begin{align*}
\text{Auramine O}^2 & \quad 3.0 \text{ gm.} \\
\text{Glycerol} & \quad 70.0 \text{ ml.} \\
\text{Phenol (liquefied)} & \quad 32.0 \text{ ml.} \\
\text{Distilled water} & \quad 900.0 \text{ ml.}
\end{align*}
\]

Filter through cotton in a glass funnel several times.

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1Corning Glass Works, 1946 Crystal Street, Corning, New York.
2Matheson, Coleman, and Bell, Division of Matheson Company, Incorporated, 333 Patterson Plank Road, East Rutherford, New Jersey.
The non-fluorescent mounting medium consisted of the following ingredients:  

Styron 666\(^1\) 10.0 gm.  
(K 27, clear, No. 71 granulation)  
Dibutyl phthalate 5.0 ml.  
Xylene 35.0 ml.  

The styron was thoroughly dried in a drying oven at 75 degrees Centigrade for two hours before use. The mixture was allowed to stand overnight for 12 to 14 hours. More xylene was added if the consistency was too thick to allow for efficient mounting of the special coverslips.

**Fluorescence Microscopy**

The microscopy system was a basic Ortholux research microscope\(^2\) with an Osram high pressure, mercury vapor, 200 watt lamp (HBO 200 w.) enclosed within a housing. The following accessories\(^2\) were used: BG 38 heat absorbing filter, OG 12 exciter filter (primary filter), OG 1 barrier (secondary filter), dry darkfield condenser, No. 85, N.A. 0.80, periplan eyepieces (6X) with soft rubber eyecups, 12.5X apochromatic dry objective lens, 25X apochromatic dry objective lens, and 40X fluorite dry objective.

**RESULTS**

This evaluation was conducted on 1,737 submissions. Group I consisted of 1,111 cases of tissues classified histopathologically as compatible with tuberculosis. Group II was composed of 327 submissions that were histopathologically negative. Group III consisted of 296 submissions containing lesions histopathologically indicative of both granulomatous and non-granulomatous disease conditions including some suggestive of tuberculosis. The controls consisted of two experimentally-produced cases of *Mycobacterium bovis* infection and one negative animal.

Fluorescing mycobacteria appeared as brilliant golden-yellow or yellow bacilli against a darkened background that was either pastel green or gray in appearance. Most mycobacteria measured two to five microns in length and 0.2 to 1.5 microns in width and were generally present as narrow rods or short coccobacilli which were either homogeneous or finely granular in appearance, sharply delineated in outline, and brilliantly fluorescent. Fluorescing mycobacteria contrasted with the background which greatly facilitated the demonstration of even a single bacillus. Mycobacteria were easily detected at low magnification (125 to 250 diameters) by their dispersion halo, even when slightly out of focus.

The results were correlated in all instances between the findings of the group categories previously described utilizing hematoxylin and eosin as

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\(^1\)Dow Chemical Company, 1000 Main Street, Midland, Michigan.  
\(^2\)E. Leitz, Incorporated, 468 Park Avenue, South, New York, New York.
well as new-fuchsin staining, and the fluorescent technique incorporating the modified auramine O procedure.

**GROUP I**

Mycobacteria were detected by the modified auramine O staining technique in all 1,111 tuberculous submissions. Fluorescing mycobacteria stained with auramine O were easily demonstrated in these tuberculous lesions.

**GROUP II**

Group II consisted of 327 submissions containing no acid-fast bacilli. All were negative for mycobacteria by fluorescent staining with auramine O.

**GROUP III**

All 296 submissions in this group contained microscopic lesions either of granulomatous or of non-granulomatous disease conditions upon routine histopathologic examination.

Although acid-fast bacilli were not disclosed by conventional (new-fuchsin) staining procedures in any of the 296 submissions, 23 were positive for mycobacteria by fluorescent staining with auramine O. These were diagnosed histopathologically as follows: 19 were found to have lesions suggestive of tuberculosis, three were found to have lesions suggestive of parasitism, and one was found to have lesions suggestive of subacute bronchopneumonia.

**CONTROLS**

Fluorescing mycobacteria stained with auramine O were readily demonstrated in several lesions of tuberculosis from two experimentally-produced bovine infections with *Mycobacterium bovis*. (Figure 1).

No mycobacteria were evident within representative tissues with fluorescent staining using auramine O in the negative control animal.

**DISCUSSION**

The fluorescent staining procedure employed in this investigation proved to be a useful aid for detecting mycobacteria in tissue sections. Using this technique the tissue background faded out to a large extent and bothersome morphological tissue details were minimized so that the mycobacteria were easily observed. This proved to be advantageous in several cases.

Good results were attributed in part to the adaptation of some specific features of a number of other non-related fluorescent staining techniques in current use. The specific features that were considered the salient points of many procedures are outlined as follows: the use of a strong acid solution for more complete tissue decolorization of excess, tissue-retained auramine O; the use of ferric chloride solution to "quench" extraneous,
non-specific fluorescence; and the use of a recently developed non-fluorescent mounting medium, Styrion 666 in dibutyl phthalate, to eliminate extraneous fluorescence attributed to some mounting media.

Some of the techniques had to be modified somewhat so that they could be used in this investigation. These modifications were centered around the adaptation of fluorescent staining to bovine lymph node tissues and the strict control of the water content in tissue sections. Both modifications were achieved by thorough deparaffinization and dehydration procedures.

The problem of fluorescing artifacts was carefully considered. This problem was practically eliminated by the "quenching" of extraneous fluorescence with ten percent ferric chloride. Moreover, artifacts were not a problem when the following criteria were carefully applied. Typical size of acid-fast bacilli was a prime consideration; most acid-fast organisms measured two to five microns in length and 0.2 to 1.5 microns in width. Generally speaking, most artifacts were larger. Typical form was another consideration; acid-fast bacilli were generally present as narrow rods or short, regular coccobaccilli which were sometimes curved whereas most

Figure 1. Bovine Tuberculosis. Fluorescing mycobacteria in a tuberculous lesion from an experimentally-produced bovine infection with *Mycobacterium bovis*. Auramine O stain—400 diameters magnification.
artifacts were plump, irregular masses. Typical structure was yet another consideration; acid-fast bacilli were either homogeneous or granular in appearance. This was in contradistinction to artifacts which were either heterogeneous or amorphous in character. Sharp delineation of acid-fast bacilli was an important differential feature; artifacts had fuzzy, ill-defined borders. Most acid-fast bacilli were strong and brilliant in fluorescence and many artifacts were weak and variable in fluorescence.

The fluorescent staining procedure incorporating auramine O was efficient, economical, and highly advantageous compared to acid-fast staining procedures. The fluorescing organisms were more easily seen, appearing as brilliant golden-yellow or yellow bacilli or coccobacilli against a darkened background. This greatly facilitated the demonstration of a single bacillus. The organisms were detected at low magnification by the dispersion halo, even when slightly out of focus.

Oil immersion objectives were not employed for fluorescence microscopy. The fluorescing bacilli were searched for with the medium dry (25X), or even the low dry (12.5X) objectives and then conclusively identified with the medium dry (25X) or the high dry (40X) objectives. Therefore, it is apparent that a much larger area may be covered, not only in width but also in depth.

More positive cases of tuberculosis were found with the fluorescent method because its sensitivity exceeded that of any other microscopic technique with which it has been compared.\textsuperscript{11,15,16} This increased sensitivity was explained by a number of observations that are discussed in the following paragraphs.

More bacilli were stained by the fluorescent dyes, including auramine O, than by carbol-fuchsin as shown in ruled fields of identical slides successively stained with auramine O after destaining the conventional acid-fast techniques.\textsuperscript{12}

Mycolic acid, the principal acid-fast component of tubercle bacilli, combined more readily with auramine O than with carbol-fuchsin and, in the former combination, resisted destaining for a much longer time. Thus, it was likely that some of the organisms demonstrated by fluorescence microscopy included a larger number of bacilli with lower than normal mycolic acid content, such as saprophytic mycobacteria, young tubercle bacilli, and dead tubercle bacilli.\textsuperscript{8,10,12}

Finally, the larger microscopic area under observation and the comparative ease with which the fluorescent bacilli were detected contributed to its increased sensitivity.

Staining experiments with suitable acid decolorization steps, indicated that the fluorochrome, auramine O, was specific for the acid-fast group of bacilli.\textsuperscript{8,10,12}

A problem encountered in this investigation was the fading of the fluorescent bacilli in the tissue sections. Subjectively speaking, it was apparent that the auramine O fluorescence was noticeably fading after six days. A cursory review of slides that were at least 60 days old revealed
almost a complete loss of fluorescence. This has been mentioned by others as a problem.

Other practical difficulties were evident in the fluorescent staining method. Fluorescence microscopy required a certain degree of skill; this skill was gained only after continual utilization of the method. For this reason complete confidence was not easily and quickly gained. Any change-over from conventional staining of acid-fast bacilli to fluorescent staining should be made over a considerable length of time. Allowing for gradual adjustment, efficiency will improve considerably.

Focusing was another difficulty that has been encountered in this study. This was almost totally attributable to the dark background of the dark-field. However, it has been found that by carefully focusing at the edge of the lesion or section under consideration that this difficulty was lessened. Also, it had been noted that by working in a darkened room, or a room with subdued lighting, focusing was less difficult. It was important to allow the light source to perform to full capacity before beginning the examination.

SUMMARY

An investigation was conducted to evaluate fluorescent staining of acid-fast bacilli in bovine tissues using auramine O dye. Tissues from 1,737 submissions were investigated. Group I consisted of 1,111 submissions classified as compatible with tuberculosis upon using routine histopathologic methods. Mycobacteria were detected by auramine O staining in all submissions. Group II was composed of 327 submissions that were histopathologically negative. Mycobacteria were not demonstrated by auramine O staining in this group. Group III consisted of 296 submissions containing lesions histopathologically indicative of both granulomatous and non-granulomatous disease conditions including some suggestive tuberculosis. Fluorescing mycobacteria were observed in 23 submissions. The controls consisted of two cattle experimentally infected with Mycobacterium bovis and one negative control animal. Mycobacteria were detected by fluorescent staining in the lesions of the two tuberculous control cattle but not in the negative control animal.

REFERENCES


GENETIC BLOOD TYPES AND SOME SEROLOGICAL TESTS

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Although the title of this item on the program refers to the subject of genetics, I want to make it very clear that I am not a geneticist in any sense of the word. Having been in diagnostic laboratories most of my working years, my primary interest is accurate diagnoses and methods of arriving at them.

A few years ago I became interested in the laboratory diagnosis of anaplasmosis. After Theiler’s recognition and report¹ in 1910 on the causative agent, diagnostic procedures for some years relied on stained blood films and the complement fixation (CF) serological test, or on clinical evidence. A few other procedures such as Boynton’s² water-serum test may have been tried from time to time but never gained much acceptance. Then in 1962, the capillary tube agglutination test (CA) was introduced. It had the potential for eliminating some of the disadvantages of the CF test but it created some controversy. Its use has gradually increased, sometimes being used as a screening procedure prior to CF, but the CF procedure finds itself being used less and less.

The comprehensive and valuable report of the USLSA Committee on Anaplasmosis in 1966³ recommends that the CA test be recognized as an official test, although the report also points out that false positive CF test results occur due to eperythrozoonosis.

In 1964, animal inoculation tests at the Iowa Veterinary Diagnostic Laboratory, with blood from a positive reactor to the CA test, led to the suspicion that the serological test results had not been due to anaplasmosis. The animal tests were not sufficiently convincing at this time so they were repeated in 1965 with blood from another positive reactor to the standard CA test. From this test much more definite evidence that the resulting infection was due to eperythrozoonosis was secured. The occurrence of positive CA test results with swine sera from cases of swine eperythrozoonosis has been observed since 1963.

In attempting to develop some means of distinguishing between anaplasmosis and eperythrozoonosis positive tests without changing the technique of the CA test, a serum dilution system was adopted. Using serum from cases which gave positive results on the routine test, the test was repeated with undiluted serum and with a portion which was diluted in a two fold series, starting with a 1 to 1 mixture with normal saline and extending to an including a 1 in 32 dilution. After some experience with this retest program the routine procedure at the Iowa laboratory was changed only to the extent of including a dilution of 1 to 4 with the normal single tube test. Test result reports, of course, still depended only on the undiluted, single tube reaction as recommended for the standard CA procedure. Some encouraging results of the dilution program have been recorded.
Illustrative samples of some test results can give a quicker and better report than can be written, so if we start with the first slide I believe one or two causes of false positive CA tests can be seen.

Slide 1. Case 5031—In case someone is not familiar with the CA test, this is just a sample of a good positive reaction. Counting from left to right, the first tube constitutes the standard CA test and is the sole criterion for reporting test results. The second tube is the 1 to 1 dilution with normal saline and the other are successive 2 fold dilutions. Next slide please.

Slide 2. This is a Giemsa stained film to illustrate the physical character of the CA antigen itself. There are many anaplasma bodies present, but there is also other particulate matter of varying size and shape. Since the antigen is prepared from anaplasma infected blood cells the irregular material is assumed to be cell fragments. Next slide.

Slide 3. This is also CA antigen but it is stained with acridine orange. Personally, I prefer this stain over Giemsa for this material. There is more differential contrast because of normal tissue fluorescence. The picture isn't quite clear enough but under the microscope many anaplasma can be distinguished both free of cellular material and scattered thru the large and small cellular fragments. What does complement fixation antigen look like with these same stains? Next slide please.


Slide 5. CF antigen and AO stain. Preparation of the antigen starts with blood cells, so the two antigens contain cell fragments and have some physical properties in common. Next slide please.

Slide 6. This is strictly the result of curiosity. The two antigens are for use in entirely different test procedures, but if CA positive serum is used with CF antigen in the capillary tube procedure, positive test results occur. In the very few instances that I have done this, the CF antigen gave a higher titer than the CA antigen. But to get back to a more normal use of CA antigen, what does the dilution retest procedure show with serum from a case of anaplasmosis. The first slide we saw showed dilution reactions but that was from a routine field test of previous years, and the next slide is from a recent case on which I could get supporting information.

Slide 7. (4842)—This is the positive routine test with undiluted and 1 to 4 diluted serum. Next slide please.

Slide 8. (4842)—This is the retest with undiluted serum and dilutions from 1 to 1 thru 1 in 32. This was a case from the field and the practitioners description of the clinical signs, plus a laboratory examination of stained blood films which were available, left very little doubt that this was a case of anaplasmosis. I'll ask you to remember this picture because we will come back to this case in a few minutes. This is a good example of a test reaction due to anaplasmosis so what does the test reaction from a good case of bovine eperythrozoonosis look like? Next slide please.

Slide 9. (3028)—This test is from a bovine field case. The routine CA test, shown in the tube on the left, gave a good positive reaction. The
case was thoroughly investigated by members of the Diagnostic Laboratory staff, both in the field and with specimen material brought to the laboratory. Some of the clinical evidence was suggestive of either anaplasmosis or eperythrozoonosis but other factors were suggestive of some type of toxemia. After completing laboratory tests and examinations, including a hematocrit of 21.5%, the diagnosis was primary eperythrozoonosis complicated by bacterial pneumonia. The significant point about the serological test result is the fact that the positive reaction occurred only with undiluted serum. (Put this slide aside and we'll go back to it.)

These examples of relatively high titers with serum from anaplasmosis infected cattle, and very little if any dilution titer with serum from cattle affected with eperythrozoonosis, are fairly consistent in routine CA testing. But this titer difference alone does not exclude the possibility of the positive tests observed under standard CA procedures, or even very low dilutions, being due to causes other than either anaplasmosis or eperythrozoonosis. One of the other possible causes could be genetic blood types, and this possibility can be investigated without making any change in standard CA test methods. During the past 50 years the geneticists have made much progress in clarifying inherited blood factors. If we may have the next slide, one of the more generally known interrelationships is outlined.

**Slide 10.** Human A system—cells agglutinated by O & B sera.
Swine A system—cells agglutinated by some natural anti A sera.
Sheep R system—cells agglutinated by some natural anti R sera.
Bovine J system—cells agglutinated by some natural anti J sera.
Next slide please.

**Slide 11.** This is the positive test result from the use of CA antigen and normal human serum from a known blood type B individual. Next slide.

**Slide 12.** This positive test resulted from the use of normal human serum from a known blood type O individual. These two slides indicate that the CA antigen was prepared from blood cells of one or more animals whose blood type was J positive. However, the slides do not mean that all human O and B sera will give positive test results such as these. If the CA antigen contains J positive substance on its cell fragments, it should be agglutinated by some J negative sera. Next slide.

**Slide 13.** (2848-28)—This shows the positive CA test resulting from the use of serum from a known J negative cow. The cow is in a small herd that has been under the close care of a veterinarian for some time and has never been suspected of having anaplasmosis. A portion of this serum sample was also tested at another laboratory by the CF test and reported as showing a 2 plus suspicious reaction. Another portion of the serum was adsorbed with Formocells* (either bovine or human) coated with commercial blood type A substance.** This product is

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* Difco Laboratories, Detroit, Michigan.
** Blood Group Specific Substance A. Michael Reese Research Foundation, Chicago, Illinois.
prepared from swine stomachs for us in human blood transfusions. Next slide.

**Slide 14.** (2848-28)—Here is the same positive test alongside the negative result that followed testing of the adsorbed serum. The agglutinins active in this case are evidently the genetically controlled anti J. Next slide please.

**Slide 15.** (4463-15)—This is another J negative individual with an apparent false positive test result that disappeared after adsorption. Next slide.

**Slide 16.** (4842)—Now we'll go back to the well supported case of anaplasmosis whose routine and dilution retest results were pictured in slides 7 and 8. This slide shows the test results with a portion of serum after adsorption in the same manner as used in the two immediately preceding pictures. The animal involved in this case was a J negative type but obviously, the full dilution range agglutination here is due to active agglutinins other than anti J. Next slide.

**Slide 17.** (4374)—Here are test results with a bovine serum sample submitted by a practitioner. The blood type of the individual from which the sample was secured is not known. The routine test results are shown on the left. There is a good positive reaction in the undiluted tube, and, although it can barely be detected in the picture, there was sufficient fine agglutination in the 1 to 4 dilution that it could be classified as a suspicious reaction. The right hand pair of tubes shows the test results after adsorption of the serum. The fine agglutination in the 1 to 4 dilution has disappeared and, although there is still a good positive appearance to the undiluted test, it seems to be less pronounced, by perhaps 50%, than the unadsorbed test. The normal assumption in interpreting these test results would be to regard them as due to anaplasmosis but there is a possibility that they might be due to eperythrozoonosis. Although the blood type is unknown there is some visual evidence that agglutinins with different activities could be involved. If the serum was from a J negative animal, adsorption would remove the J type agglutinins but leave agglutinins which are active in either anaplasmosis or eperythrozoonosis. Continuing the speculation, consider the CA antigen for a minute. Two antigenic fractions have been illustrated. The primary one is hopefully anaplasma organisms, but they apparently have enough relationship to eperythrozoonata that agglutinins for one also reacts with the other. This kind of relationship usually means that there will be some difference in the agglutination titer. The second antigenic fraction is J positive substance. If the bovine serum to be tested with this CA antigen happens to be from certain J negative individuals we would have a condition of increased titer due to the combined effect of serum antibodies reacting with both antigenic fractions. Now, if we can have that slide of the eperythrozoonosis case that was laid aside earlier, the negative test shown on the right, is the result after adsorbing a portion of the serum from this case. In this particular case, adsorption of the serum with A substance removed enough of the genetic antibody that standard test pro-
procedure gave clearly negative results, so with the materials used it is impossible to tell whether or not either anaplasmosis or eperythrozoonosis antibody is present. On the other hand, in the immediately preceding case, adsorption still left agglutinins to give a positive test but it is not clear whether it is due to anaplasmosis or to eperythrozoonosis. Next slide.

Slide 18. (3413 3/7/65)—Let's consider another problem that occurs with the CA test. This slide shows the routine test results of a test made March 7, 1967. At that time I was going to be away from the laboratory for a couple of weeks so instead of making the dilution retest right then, the serum was left in a freezer until I returned. It was a month later on April 7, 1967, before I did the retest and the next slide shows the results.

Slide 19. The point I want to make with these two slides is the time involved. If portions of the same serum sample from an animal affected with anaplasmosis, (or for that matter with Eperythrozoonosis) gives a positive reaction on the first of two tests, the second test would usually be expected to give positive results also, at least within a reasonable time limit. The serum of this case did just that. Next slide please.

Slide 20. (5328-5) (December 1965)—Here is an older case that didn't behave like the previous one. This was a field case that gave positive test results on the routine procedure done on December 16, 1965. The two tubes of the routine test were 48 hours old when this picture was taken but heavy clumping can still be seen in the 1 to 4 dilution. The remaining tubes show completely negative results with a portion of the serum used for the dilution retest 24 hours after the routine test. Similar results have been observed with some frequency during the past two years. Next slide please.

Slide 21. (4463-9)—A known J positive serum was used in this case with positive routine test results. With J positive antigen and J positive serum the agglutination should not be due to genetic factors. Agglutination occurred only with undiluted serum. To show that the agglutination was not under genetic influence and because of the unpredictable occurrence of quick disappearance of the agglutination, the routine and adsorption tests were made on the same day, August 24, 1967. Both tests were positive when read on August 25 as shown in this picture. Immediately after reading these tests I used some of the original undiluted and non adsorbed serum to set up a retest to be read the following morning, August 26. The next slide.

Slide 22. shows the negative test result that occurred.

There are some additional facts that can be added to the pictorial evidence. With one exception, the cases illustrated where the blood type was known, were individual animals in the small herd which I mentioned previously as being under the direct supervision and care of a veterinarian. The single exception was case 4842 which was certainly anaplasmosis. The small herd consists of 18 animals and includes both J positive and J negative individuals. Complete herd tests were made
on June 3, 1967, and August 23, 1967, with both CA and CF tests. No positive reactions were reported on the CF tests, however, on the June test animal 28 was reported as a 2 plus suspect. This is a J negative individual and earlier we saw the positive CA test that became negative after adsorption of the serum. On the August CF test this animal gave negative test results. Again in the August CF test one individual (#27) was reported as a 1 plus suspect. This animal is a J positive type and its CA test at that time was negative. On the other hand, the CA test in June gave 4 positive results, 2 of which (including #27) are J positives and 2 are J negatives. The August CA test gave 4 positive test results, including both J positive and J negative individuals, but only one of which had also been positive on the previous June test, and this one happened to be a J negative type. The clinical history of this herd is also interesting but would take too much time to detail. At the time of the August tests, blood films stained by both Giemsa and acridine orange methods were examined and every individual was found to show the presence of blood parasites in varying numbers. In other words, the entire herd is infected with Eperythrozoonata but not anaplasmosis, as indicated by the negative CF tests. This is reflected in the erratic and inconsistent CA test results which, on two tests less than three months apart, gave positive reactions with 7 of the 18 individuals, or almost 40%. There is still another side to the eperythrozoonosis positive CA tests and if we can have the next slide, we'll take just a brief look.

*Slide 23.* (3435)—I mentioned earlier that positive CA test results with serum from cases of swine eperythrozoonosis have been observed since 1963. The two tubes in this picture show positive test results with undiluted serum only from each of two 6 week old pigs affected with eperythrozoonosis. One of the pigs was an A positive blood type and the other was an A negative type. Next slide please.

*Slide 24.* (4260) This is the dilution retest of serum from another pig with clinical and pathologic evidence of eperythrozoonosis. The titer is at least 1 in 8. Next slide.

*Slide 25.* (4260) This shows the dilution test result after adsorption of a portion of serum from the same case. This was a routine field case and there was no opportunity to get the blood type, but whether it was A positive or A negative, adsorption had no effect on the titer which is still 1 in 8 in this picture.

An inference that can be drawn from the eperythrozoonosis positive tests of both cattle and swine is that with CA antigen and methods, bovine sera reactions will seldom, if ever, be detectable in a dilution of 1 to 4 or higher, while swine agglutinins will usually give good positive reactions with the 1 to 4 dilution or higher. (There might be a complicated relationship with genetic factors in this difference.)

From the evidence so far, I think two conclusions are warranted. First, bovine eperythrozoonosis can and does cause false positive CA test reactions. Second, some genetic blood type relationships or com-
Gene'l'lc blood types and do influence CA test reactions. Perhaps something can be done to change this situation. Next slide please.

Slide 26. This is a summary of routine CA tests made at the Iowa Veterinary Diagnostic Laboratory during the past several months. A total of 1263 tests were made. 158 or 12.5% were reported as positive for anaplasmosis according to standard test results. 80 of the positive tests showed agglutination both undiluted and in a 1 to 4 dilution. This is 6.3% of the total tests and approximately 50% of all the positive tests. The remaining 78 reactors or 6.1% of the total tests gave positive reactions only if the serum was not diluted. Also, 13 or 1.0% of the total tests were negative unless they were diluted. Considering only the 78 undiluted positive reactions and using only the points we have discussed, there are four good reasons to account for such a reaction. There may be other explanations but for the present let's consider them as unknown.

1. They may occur in convalescent or carrier states.
2. They may occur in an early stage of anaplasma infection and if retested later would show a higher titer.
3. They may be due to eperythrozoonosis and if retested later might be either negative or show no increase in titer.
4. They may be due to genetic blood factors alone or to a combination of genetic factors and either anaplasma or eperythrozoonata infection.

Personally, I would discount the second of these explanations. In most of these situations a serological titer of 1 to 4 or higher has developed by the time clinical evidence leads to a suspicion and test for anaplasmosis. If we estimate that only 5 of the 78 are not anaplasmosis, I think it would be conservative. In connection with the third explanation, I think the incidence of bovine eperythrozoonosis, in Iowa at least, is much higher than that of anaplasmosis. So, on the basis of incidence, I would estimate that more than half of the 78 tests were probably due to eperythrozoonosis. But to stay on the conservative side let me suggest that only 28 are not anaplasmosis.

Some of the tests will involve genetic blood factors under present conditions of antigen preparation. The number of such tests will vary somewhat from month to month but suppose only 4 of the total 78 are due to genetic factors.

So, from three of the four explanations for the occurrence of only undiluted reactions, I think I have conservatively estimated a total of 37 or slightly over 47% of the standard positive tests that could have been false positives during the period of approximately 15 months covered by the summary of CA tests in Iowa. On the basis of total reactors (158), the estimated numbers of false results will account for 23.4% of them, with 17.6% caused by eperythrozoonosis and only 2.5% due to genetic factors.

The use of J negative type individuals for antigen preparation would eliminate agglutination due to this system of genetic blood types, but more study of eperythrozoonosis will be necessary to eliminate the diagnostic errors it causes.
The assistance of Dr. Wilmer J. Miller, Department of Genetics, Iowa State University, and Mr. Derald E. Kimm, Animal Science Antigenic Laboratory, Iowa State University, in performing blood typing tests and for their interest and constructive suggestions is gratefully acknowledged.

REFERENCES

CANINE BRUCELLOSIS: ISOLATION, DIAGNOSIS, TRANSMISSION

by L. E. CARMICHAEL*

A gram-negative coccobacillary organism recently has been recognized as the cause of widespread abortions, whelping failures and epididymitis in dogs located throughout the United States. At the present time the disease appears to be most prevalent in breeding kennels of Beagles, although dogs of other breeds also have been found infected. Abortions and infertility problems in kennels of dogs now known infected by the canine abortion organism have been observed by dog breeders at least since 1963, however serious outbreaks apparently were not noted until 1964-65. Laboratory studies began only recently. Isolations of a brucella organism have been made in our laboratory from placental and fetal tissues of aborted pups or from blood of infected bitches that came from eight states. The disease has been diagnosed in 37 states by serological tests of bitches that aborted. All isolates had virtually identical cultural and biochemical characteristics and they resembled the genus Brucella. Our type strain (RM-666) was isolated in June 1966 from an outbreak of abortions in New Jersey and has been studied in detail in several laboratories. It has been deposited with the American Type Culture Collection (ATCC No. 23365). Additional organisms with similar characteristics have been reported isolated from aborted fetuses in West Virginia, South Carolina, Texas, Michigan and Florida.

Detailed bacteriological and serological studies on strain RM-666 indicated that it fits most closely into the genus Brucella. Studies on water soluble antigens obtained by ultrasonic treatment and examined by immunoelectrophoresis and gel-diffusion tests also have shown near identity with reactions occurring between strain RM-666 and other members of the genus Brucella, but not with other gram-negative species within the family Brucellaceae.

Since primary isolates on solid media always have been mucoid, serological comparisons for taxonomic purposes with recognized species of smooth Brucella have been difficult to evaluate. Morphologically, biochemically and metabolically the canine organism closely resembles B. suis biotype-3, but its antigenic structure is more like B. ovis. The name Brucella canis has been suggested for the bacterium, however the question whether the canine organism should be designated a new species, or B. suis biotype-5, can be decided only after further study by the Subcommittee on the Taxonomy of Brucella.

Clinical and pathological studies indicated remarkable similarity between the canine disease and brucellosis in other species, however the canine brucella caused only mild signs of disease and lesions were confined

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primarily to reticuloendothelial and reproductive tract tissues. The disease in dogs is readily transmitted, especially among dogs closely kenneled. Salient features observed in female dogs consisted of abortions at approximately 40-50 days gestation, early undetected embryonic deaths ("conception failures"), prolonged vaginal discharges following an abortion, generalized lymphadenitis with enlargement of lymph nodes and splenitis. In the male, in addition to lymphatic involvement, there may be epididymitis, prostatitis, dermatitis of the scrotum and testicular atrophy, which often occurred unilaterally. Many infected animals appeared without visible signs of illness, although loss of vigor and lassitude often has been reported by owners of infected dogs used for field-trial purposes. Persistent bacteremia commonly lasting more than 1 year was a characteristic finding in dogs infected orally or parenterally.8

There has been one confirmed human infection. Serums from owners of kennels that experienced several abortions, and also from laboratory personnel working with the disease for more than a year, however, were uniformly negative to agglutination tests.

This paper reports methods used in our laboratory for isolation, identification and serological diagnosis of canine brucellosis. Known routes of transmission obtained from laboratory studies are presented along with patterns of spread within kennels deduced from field reports submitted by dog breeders and veterinarians.

ISOLATION AND IDENTIFICATION

As with other infectious diseases, the most satisfactory method of diagnosis is achieved by isolation and identification of the causal organism. Methods found useful for brucellosis diagnosis in other species1,12 also are applicable to the canine disease. Methods for isolation and sero-diagnosis, reported in the following sections, have proved reliable and not difficult to perform. Although histopathological methods for demonstration of intracellular gram-negative organisms by the Barbito-Lopez or Brown and Brenn stains have been useful in pathological studies,4 we do not employ them for routine diagnosis.

Tissues of choice—Isolation of B. canis from aborted fetal and maternal tissues has consistently been made from cultures of placenta, allantoic fluid, lung, liver, spleen and lymph node. Organisms may be cultured from tissues of adult dogs that are rich in reticuloendothelial cells; lymph nodes and spleen cultures of infected dogs generally yield abundant growth. Vaginal discharges from bitches that had recently aborted usually contain large numbers of bacteria. Blood cultures from infected dogs have been positive for as long as 15 months. In blood, organisms were found associated entirely with leukocytes. Although brucellae were isolated from the epididymides and prostate glands of 12/12 infected males, the rate of recovery from semen samples obtained by manual ejaculation has been very low.
Culture methods—The following media have been found satisfactory for cultivation of the canine brucella:

1. Brucella Broth or Agar (Albimi, Flushing, New York)\textsuperscript{8,4}
2. Tryptose Broth or Agar (Difco, Detroit, Michigan)\textsuperscript{3,10}
3. Horse or Cow Blood Agar\textsuperscript{3,7,8,10,14}
4. Trypticase Soy Agar (BBL, Baltimore, Maryland)\textsuperscript{9}

For culture of blood or fluid specimens we routinely employ Brucella Broth (Albimi) containing 1% of sodium citrate as an anticoagulant. Approximately 2 ml of blood, taken aseptically, is added to 5 ml of broth. After aerobic incubation at 37 C for 4 or 5 days, blood cultures are streaked onto solid media (Brucella Broth containing 1.5% agar). Streaked plates then are incubated aerobically for at least 3 days. If typical colonies are not apparent macroscopically by this time, an additional test of the original broth culture is made. Tissue specimens are streaked directly on solid media and incubated aerobically at 37 C. Colonies usually are visible to the naked eye within 48 hours, however plates not showing growth by this time should be held for 3 or 4 days before discarding as negative. Since specimens often are highly contaminated when received at the laboratory, antibiotics should be added to media to be used for culture. Effective control of contaminants is achieved by adding 25,000 units of bacitracin, 4,500 units of polymyxin B sulfate, and 100 mg. of cyclohexamide (Actidione, Upjohn Co., Kalamazoo, Michigan) to each liter of medium.\textsuperscript{11} These do not inhibit the canine abortion organism.

Identification—Identification is made by establishing characteristics of the organisms as typical of the genus \textit{Brucella} according to criteria set forth by the Subcommittee on Taxonomy of the Genus \textit{Brucella}.\textsuperscript{13} Culturally, \textit{B. canis} is aerobic, grows optimally at 37 C and produces a grayish-white soft growth which is visible after 36-48 hours and changes to a highly mucoid form within about 5 to 7 days. Mucoid growth tends to adhere to agar surfaces. Colonies are 1 to 1.5 mm in size at maturity, with “dwarf” colonies common on primary isolation. In broth, there is moderate turbidity within 48 hours and, after several days growth, a ropy sediment is present. The organisms are gram-negative, small, rod-shaped cells 0.5 by 0.5 to 2 microns in size. Fresh isolates appear more coccoid. In stained preparations, organisms may occur singly, in pairs or in groups. The organism tends to retain the gram stain to a greater degree than the gram-negative enteric bacteria, similar to other brucellae. There is no motility. Additional characteristics are listed in Table I. To assist in preliminary identification and differentiation of the canine brucellae a slide agglutination test using monospecific serum should be done. Antiserum to other \textit{Brucella} species does not react strongly, if at all, with the canine organism, although serums prepared against rough brucellae\textsuperscript{9} and \textit{B. ovis}\textsuperscript{9} will agglutinate the canine agent (Tables II and III). Antiserum prepared against the canine organism which reacts strongly with homologous antigen may react slightly with \textit{Bordetella bronchiseptica} and \textit{B. suis}, and strongly with \textit{B. ovis} antigens.
# TABLE I

**Comparisons Between Canine Organism and the Genus Brucella**

<table>
<thead>
<tr>
<th>TEST</th>
<th>Canine Organism</th>
<th>B. Abortus</th>
<th>B. Suis-1</th>
<th>B. Suis-3</th>
<th>B. Melitensis</th>
<th>B. Ovis</th>
<th>Bordetella Bronchiseptica</th>
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<td>+ or −</td>
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<td>−</td>
<td>+</td>
<td>−</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>Phage lysis*</td>
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<td></td>
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<td>RTD</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>−</td>
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<td>10⁴ × RTD</td>
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<td>+</td>
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<td>Hemolysis of blood agar</td>
<td></td>
<td>−†</td>
<td>−</td>
<td>−</td>
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<td>+</td>
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<tr>
<td>Litmus milk</td>
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<td>−</td>
<td>−</td>
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<td>Growth of agar containing</td>
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<td></td>
<td></td>
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<td>1:50,000</td>
<td>−</td>
<td>+++++</td>
<td>−</td>
<td>+++++</td>
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<td>+++++</td>
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<td>−</td>
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<td>1:25,000</td>
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<td>−</td>
<td>+++++</td>
<td>++++</td>
<td>−</td>
<td>+++++</td>
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</tr>
<tr>
<td>1:50,000</td>
<td>+++++</td>
<td>−</td>
<td>++++</td>
<td>+++++</td>
<td>+</td>
<td>+++++</td>
<td>+</td>
</tr>
</tbody>
</table>

*Jones et al. (9).
†Partial hemolysis on horse or cow blood agar after 72 hours.
TABLE II
SEROLOGICAL COMPARISONS OF BRUCELLA BY AGGLUTINATION TESTS

<table>
<thead>
<tr>
<th>ANTIGEN</th>
<th>Canine Brucella</th>
<th>B. Abortus</th>
<th>B. Melitensis</th>
<th>B. Suis</th>
<th>B. Bord. Bronch.</th>
<th>B. Ovis</th>
<th>A. Equuli</th>
<th>B. Abortus (45/20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine brucella (RM-666)</td>
<td>+4†</td>
<td>-</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>+4</td>
<td>+3</td>
<td>+4</td>
</tr>
<tr>
<td>B. abortus (544)</td>
<td>-</td>
<td>+4</td>
<td>±</td>
<td>+4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>B. melitensis (16M)</td>
<td>±</td>
<td>-</td>
<td>±</td>
<td>+3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>B. suis (1330)</td>
<td>+</td>
<td>+4</td>
<td>-</td>
<td>+4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Bordetella bronchiseptica</td>
<td>+2</td>
<td>-</td>
<td>±</td>
<td>+4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>B. ovis (1182)</td>
<td>+4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>A. equuli</td>
<td>-</td>
<td>...</td>
<td>.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>B. abortus (45/20)</td>
<td>+4</td>
<td>±</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>+2</td>
<td>...</td>
<td>+4</td>
</tr>
</tbody>
</table>

*Canine brucella antiserum was prepared in dogs; B. abortus (A), B. melitensis (M) monospecific serums prepared in rabbits and kindly supplied by Dr. B. Y. Deyoe, NADL, Ames, Iowa. B. suis serum came from an inoculated sow; Bordetella bronchiseptica, B. ovis and A. equuli serum were prepared in rabbits; B. abortus (45/20) ("Dupavac") antiserum was prepared in dogs. "Dupavac" was kindly supplied by Dr. J. M. Williams, Philips-Roxane, St. Joseph, Missouri.

†Data indicate comparative degree of cross-reactions based on quantitative tests presented elsewhere (3). Plus-2, for example, indicates a titer approximately equal to 50 percent that obtained with homologous antigen. Plus-minus indicates partial agglutination.

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<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Homologous Titer (Unabsorbed)</th>
<th>Titer of Serums Absorbed with Heat Killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. RM-666 (canine)</td>
<td>1000*</td>
<td>RM-666</td>
</tr>
<tr>
<td>2. B. abortus (A)</td>
<td>500</td>
<td>Bord. Bronch.</td>
</tr>
<tr>
<td>3. B. melitensis (M)</td>
<td>250</td>
<td>B. Abortus</td>
</tr>
<tr>
<td>4. Bordetella bronchiseptica</td>
<td>1000</td>
<td>B. Mel.</td>
</tr>
<tr>
<td>5. B. ovis</td>
<td>500</td>
<td>B. Ovis</td>
</tr>
</tbody>
</table>

* Titer equals 1/dilution of serum giving at least +2 agglutination.
CANINE BRUCELLOSIS

AGGLUTINATION TEST PROCEDURE

Following exposure to approximately $10^8$ organisms given orally, dogs generally developed bacteremia within 1 to 3 weeks. Coincident with the onset of bacteremia, lymph nodes were palpable and antibody could be detected by the agglutination test. In a study on the relationship between \textit{B. canis} agglutination titers and blood isolation frequencies in a kennel of 166 dogs, organisms were isolated from 55/57 dogs with titers 1:100 or greater, from 3/13 with titers of 1:50 and from 1/10 dogs with titers of 1:25. No isolations were made from 86 serologically-negative animals.\textsuperscript{4} Of twelve infected dogs which were kept in isolation for more than a year, four became negative to repeated blood cultures between 11 and 13 months. Their antibody titers commenced to decline, but have remained at 1:125-1:250 during the 2-3 month period since bacteremia no longer could be detected.

Agglutination tests were performed on 366 dogs located in kennels in Georgia, Illinois and New York who had not experienced abortions or unusual reproductive problems and none had positive titers at 1:50 serum dilutions or greater. Several serums, however, partially agglutinated the \textit{B. canis} antigen at 1:25 dilutions and lower. Similar results were obtained from 139 “family dog” serum samples that had been submitted for distemper antibody tests to the Diagnostic Laboratory, New York State Veterinary College.

Agglutinins were not detected at a 1:50 dilution in any of 53 arctic fox or 91 wolf serums sent by Dr. L. Choquette, ADRI, Hull, Canada, nor were they found in any of 32 coyote or 24 Utah jackrabbit serums sent by Dr. D. O. Trainer, Madison, Wisconsin. Significant titers were found, however, in 2/24 Texas cottontail rabbit and in 8/20 Canadian goose serums sent by Dr. Trainer. It is possible that they represented heterologous reactions since \textit{Pasteurella multocida} antiserum was shown by Diaz et al.\textsuperscript{6} to agglutinate the canine organism at low dilutions and other gram-negative bacteria may react in a similar manner.

The agglutination test procedure presently employed at the Veterinary Virus Research Institute is as follows:

\textbf{Serums:} Clear, unhemolyzed serums are essential. Whole blood cannot be sent because dog erythrocytes are fragile and readily hemolyze.

\textbf{Antigen:} The canine brucella organism (strain RM-666; ATCC \# 23365) is used at the 2nd and 3rd transfer on solid media. Antigen is prepared from 72-hour tryptose or brucella (Albimi) agar cultures grown in Roux bottles. Cultures tend to be mucoid and adhere to agar, therefore glass beads should be included to spread and harvest inocula. Organisms are removed with phosphate-buffered saline (pH 7.2, 0.15M) by gently rocking glass beads. Usually about 25 ml of PBS is used/Roux bottle. The suspension is then filtered through 6 layers of gauze and washed twice with PBS. The suspension should be heated at 56-60 C for 1 hour shortly after harvesting to prevent its
becoming a viscous gel-like material. The heated suspension is stored as concentrated stock. Merthiolate (0.01%) is added as preservative. For use, concentrated stock is diluted to an optical density of 0.15 (procedure I below) or 0.08 (procedure II below) at 420 microns (Lumintron photometer). Antigen has remained stable for at least 6 months.

Test Procedure:

I. Serial dilution method: 2-fold dilutions of serum in volumes of 1 ml are made in PBS, starting at 1:25 dilution. Antigen (OD = 0.15 at 420 microns, 1 ml/tube) is added to the serum dilutions and to an antigen control tube containing 1 ml PBS. Tubes are well-shaken and incubated at 50-52°C in a water bath. Temperatures in excess of 45°C are important, but should not be greater than 55°C.

II. Graded-pipet method: Graded amounts of serum from a 0.2 ml pipet (0.04, 0.02 ml . . .) are added to tubes directly. Antigen (OD = 0.08 at 420 microns, 2 ml) is then added. Tubes are shaken and incubated at 50-52°C, as above. There has been little difference in parallel tests using both methods.

A positive serum control is run with each test series. Reference sera are available from the Veterinary Virus Research Institute, Cornell University, Ithaca, New York 14850.

Initial readings may be made after 24 hours incubation, but final readings are not made until 48 hours. Little difference has been noted between readings except in certain “early” serums, where reactions are clearer at the later reading.

Reading and Interpretation:

Agglutination is rather mucoid and reactions are graded in the usual way from +4 to negative, depending on degree of agglutination. Comparisons are made with the control serum included with each test series.

Readings are not presently regarded as “positive” unless there is at least 2+ agglutination at a 1:100 serum dilution. Titers are recorded as the highest serum dilution giving 2+ or greater agglutination. Occasional prozones may occur with serums from dogs infected less than a month. Organisms generally can be isolated from the blood of animals with titers of 1:100 or greater.

TRANSMISSION

Laboratory studies: Various routes of inoculation have proved successful in establishing infection in dogs. These include intravenous, subcutaneous, oral, conjunctival, intravaginal and contact exposure. *Approximately 10^8
organisms were inoculated, except in those dogs exposed by contact to bitches that had recently aborted. The period between inoculation and the onset of bacteremia varied from 4 days (intravenous inoculation) to 3 weeks (oral inoculation). Three dogs inoculated intravaginally all became bacteremic within 1 week. Four susceptible dogs placed in contact with a bitch that had aborted 2 days previously became bacteremic 1 to 3 weeks later. Two of three females in heat placed in an isolation unit with an infected male that had epididymitis became infected within 3 weeks following observed matings. Both bitches expelled necrotic placental tissue and small embryonic buds about 1 cm long, indicating that abortions had occurred at about 3 weeks gestation. Such occurrences in the field would probably have been considered "conception failures." Vertical transmission was demonstrated. In two instances infected bitches gave birth both to normal-appearing and to weak pups. All puppies from one dam survived, yet they were bacteremic when tested at 1 week of age. Three of five pups from the other litter died at 2 days of age and brucella organisms were recovered in pure culture from their blood and internal organs; the two pups that survived appeared normal, though they were bacteremic until sacrificed at 4 months of age and had enlarged lymph nodes.

Prior to breeding, spread did not appear to occur readily between infected and non-infected dogs. Three infected 6-month-old dogs (two males; one female) allowed to co-habit for 10 months with two uninfected female littermates in an isolation unit did not transmit the infection until estrus was observed. Both females were found bacteremic 1 month later and they had enlarged lymph nodes. Infection was presumed to have occurred by venereal transfer, although organisms could not be isolated by culture of semen from the infected males. Cultures were obtained however at autopsy from the prostate glands and epididymides of the two chronically infected males. We have not been successful in recovering organisms from urine obtained directly from the bladder.

It appears from these limited studies that virtually all mucus membrane surfaces are susceptible to the canine Brucella in a manner similar to brucella infections in other species.

Field studies: Field data was obtained primarily from three large commercial breeding kennels and from reports submitted in response to a questionnaire sent to more than 250 Beagle breeders. Infection rates always were highest in kennels where dogs were housed in groups, or where dogs were placed in common whelping areas 2 weeks or so prior to whelping. In kennels that housed dogs individually, infection rates were much lower, abortions were sporadic and the disease was insidious. Abortions that occurred often were in dogs in cages immediately adjacent animals that had previously aborted or who had failed to whelp at the predicted time. In one kennel there was evidence that infection had been transmitted manually by animal attendants during weekly vulvar examinations of bitches for estrus. The incidence of abortions in two breeding kennels increased over a period of 12 months from 2% to 25-40%. Observed abortions, however, appear to be only a portion of the problem in kennels with established in-
fections since reproductive failures, which probably are early undetected embryonic deaths, seem to be a major manifestation of infection in bitches.4,14

SUMMARY REMARKS

Canine brucellosis is usually first noticed and should be suspected when abortions of unusual frequency occur, or if bitches repeatedly fail to whelp after apparently successful matings. Males may appear normal, but epididymitis, scrotal dermatitis and testicular atrophy often have been observed. Males have been known to become sterile. At the present time the disease appears most common in the Beagle breed, but there is no apparent difference in breed susceptibility. The disease appears to be most prevalent in kennels that participate in field-trials or those that constantly introduce or exchange dogs. Canine brucellosis at present does not seem to be a problem of the family dog. The disease is widespread throughout the United States and at the present time no workable control procedure is available that would be acceptable to most dog owners.

Diagnosis is readily accomplished by isolation of the organism from infected tissues and blood. Infected animals may be identified by serum agglutination tests. Procedures for isolation and identification of \textit{B. canis} are described. At the present time, agglutination tests are being performed for veterinarians by the Diagnostic Laboratory, N. Y. S. Veterinary College, Ithaca, New York 14850. Clear, unhemolyzed serum is essential for reliable test results.

This work was supported in part by The John M. Olin Foundation and by U.S.P.H.S. grant AI-07516-01.

REFERENCES


SALMONELLOSIS IN ARIZONA PENFED CATTLE

Ned W. Rokey, BS, DVM
and
Homer G. Erling, BS

Bovine salmonellosis has been recognized in foreign countries since the turn of the century and in the United States as early as 1916. Until as late as ten years ago the disease was considered to occur only sporadically in cattle in the United States. Since that time increasing numbers of reports have appeared in the literature.

This paper deals with the occurrence and diagnosis of salmonellosis in Arizona penfed cattle. Data cited in this paper were derived from penfed cattle located primarily in Maricopa County, Arizona. Cattle were of cosmopolitan origin, one to two years of age, mixed breeds, and included both steers and heifers.

Serologic studies were conducted on a group of ten pilot animals selected at random from a pen of two hundred cattle. Serum samples were collected on the day of arrival (day "O") at the premises and again on the 27th day following arrival. Serologic tests were conducted for Infectious Bovine Rhinotracheitis (IBR), Bovine Virus Diarrhea (BVD), and Para-influenza 3 (PI3). Rectal temperatures were recorded daily.

Procedures for isolation of Salmonella were those previously reported, with the exception that tetrothionate broth was used as the enrichment media, and were those generally accepted for the isolation of Enterobacteriaceae. Tissues selected for culture were generally lung, liver, gall bladder, and mesenteric lymph nodes. Other tissues were cultured on a less regular basis. Tissues were incubated in enrichment media, streaked to SS, MacConkey's and/or Brilliant Green agar plates. Selected colonies were picked to TSI. Those isolates with biochemical properties characteristic of Salmonella were typed to group and species.

An animal was considered infected if Salmonella was isolated from one or more tissues. All isolations cited in this report were from tissue sources.

CLINICAL SIGNS AND GROSS PATHOLOGIC LESIONS

Clinical signs observed in affected animals were respiratory distress and varying degrees of gastroenteric involvement. Diarrhea was observed inconsistently. At necropsy, a peculiarly demarcated pneumatic process was observed regularly in the lungs. The pneumatic process was clearly defined with normal and pathologic areas clearly demarcated. In some animals, focal suppurative areas occurred throughout the lung. Varying degrees of gastritis and enteritis were usually observed. Gastric lesions consisted of erosion of the mucosa of the abomasum to catarrhal and/or hemorrhagic processes. Intestinal lesions were inconsistent. In some animals, hemorrhagic

*Pitman-Moore Laboratories, Indianapolis, Indiana.
**Arizona State Health Department, Laboratory Division, Phoenix, Arizona.
enteritis was manifested by occurrence of free blood clots in the intestinal lumen. Animals so affected exhibited a watery diarrhea with free blood clots in the feces. In contrast, some infected animals exhibited no evidence of enteritis nor was there visual evidence of a diarrhea at the time of necropsy. Involvement of regional lymph nodes was variable. Enlargement of mesenteric lymph nodes and Peyer's patches were readily demonstrable in some infected animals while in others there appeared to be no gross pathologic changes.

Necropsies were performed and microbiologic examinations were made of tissues from 215 animals. Results of these microbiologic examinations revealed that 67 animals, or approximately 31 percent, were harboring one or more *Salmonella* serotypes. (see Table I)

**TABLE I**

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Number Examined</th>
<th>Number Positive</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960</td>
<td>24</td>
<td>6</td>
<td>25.0</td>
</tr>
<tr>
<td>1961</td>
<td>35</td>
<td>13</td>
<td>37.1</td>
</tr>
<tr>
<td>1962</td>
<td>45</td>
<td>16</td>
<td>35.6</td>
</tr>
<tr>
<td>1963</td>
<td>23</td>
<td>7</td>
<td>30.4</td>
</tr>
<tr>
<td>1964</td>
<td>28</td>
<td>8</td>
<td>28.6</td>
</tr>
<tr>
<td>1965</td>
<td>35</td>
<td>12</td>
<td>34.3</td>
</tr>
<tr>
<td>1966</td>
<td>25</td>
<td>5</td>
<td>20.0</td>
</tr>
<tr>
<td>Total</td>
<td>215</td>
<td>67</td>
<td>31.2</td>
</tr>
</tbody>
</table>

On an annual basis the lowest ratio of salmonellae-infected to non-infected animals was observed in 1966 when 20 percent of the total number of cattle examined were found to be infected. The highest rate of infection was observed in 1961 when approximately 37 percent of the animals examined, 13 of 35, were harboring *Salmonella*.

Seasonal occurrence, based on the total number of cattle examined, is shown in Table II.

Salmonellae-infected animals were detected principally during the fall and winter months, September-February. The highest number of infected animals was found during October when 18 of 29, approximately 62 percent, of the animals examined were harboring *Salmonella*. Infection rates in January, February, August, and September were 58.4, 44.4, 37.5, and 36.0 percent, respectively. No cases were detected in March and June. Total infection rate of animals examined during March-August period was 14.1 percent as compared to 39.6 percent during the September-February period. Infection rates in the four quarters, commencing in March, were 15.4, 12.5,
39.3, and 39.6 percent. Rates in the fall and winter quarters were markedly higher than those observed in the spring and summer.

The sources of isolation and recovery rates of *Salmonella* from various tissues of 67 salmonellae-infected animals are shown in Table III.

**TABLE II**

MONTHLY AND SEASONAL INCIDENCE OF SALMONELLOSIS

<table>
<thead>
<tr>
<th>Month</th>
<th>Number Examined</th>
<th>Number Positive</th>
<th>Percent Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>14</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>April</td>
<td>8</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>May</td>
<td>17</td>
<td>5</td>
<td>29.4</td>
</tr>
<tr>
<td>June</td>
<td>10</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>July</td>
<td>14</td>
<td>1</td>
<td>7.1</td>
</tr>
<tr>
<td>August</td>
<td>8</td>
<td>3</td>
<td>37.5</td>
</tr>
<tr>
<td>September</td>
<td>25</td>
<td>9</td>
<td>36.0</td>
</tr>
<tr>
<td>October</td>
<td>29</td>
<td>18</td>
<td>62.1</td>
</tr>
<tr>
<td>November</td>
<td>35</td>
<td>8</td>
<td>22.9</td>
</tr>
<tr>
<td>December</td>
<td>20</td>
<td>4</td>
<td>20.0</td>
</tr>
<tr>
<td>January</td>
<td>17</td>
<td>10</td>
<td>58.8</td>
</tr>
<tr>
<td>February</td>
<td>18</td>
<td>8</td>
<td>44.4</td>
</tr>
<tr>
<td>Total</td>
<td>215</td>
<td>67</td>
<td>31.2</td>
</tr>
</tbody>
</table>

Recovery rates from gall bladder and mesenteric lymph node cultures were comparable reflecting a success of 75.0 and 73.8 percent, respectively; spleen and kidney cultures had identical recovery rates of 66.7 percent. Comparable recovery rates were shown also for liver and lung cultures, 60.7 and 58.8 percent, respectively. Of the total compilation of tissues tried, *Salmonella* was isolated 167 times in 261 attempts for a success rate of approximately 64 percent.

A total of 77 isolations representing twelve separate serotypes was made from 67 of the 215 animals examined.

Two serotypes, *Salmonella newport* with 32 isolations and *Salmonella typhimurium* with 17 isolations, were encountered most frequently. These
TABLE III

Tissue Source of Salmonella Isolations
From 67 Natural-Infected Cattle
1960-66 Inclusive

<table>
<thead>
<tr>
<th>Tissue Source</th>
<th>Number Cultured</th>
<th>Number Positive</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gall bladder</td>
<td>24</td>
<td>18</td>
<td>75.0</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>42</td>
<td>31</td>
<td>73.8</td>
</tr>
<tr>
<td>Spleen</td>
<td>27</td>
<td>18</td>
<td>66.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>21</td>
<td>14</td>
<td>66.7</td>
</tr>
<tr>
<td>Liver</td>
<td>61</td>
<td>37</td>
<td>60.7</td>
</tr>
<tr>
<td>Lung</td>
<td>51</td>
<td>30</td>
<td>58.8</td>
</tr>
<tr>
<td>Other*</td>
<td>35</td>
<td>19</td>
<td>54.3</td>
</tr>
</tbody>
</table>

*Bronchial, cervical, pulmonary, mediastinal, prefemoral, gastric, and celiac lymph nodes; adrenal gland; blood; and urine.

TABLE IV

Distribution of 77 Salmonella Isolations
1960-66 Inclusive

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number Isolations</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. anatum</td>
<td>5</td>
<td>6.5</td>
</tr>
<tr>
<td>S. derby</td>
<td>3</td>
<td>3.9</td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>3</td>
<td>3.9</td>
</tr>
<tr>
<td>S. newport</td>
<td>32</td>
<td>41.6</td>
</tr>
<tr>
<td>S. st paul</td>
<td>8</td>
<td>10.4</td>
</tr>
<tr>
<td>S. typhimurium (15) var copenhagen ($)</td>
<td>17</td>
<td>22.0</td>
</tr>
<tr>
<td>Other*</td>
<td>9</td>
<td>11.7</td>
</tr>
</tbody>
</table>

*S. blockley, S. dublin, S. infantis: 2 each.
S. kentucky, S. taksony, S. tennessee: 1 each.

Two serotypes accounted for approximately 63.6 percent of the total number of isolations. Multiple salmonellae infections were encountered in 11 of the 67 animals and reflected 16 percent of the infected animals and approximately 0.5 percent of the total number of animals examined, 215.

SEROLOGIC RESULTS

Serologic responses of ten pilot animals from a pen of two hundred are shown in Table V.

Serologic examinations of serums failed to reveal presence of significant
C.F. titers for IBR. Results of C.F. tests of serums for BVD revealed that seven of the ten animals had negative C.F. titers on day “0” while at day 27 all animals tested had positive C.F. titers. Titers of the three animals that were positive day “0” had increased titers in the convalescent serum. Parainfluenza 3 H.A. titers of 1:320 or higher were demonstrated in all serums tested at day 27.

Animals numbered 3 and 8 died during the 43-day test period; Salmonella st paul was isolated from tissues of both animals.

TABLE V
SEROLOGIC RESULTS
10 Penfed Steers

<table>
<thead>
<tr>
<th>ANIMAL NUMBER</th>
<th>IBR C.F. TITER</th>
<th>BVD C.F. TITER</th>
<th>PI3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 27*</td>
<td>Day 0</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>....</td>
<td>1:100</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1:2</td>
<td>....</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>....</td>
<td>1:10</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>1:2</td>
<td>....</td>
</tr>
<tr>
<td>6</td>
<td></td>
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<td>1:100</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>10</td>
<td>&lt;1:2 &gt;1:16</td>
<td>....</td>
<td>&gt;1:10</td>
</tr>
</tbody>
</table>

*Convalescent sera 10-6.

INCIDENCE AND OCCURRENCE

It is difficult to define the incidence of salmonellae infection in man and animals. The occurrence of salmonellosis in penfed cattle is no exception. Although reflecting a comparatively small sample, the studies cited indicate a constant level of salmonellosis occurs in penfed cattle and the disease probably occurs on a year-round basis. Failure to demonstrate the infection in animals in the months of March and June was probably the result of insufficient samples. High rates of morbidity in the pens from which these animals originated would possibly support a hypothesis of an even higher rate of infection represented by non-fatal cases. Moreover, many of the animals examined had received liberal and prolonged medication with antibiotics and other chemotherapeutic agents—a situation which could conceivably render tissues microbiologically sterile and represent or reflect false “negative” microbiologic findings.

Salmonellosis is believed to be endemic in penfed cattle in this area. If consideration is given to the origin of cattle and the similarity of pen feeding operations, the same situation probably exists throughout the southwest, but has not been detected or, at least, has not been reported. Salmonellae-
infected animals were detected in all major pen feeding operations sampled. When four or more animals from a given operation were examined, without exception, *Salmonella* was isolated from the tissues of one or more animals.

**PATHOGENESIS**

Pathogenesis of salmonellae infection, although studied extensively since the turn of the century, remains vague. The exact mechanism of infection is not clearly understood. Even though the genus *Salmonella* is pathogenic by definition, a great deal of controversy exists concerning the exact role the organism may play in the manifestation of disease. This controversy probably had its inception about 1903 when a filterable virus, rather than the bacterium now known as *Salmonella choleraesuis*, was identified as the causative agent of hog cholera. Following the identification of hog cholera virus, the *Salmonella* genus, with few species excepted (notably *Salmonella typhi*, the causative agent of typhoid fever of man) was largely relegated the role of a secondary invader; a concept that, in some quarters, is still in existence today. Moreover, researchers have been unable to satisfactorily reproduce the disease syndrome in experimentally infected animals without interjecting such factors as stress, nutritional changes, intercurrent viral infections, and other physical insults.

Manifestation of salmonellosis in man is believed to be directly associated with the number of *Salmonellae* organisms ingested or the size of the dose. It is extremely doubtful that a similar situation occurs in cattle, or that it does, in fact, exist in man. Although clinical salmonellosis has been experimentally induced in young calves, the number of challenging organisms is unrealistically greater than the contamination generally encountered in feed and water supplies or other possible sources of infection. Experimental reproduction of salmonellosis in semi-mature cattle would be considered to be even more difficult.

Isolation of *Salmonella* from a wide variety of tissues of naturally-infected cattle indicates that the disease in cattle is septicemic rather than simple gastrointestinal infection. Intercurrent viral infections, such as BVD, IBR, PI, and perhaps many others as yet unidentified, are known to exist concurrently with bovine salmonellosis but their inter-relationships have not been clearly defined or well understood. It would be remiss not to consider the relationship of viral infections in a discussion of salmonellosis in penfed cattle. In the work cited, there was substantial evidence that at least two intercurrent viral infections were present in the ten pilot animals cited and more probably throughout the entire pen. Morbidity in this particular pen of two hundred cattle was approximately 65 percent with an overall mortality of 4 percent. Clinical signs were respiratory distress, inappetence, and diarrhea. Similar clinical signs were observed in the ten pilot animals.

**SIGNIFICANCE OF SALMONELLA SEROTYPES**

Twelve separate *Salmonella* serotypes were identified in 67 infected cattle. Definition of specific *Salmonella* serotypes is of significant impor-
tance in epidemiologic considerations of salmonellosis; however, in animals, definition of Salmonella serotypes, with the possible exception of Salmonella dublin in calves, is perhaps of little importance in determining or rendering a diagnosis or in the recommendation of treatment regimes. No evidence could be found to associate a particular set of clinical signs or pathologic changes with a specific Salmonella serotype nor was there evidence that a particular Salmonella serotype had an affinity for any specific tissue. Insofar as Salmonella dublin is concerned, the organism may be held responsible for a distinct entity in young calves.\textsuperscript{11}

**EPIDEMIOLOGY**

The epidemiologic relationships of Salmonella in penfed cattle are a matter of speculation. Cattle involved in these studies originated principally from the southern and southeast United States. Some feeding establishments had cattle from as many as nine states and Mexico. There can be little doubt that at least some of these cattle were salmonellae carriers prior to movement since Salmonella was isolated from tissues of animals dead on arrival at the feeding pens. These animals doubtlessly serve as a source of intransit exposure to other cattle in the shipment as well as to cattle already on the premises constituting a continuing reservoir of salmonellae infection.

Investigation of feed and water supplies, feral animals and birds—considered as classic reservoirs of salmonellae infection—failed to yield substantial information to support these agents as reservoirs of infection in the studies cited. Salmonella could not be demonstrated from the tissues of one hundred feral pigeons collected over a ten-week period at a feeding establishment where salmonellosis was a serious and continuing problem.\textsuperscript{12} Although future studies may reveal other possible reservoirs of infection, it is believed that cattle themselves are the major source of salmonellae infection.

Salmonellosis is not only held responsible for contributing to the singular economic losses to the livestock industry but is also considered to have potentially serious regulatory implications. Although federal regulations strictly prohibit interstate movement of diseased animals, salmonellae-infected cattle are moved without restriction due to the lack of adequate technology to detect latently infected animals.

**SEASONAL OCCURRENCE AND PUBLIC HEALTH**

Seasonal occurrence of salmonellosis in man, with a preponderance of the cases occurring during the fall and winter months, is well documented\textsuperscript{1} but not clearly understood. A similar seasonal trend also occurred in the penfed cattle studied. (see Table 6)

Fifty-five (55) of the 67 cases in animals were detected during the six-month period, September-February, or approximately 85 percent of the total cases. Ten cases were detected during the March-August period; a 5:1 ratio for the two 6-month periods. The total number of animals ex-
amined in the comparable six-month periods was 144 and 79, or a ratio of 2:1.

Source of cattle, population densities, and other husbandry practices, and associated with local weather conditions are believed to be important factors that may influence the seasonal occurrence of salmonellosis. Due to favorable ambient temperatures, feeding pen densities are highest during the fall and winter months. Maximum densities in conjunction with fall and

| TABLE VI |
| MONTHLY AND SEASONAL DISTRIBUTION OF 67 SALMONELLA CASES |
| 1960-66 Inclusive |

<table>
<thead>
<tr>
<th>Monthly</th>
<th>Seasonal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
</tr>
<tr>
<td>March</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>1</td>
</tr>
<tr>
<td>May</td>
<td>5</td>
</tr>
<tr>
<td>June</td>
<td>0</td>
</tr>
<tr>
<td>July</td>
<td>1</td>
</tr>
<tr>
<td>August</td>
<td>3</td>
</tr>
</tbody>
</table>

9 | 15.0 |

| September | 9 | 13.4 |  |
| October   | 18 | 26.9 |  |
| November  | 8 | 11.9 | 35 | 52.2 |
| December  | 4 | 6.0 |  |
| January   | 10 | 14.9 |  |
| February  | 8 | 11.9 | 22 | 32.8 |

57 | 85.0 |

Total | 67 | 67 |

winter rains, and accompanied by wide variations in maximum-minimum day-night temperatures, are favorable influences on the manifestation and spread of not only salmonellosis but other infectious diseases as well. In contrast, summertime feeding pen capacities are approximately two-thirds of those in the winter due to shade requirements. Although subject to some variation, both day and night summertime temperatures are unreasonably high.

The apparent high rate of salmonellosis in penfed cattle can probably
be attributed to recent sophistications in pen feeding management and husbandry practices. Pen feeding of relatively young animals of cosmopolitan origin further compounds the problem. Factors such as mass movement of cattle, ultra-high densities and mass concentration of animals, highly concentrated feeding rations, widespread use of growth promotants, and mass treatment of disease may have resulted in development of a sociobiological environment ideally suited to rapid dissemination and buildup of infectious disease processes in pen feeding operations.

In contrast to the treatment of individual animals common a few years ago, *en masse* treatment with a wide variety of antibiotics is now common practice in pen feeding operations. Unfortunately, in some instances little consideration or regard is given to limitations and regulations concerning minimum dose, duration of treatment, and drug choice.

Emergence of antibiotic-resistant bacteria is of considered importance. Antibiotic sensitivity tests of 117 *Salmonella typhimurium* isolates revealed that 59, or approximately 50 percent, were resistant to oxytetracycline. Approximately 35 percent of 114 *Salmonella newport* isolates were similarly resistant. The salmonellae cultures tested were primarily from cattle.

Emergence of antibiotic-resistant *Salmonella*—and other pathogenic bacteria as well—is of considered economic importance from the standpoint of treatment response. Perhaps of far greater importance is the potentially serious public health hazard posed in the event that antibiotic-resistant *Salmonellae* become established in human populations.

**SUMMARY**

*Salmonellae* were isolated from one or more tissues of 67, approximately 31 percent, of 215 penfed cattle examined during the period of 1960-66. Gross pathologic changes consisted of a pneumonic-enteric disease syndrome. High rates of morbidity-mortality were associated with the disease syndrome. Salmonellae-infected cattle were detected principally during the fall and winter months with the peak of infection occurring in the month of October. Seventy-seven (77) salmonellae isolations were made and represented twelve separate serotypes. *Salmonella typhimurium* and *Salmonella newport* were the serotypes encountered most frequently. The disease was readily demonstrable as a septicemia and *Salmonellae* were isolated from a wide variety of tissues and organs.

Although the true incidence of salmonellosis was not defined, results indicate that the disease is endemic in pen feeding operations in this area. Investigations failed to establish feed and water supplies, feral birds or animals as significant reservoirs of infection and indicate that cattle themselves probably serve as a primary reservoir of salmonellae infection in pen feeding operations.

Dr. Rokey is pathologist in charge of the Animal Pathology Laboratory, University of Arizona Experiment Farm, Mesa, Arizona.

Mr. Erling is research associate, Animal Pathology Laboratory, University of Arizona Experiment Farm, Mesa, Arizona.
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Arizona Agriculture Experiment Station Technical Paper No. 1280.

REFERENCES

NECROTIC LARYNGITIS IN FEEDLOT CATTLE

BY DR. L. N. BROWN

CASE REPORT NUMBER 1

An outbreak of necrotic laryngitis occurred in a contract feeding establishment in eastern Nebraska in the fall of 1966. Over seventeen thousand cattle of diverse origin and with different ownership were involved. More than seventy cattle were lost in less than a week’s time, and thirty were confined to the sick bay for treatment. In the cool mornings of early September, the cattle showed no apparent signs of illness. However, with unseasonably warm midday temperatures, signs of acute respiratory distress became increasingly evident throughout the feedlots. The disease occurred in different lots of cattle irrespective of IBR and shipping fever vaccination. There was no indication of upper respiratory infection, foot rot or diarrhea in any of the cattle.

Post mortem examinations were performed on sixteen cattle by staff members of the Iowa State University Veterinary Diagnostic Laboratory. Cattle that had not been moved from the lots had lesions restricted to the larynx, and had apparently died of suffocation. Cattle that had been cut from the lots and herded to the sick bay for treatment had healing laryngeal lesions, but were dying of acute gangrenous pneumonia, apparently caused by inhalation of dislodged pieces of necrotic exudate.

The true and primary disease condition had escaped detection by a number of veterinary consultants and feed salesmen during the preceding ten-day period. Although diagnosis of the condition was made by adequate post mortem examinations, the factors contributing to the relatively high incidence of necrotic laryngitis as a primary disease syndrome remain unknown.

CASE REPORT NUMBER 2

This case of necrotic laryngitis occurred in midwinter in northwest Iowa, and unfortunately was associated with the death of over 50 percent of 400 feeder calves. Soon after arrival in the feedlot, a number of these calves had been treated for shipping fever. Later, at least one case of thrombo embolic meningoencephalitis was confirmed by isolation of the etiological agent from affected tissues. As calves continued to sicken, necrotic laryngitis was then detected by post mortem examination of several animals. The excessive mortality in this herd occurred approximately 10 days following vaccination of the sick cattle with a modified live IBR virus vaccine. Post mortem examination of three calves performed sixteen days subsequent to vaccination, revealed extensive laryngeal, tracheal and pneumatic lesions. IBR virus was isolated from the nares of these calves.

Because the yards had initially been overcrowded, a portion of these cattle had been gate cut on arrival into nearby river bottom pasture. None

Presented at the National Conference of Veterinary Laboratory Diagnosticians in Phoenix, Arizona, October 16-17, 1967.

538
of the pastured calves became ill. Neither however, did four native 4-H Club calves kept in the sick pen in contact with the sickest of the feeders.

CASE REPORT NUMBER 3

A case of necrotic laryngitis occurred in 160 head of fat Angus cattle in central Iowa in the spring of 1967. Well over 50 percent of the cattle had a purulent nasal discharge, had a mild diarrhea, were depressed and off feed. Ten or twelve had a distinct fetid odor to their breath and exhibited marked dyspnea. One steer presented for necropsy was found to have extensive necrotic tracheitis, severe pneumonia and a mild catarrhal enteritis. IBR virus was isolated from this animal. Paired serum samples (collected 14 days apart) from five sick cattle exhibited a significant rise in specific antibody titer to IBR. BVD titers remained negative or unchanged in four out of the five. There was no change in the level of MP 3 neutralizing antibodies. Apparently an IBR infection existed in these cattle concurrently with, or prior to, the occurrence of necrotic laryngitis.

DISCUSSION

Three cases of necrotic laryngitis have been selected for presentation. In the first, necrotic laryngitis certainly appears to have been the primary disease syndrome which occurred in both IBR vaccinated and unvaccinated cattle, none of which exhibited clinical evidence of IBR infection. In the second, vaccination with IBR virus may have been associated with a sharply increased mortality rate in the sick calves. In the third case, IBR infection was apparently involved.

While necrotic laryngitis and infectious bovine rhinotracheitis may occur simultaneously, the two diseases are not one and the same. Unfortunately, veterinarians in the Midwest too often erroneously attribute the lesions of necrotic laryngitis and tracheitis to the IBR virus, and treat the two diseases as the same syndrome. Certainly in terms of death loss alone, necrotic laryngitis is responsible for a far greater economic loss than is the uncomplicated virus disease. Recent experience in Iowa would also indicate that necrotic laryngitis is occurring with increasing frequency.

CONCLUSIONS

In an industry where individual treatment of sick animals is rapidly becoming less practical, adequate preventive measures are of utmost significance for profitable cattle feeding operations. However, preventive medicine must first depend on an adequate and definitive diagnosis of the disease one is attempting to control, and is equally dependent on a complete understanding of the etiology and pathogenesis of that disease.

Since necrotic laryngitis has not been successfully reproduced experimentally, the primary etiology and pathogenesis of this disease are very poorly understood. Only those in the position to investigate the circumstances contributing to the naturally occurring disease can add to our deficient knowledge. Who is in a position of greater challenge than the veterinary diagnostician?
Bovine cysticercosis is extremely common in many parts of the world. Estimates of infection rates are usually based on slaughter data and, as would be expected, vary widely from one country or investigator to another. Even so, they have been shown conclusively to exceed 50 percent in some countries in Africa and 10 percent in certain European countries. Even some countries in the Western Hemisphere report a widespread incidence of infection. This is somewhat surprising when one considers that the life cycle of the causative parasite, Taenia saginata, has been known for many years and that its ultimate control might come under the heading of proper disposal of human waste material. Unless cattle can ultimately ingest viable eggs of the tapeworm which are only shed in human excreta, bovine cysticercosis could not occur. The obvious answer then would be to positively prevent the contamination of bovine food and water with human excreta. In the case of some countries it appears that adequate emphasis has not been placed on the need for even the most basic sanitary measures.

When compared to the high infection rates in some foreign countries, the United States has a relatively low incidence of infection. On the basis of the total number of cattle slaughtered annually in this country, the number of infected animals probably does not exceed 0.1 percent. This may not seem like a significant number unless it is realized that if the source of infection is present for one animal in a herd it is also available to the others in the herd. Infection rates in some herds may exceed 40 percent. In the face of such numbers, an infected herd may well be the cause of economic ruin for a single producer.

Even within the United States, regional differences in the incidence of infection are considerable, with the result that some parts of the country experience more difficulty with the problem than others. The southern border states adjacent to Mexico undoubtedly have a higher incidence of infection than many states farther north. The exact reasons for this are not completely defined but must include such factors as the proximity to Mexico where widespread infection is known to occur and the use of numerous individuals from Mexico in handling cattle. An obvious additional factor is that at least some of the cattle imported from Mexico may be infected before they reach this country.

In Arizona, most of the feedlots are large and they may cover many acres. Frequently, only a single sanitary facility is provided with the result that workers will sometimes defecate in the cattle pens rather than walk...
or ride a considerable distance to the bathroom. Such situations can event-
ually result in tapeworm eggs being available to and ingested by a few or
a large number of cattle. In a recent outbreak in Arizona a single worker
found to be infected with an adult *T. saginata* admitted having defecated
in the water supply and on the haystack. The resulting infection was found
to be extensive in the approximately 6,000 head of cattle in the feedlot and
the owner suffered serious financial losses. Through the last few years, the
author has received numerous reports of similar occurrences attesting to the
fact that bovine cysticercosis remains in this country as an ever present
hazard to the livestock industry and an occasional public health menace to
human population.

It is obvious that the most logical means of preventing cysticercosis in
cattle is to prevent the contamination of cattle feed and water with human
excreta. Unfortunately, this has not and would not in the near future appear
to be an attainable goal. We, as humans, tend to become complacent about
any problem which is not continually before us in major proportions.

Since the most logical means of control—sanitation—is not likely to
eliminate bovine cysticercosis, it remains that we must attempt to prevent
the infection of humans with the tapeworm. This means diagnosing the
condition in cattle and preventing the consumption of infected beef by the
human populace. In the past and at present this is accomplished through
the inspection of carcasses in slaughter houses. Generally speaking, this
inspection is effective and prevents the large majority of infected animals
from reaching the consumer’s table. Unfortunately, at least in some situa-
tions, the physical inspection procedure is inadequate with the result that
infected beef can be consumed by an unsuspecting public. This statement
is not a condemnation of the meat inspection procedure as has been pointed
out but is simply an honest evaluation. When one realizes that cysticerci
are only approximately three-eighths inch in diameter and may not even be
found in the normal predilection sites (head muscles, heart, and diaphragm)
but may be found in other muscles of the animals body, it becomes apparent
that no practical physical examination can be completely accurate.

Various authors have suggested that cattle infected with cysticer-
cosis are immune to reinfection and that demonstrable circulating antibodies
are present in the serum. These findings coupled with the apparent inade-
quacy of physical examination procedures suggested that immunological
techniques might eventually provide more reliable diagnostic procedures.
Initial efforts utilizing antigens prepared from adult and larval *T. saginata*
in immunodiffusion studies showed that those from the adult tapeworms
were superior for diagnostic test purposes than those prepared from cys-
ticerci. Because of this fact and the relative ease of acquisition of adult *T.
sagitata* in quantity of total material over that possible with cysticerci, all
antigens utilized by this author were prepared from adult worms.

**ANTIGEN PREPARATION**

Adult tapeworms came from a wide variety of sources but were usually
the result of therapeutic treatment of infected individuals. Upon acquisition,
each tapeworm was washed repeatedly in glycine-buffered saline to remove all traces of foreign material. Eggs were removed from the tapeworm by careful dissection and subsequent repeated washing of all segments. The resulting tapeworm fragments were finely ground in a tissue grinder, dispensed in 3 ml. aliquots into tear bulbs, lyophilized, and stored at $-20^\circ$ C. When needed for antigen production, lyophilized tapeworm material was defatted with anhydrous ether, subsequently desiccated, and later reconstituted in glycine-buffered saline. Essentially, the procedure of Chaffee et al. was followed except that the final extraction in glycine-buffered saline was allowed to proceed for 18 hours.

Concentrations of antigenic protein in the resulting antigen were determined by Kjeldahl microanalytical procedures as outlined by Lang. Adjustments to the final desired concentrations were made by dilution with glycine-buffered saline. The final antigens preparation was, in some cases, lyophilized and, when needed, reconstituted to the original volume with triple glass distilled water. All antigens were merthiolated to a final concentration of 1:10,000.

Figure 1. Indurations on the neck of a steer infected with cysticercosis which are due to the intradermal injection of three dilutions of tapeworm antigen.

INTRADERMAL TESTS

Intradermal tests were the same as those as outlined by Dewhirst et al. Initially, this involved the use of antigens with varying amounts of antigenic protein as test materials, the use of several sites on the animal's body, and the lapse of varying amounts of time in which to obtain the most reliable
results. (Figure 1 shows typical reactions occurring on the neck of an infected animal from three different concentrations of the tapeworm antigen.) By utilizing numbers of known infected and noninfected cattle, it was determined that the intradermal injection of 0.1 ml of an antigen containing 0.002 mg of nitrogen/ml of antigenic protein on the side of the neck would result in an induration 18 mm or larger in diameter between one and two hours after the injection. Reactions of less than this critical diameter were usually obtained from noninfected animals. Unfortunately the use of a single division into positive or negative categories forces one, in the case of individual animals, to occasionally make decisions on minute differences. It also was apparent that an occasional infected animal did not even come close to the minimal expected reaction and, conversely, an occasional noninfected animal elicited a response beyond that of some infected animals. When utilized on a large number of animals these latter variations averaged out with the test analyses closely approximating the actual infection rates. In one such test involving a natural infection in feedlot cattle, the intradermal test had a predictive accuracy of 95 percent when viewed on a herd basis.

When analyzed on the basis of individual animals, the intradermal test as currently used falls somewhat short of this level of reliability. In the natural outbreak mentioned above the predictive accuracy was 75 percent when examined on an individual animal basis.

One might argue that such results are not good enough to warrant their use as diagnostic tools but conclusive proof has been provided\(^4\) that they are better than the physical examination procedures utilized in some slaughter houses.

**HEMAGGLUTINATION TESTS**

Test procedures for cysticercosis consisted of utilizing the microtiter system.\(^4\)\(^,\)\(^11\) The hemagglutination test proved applicable as a diagnostic tool when evaluated against known infected and noninfected animals and when utilized in a natural infection. It appears at the present time that the hemagglutination test has some of the faults of the intradermal test, i.e., a lack of consistent reliability. It does have a slightly higher predictive accuracy (85 percent) when viewed on an individual animal basis. Generally speaking, this test promises to be a valuable tool but it is considerably more difficult to obtain consistently reliable results. It is possible that anticipated refinements in antigen production may make this test the one of choice.

**GEL-DIFFUSION TESTS**

Gel-diffusion studies were conducted utilizing a modified Ouchterlony procedure. The specificity of precipitin bands occurring between the tapeworm antigen and sera from known infected cattle was determined by ascertaining if there were continuous bands of identity with precipitin bands occurring between adjacent wells containing antisera developed in rabbits.
Results of these studies showed that demonstrable specific bands occur in the sera of most infected animals. These bands first appeared at approximately 35 days post-infection and, in the case of experimentally infected animals, persisted for at least 250 days.

DISCUSSION AND SUMMARY

Antemortem diagnostic capabilities for bovine cysticercosis would be of considerable value due to the apparent inadequacy of the currently utilized diagnostic procedures in at least some inspection facilities. It is not anticipated that the techniques herein described or referred to would find widespread application in this country because of the relatively low incidence of infection. It would appear, however, that they would be applicable at least in situations of suspected or proven herd infections. Their use in foreign countries where high infection rates occur would provide more reliable diagnostic capabilities.

There is no doubt the antemortem or postmortem diagnosis of bovine cysticercosis is only a single step in the eventual eradication of this disease problem. It is far better to prevent infections from occurring in the first place than to simply rely on diagnostic procedures as the only means of protecting the consumer and the beef producer. Recommendations with regard to this entire problem were published by Dewhirst et al.4

Accurate antemortem diagnostic procedures for bovine cysticercosis appear, at the present time, to be distinct possibilities. It is anticipated that their accuracy could be increased considerably through procedures aimed at further refinements in the antigen production. Some research has been conducted on this aspect of the problem but much remains to be done.

REFERENCES


DISEASES IN CATTLE IN CONTACT WITH SWINE UNDER FEEDLOT CONDITIONS

PART I—PSEUDORABIES AND ULCERATION OF THE MUZZLE AND PERINEUM

E. J. Bicknell, D.V.M., Ph.D.

It is not uncommon, in these times, to observe feeder pigs in a cattle feedlot. While this makes for a more complete utilization of feedstuffs, there are some dangers inherent in this procedure, namely, the spread of certain diseases from apparently healthy swine to cattle. At least three disease entities have been diagnosed in feedlot cattle mixed with swine: (1) Aujeszky’s Disease, (2) Ulceration of the Muzzle and Perineum, and (3) Glossitis—Respiratory Complex, and will be discussed in this presentation.

Pseudorabies

A case of Pseudorabies in cattle is presented which was first brought to the attention of the Iowa Veterinary Diagnostic Laboratory and Veterinary Pathology Department by a practitioner in central Iowa. He was called out to examine some angus heifers that were “acting peculiarly”. The farmer had moved about 50 head of heifers from a pasture to a small feedlot in which swine were being kept. Seven days after being moved, several of the heifers were observed rubbing their hind parts on posts and fencing. The practitioner made the diagnosis of Aujeszky’s disease and called in personnel from the Iowa State Veterinary College to collect tissues for laboratory confirmation. In all cases, the vulva was the site of the intense itching. The degree of vulvar injury ranged from swelling to multiple lacerations. Three heifers were in lateral recumbancy, and comatose. In addition to the affected cattle, two pigs in the lot were ataxic and had generalized tremors.

A necropsy was performed on a heifer that had died the previous night. Brain and lumbar cord were collected, as well as nasal washings from the ataxic pigs. An interesting feature of this case was the inability to demonstrate the presence of virus by rabbit inoculation of cord and brain tissue suspension. However, a small area of CPE was observed in primary swine kidney cells, inoculated with a suspension of spinal cord tissue. Fluid from a second passage was harvested and inoculated into a rabbit. Signs of pruritis as the site of injection were observed on the 4th day postinoculation, with coma and death occurring a few hours later. Virus was not isolated from the swine nasal washings.

The referring veterinarian reported that losses occurred for 7 days after the swine had been taken from the feedlot. The overall mortality in the cattle was 50 per cent. One of the two previously described pigs was kept at the Diagnostic Laboratory for observation. There was a complete remission of signs within a month.
Ulceration of the Muzzle and Perineum

Iowa workers (1, 2) have reported on a condition which they found in cattle that were mixed with swine. They observed erosions and ulcerations on the dorso-medial margin of the external nares, muzzle, vulva and caudal fold. The morbidity rate ranged from 60-100 per cent while the mortality was 0 per cent. They further stated that there was little or no loss in condition of cattle with this disease and the lesions healed without complications within 10 days to 2 weeks after first being observed. Virus attempts by these workers have been unsuccessful.

BIBLIOGRAPHY


PART II—CASE STUDIES ON A GLOSSITIS-RESPIRATORY COMPLEX

H. A. McDaniel, D.V.M., Ph.D.; and K. C. Sherman, D.V.M.*

OBJECTIVE AND INTRODUCTION

The purpose of this paper is to describe the clinical manifestations, lesions, and microscopic changes in a glossitis-respiratory syndrome observed in feedlot cattle in contact with swine. This is similar to the glossitis reported by Wake.4 Tyler and Van Der Maaten2,3 reported a condition with similar muzzle lesions. They suggested a herpes virus as the etiologic agent and believed swine could be inapparent carriers. Some of the gross and histopathologic lesions resembled those seen in malignant catarrhal fever, foot and mouth disease, vesicular stomatitis, rinderpest, infectious bovine rhinotrachitis, blue tongue, and virus diarrhea mucosal disease complex.

Information for this report was obtained from cases in South Dakota (1), Ohio (2), Iowa (2), Pennsylvania (1), and Indiana (1).

HISTORY

Cattle in feedlots containing 150 or more animals were affected. The glossitis-respiratory complex usually occurred during the terminal phase of the feeding period, affecting animals weighing between 850 and 1,050 pounds. Husbandry practices were above average. The quality of the feeder cattle was good. Hereford, angus, and crossbred cattle were affected. In most instances the feeder cattle had been vaccinated for infectious bovine

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rhinotrachitis and bovine virus diarrhea several months prior to the appearance of this syndrome.

In every case reported to date, swine were in direct contact with the cattle. There was no evidence of a similar disease in the swine.

Outbreaks occurred during any season, but were less prevalent in the winter. The origin of the cattle did not seem to be a significant factor. The disease was observed in cattle originating from western or southeastern states.

**Clinical Signs and Lesions**

The most obvious sign was sloughing of the tip of the tongue. Frequently the tip of the tongue protruded in affected animals. (Figures 1 and 2.) Use of the tongue in prehension, mastication, and swallowing was impaired. Affected animals compensated for the impaired function of the tongue by increased use of the lips. A longer time was required to fill their mouths with food and the cattle held their heads higher while chewing and swallowing. However, animals with tongue lesions seldom completely stopped eating, but their feed consumption and rate of gain were reduced. A significant fever was not observed. Severely affected animals lost weight. Additional lesions occurred on the tongue. These included oval and linear ulcers, deep necrosis, and loss of epithelium having the appearance of a ruptured vesicle (Figure 3). Ulcers predominated on the ventral surface and deep necrosis was more prevalent on the dorsal surface.

Histories indicated the respiratory system was involved two weeks to
two months prior to development of tongue lesions. Cattle husbandrymen referred to the respiratory syndrome as "snotting" or "drooling". Cattle discharging copious catarrhal exudate from their nostrils frequently extended the tip of the tongue into the nostril to remove the exudate. The first im-

Figure 3. Bovine Glossitis-Respiratory Complex

Tongue—The involved area is sharply demarcated. Apparently a vesicle covered the dorsal tip of this tongue. Note epithelial regenration from the stratum germinativum (1), presisting papillae (2), and deeper lesion (3).

pression was that an excessive amount of tenacious, stringy saliva was coming from the mouth, but in reality most of the fluid that appeared to be saliva was initially discharge from the respiratory system. Coughing and sneezing, especially just after the animals were aroused were the only conspicuous signs of respiratory involvement. Feed consumption and rate of gain were not significantly affected by the respiratory condition.

Lesions occurred on the muzzle (Figure 4), after respiratory signs were noted and usually before tongue lesions developed. Muzzle lesions consisted of erosions and ulcers. Lesions could be found anywhere on the muzzle, but most frequently near the hairline. Some lesions were heavily encrusted while other were raw and bleeding. Frequently licking of the muzzle removed the exudate; however, less frequent licking increased the encrustation by depositing saliva, cellular debris and feed on the lesions which subsequently became dry and encrusted.

Tongue lesions, muzzle ulcerations and respiratory signs disappeared almost simultaneously three to six weeks after tongue lesions were observed. Once the disease occurred it tended to recur every three to five months.

The incidence of tongue lesions was 10-15 per cent, but occasionally
exceeded 50 per cent. Respiratory involvement was seen in 90-100 per cent, and muzzle lesions in 70-90 per cent of the affected cattle.

Mortality and nervous disturbances occurred in only one feedlot containing approximately 200 cattle. Signs of respiratory involvement preceded tongue and muzzle lesions about two weeks in this herd. Three animals died within a week after tongue lesions were noted. Several animals were somewhat belligerent. All animals in this feedlot were slaughtered within a week after tongue lesions appeared.

This disease appeared to be very contagious, affecting almost all cattle penned together. However, outbreaks have occurred in only one pen of a multi-pen feedlot even though the same swine had access to all pens.

The condition tended to recur in the same feedlots year after year. In an Ohio feedlot it occurred three years in succession even though the premises were cleaned and disinfected and kept free of cattle and swine for approximately six weeks between successive groups of animals. In Pennsylvania this stomatitis-respiratory complex occurred in one pen in 1963, 1964, and 1966, but was never noted in another pen ½ to ¾ miles away. The same personnel tended both pens. Feed was from a common source.

\[\text{Figure 4. Bovine Glossitis-Respiratory Complex} \]

\[\text{Note characteristic drooling, erosions on the muzzle, and nasal discharge.}\]

\textit{Necropsy Findings:} The mucous membranes of the upper respiratory tract were congested and contained some petechial hemorrhages. Very small ulcers were seen on close examination. Occasionally, wart-like proliferations were found in the pharynx.

The anterior cervical lymph nodes were slightly congested and edematous in a few animals. However, most of the lymph nodes appeared normal. Pneumonic lesions were not detected by gross examination or palpation. However, a slow rate of deflation, indicative of emphysema, was observed.

No significant lesions were found in the central nervous system, gastrointestinal tract, feet, skin, or other tissues.
Microscopic Lesions

Reticular tissue in the tongue, muzzle, and throughout the respiratory tract appeared to first proliferate and later became densely infiltrated with lymphoid cells.

**Tongue:** Lesions usually involved epidermis, dermis, and subepithelial tissues. Defects in the epidermis consisted of ballooning degeneration, loss of intercellular bridges, and development of cleavage lines through the superficial layers of the stratum germinativum. (Figures 5 and 6.) Oval or linear ulcers extended deep into the muscle, and often throughout the entire thickness of the tongue. (Figure 7.) The centers of the lesions were ne-
crotic and usually infiltrated with neutrophils. Extensive zones of lymphoreticular hyperplasia surrounded the necrotic areas. Hyperplasia of loose lymphoreticular tissue occurred underneath vascular endothelium, first constricting and finally occluding the lumen. (Figures 8 and 9.) It appeared the necrosis was at least partially due to infarction. Muscle bundles were surrounded and replaced by proliferating lymphoreticular tissue.

Figure 8. Bovine Glossitis-Respiratory Complex
Tongue—Muscular portion. Note blood vessel almost occluded by subendothelial proliferation of lymphoreticular tissue, degenerative changes in vessel wall and infiltrating lymphoreticular tissue.

Figure 9. Bovine Glossitis-Respiratory Complex
Muzzle—Note subendothelial infiltration of lymphoreticular tissue, and cellular infiltration into muscular layers of the vessel wall.

Figure 10. Bovine Glossitis-Respiratory Complex
Muzzle—The subepithelial lymphoreticular tissue proliferation appears to be encroaching on the epithelium. Note the focal accumulation of lymphoid cells.

Figure 11. Bovine Glossitis-Respiratory Complex
Muzzle—Dense lymphoid infiltration appears to be in the process of erupting through the epithelium.

Muzzle: Ulcers and erosions began apparently as focal lymphoreticular proliferation in subepithelial connective tissue. (Figures 10 and 11.) Ballooning degeneration of epithelial cells occurred, but cleavage and vesicle formation were not extensive. Vascular walls underwent changes consisting
of fibrinoid degeneration, intracellular edema, and swelling. (Figure 12.) Lymphoreticular tissue proliferation beneath vascular endothelium was not as extensive as in the tongue.

**Upper Respiratory Tract:** Proliferation of lymphoreticular tissue appeared to be the primary change. (Figures 13, 14, and 15.) Reticular fibers and lymphoid cells increased under the epithelium and apparently pene-

![Figure 12. Bovine Glossitis-Respiratory Complex](image12)
Muzzle Submucosa—Note fibrinoid degeneration in an arterial wall.

![Figure 13. Bovine Glossitis-Respiratory Complex](image13)
Nasal Mucosa—Note the lymphoid cells in the basal epithelial tissue, epithelial balloononing degeneration, and subepithelial lymphoreticular tissue hyperplasia.

![Figure 14. Bovine Glossitis-Respiratory Complex](image14)
Nasal Mucosa—Note the germinal center and the overlying lymphoreticular hyperplasia which appears to be erupting through the epithelium.

trated the epithelium to form small ulcers. Germinal centers were found within some of the focal concentrations of lymphoid cells. Ballooning degeneration was occasionally observed in epithelial cells. When found, only individual cells and small groups of cells were involved. Lesions were not usually confluent.

Changes in the vascular walls consisted of intracellular edema, lymph-
oid infiltration and fibrinoid degeneration. Subendothelial lymphoreticular hyperplasia was not observed.

The wart-like growths found occasionally on the mucous membrane of the pharynx were covered by respiratory epithelium with a core of proliferated lymphoreticular tissue.

**Lungs:** Lymphoreticular hyperplasia occurred beneath the respiratory epithelium of the bronchi and bronchioles, along the interlobular septa and less frequently in alveolar walls. (Figures 16, 17, and 18.) Lymphoreticular tissue proliferations often erupted through the respiratory epithelium and released numerous lymphoid cells into the lumen where they completely or partially blocked the air passage. Vascular changes were similar to those occurring in the tongue, but were not numerous.

**Central Nervous System:** Minimal perivascular cuffing and swollen Purkinje cells were detected in some, but not all, affected animals.

**Discussion**

Degenerative changes in the walls of blood vessels in the tongue, muzzle, and respiratory tract together with slight lesions in the brain were suggestive of malignant catarrhal fever, but significant lesions were not found in lymph nodes, eyes, or mouth except on the tongue. The similarity of the peribronchiolar lymphoreticular infiltration in this condition and the similar lesion found in virus pig pneumonia was of interest. Specimens from affected cattle were examined for blue tongue at the Animal Disease and Parasite Research Laboratory in Denver, Colorado. No evidence of blue tongue was found.
The vascular changes occurring in the tongue, muzzle, and respiratory system were intriguing. Subendothelial lymphoreticular tissue proliferation and vascular occlusion occurred primarily in the tongue and to a lesser extent in the muzzle and lungs. Perhaps the degree of tissue movement enhanced subendothelial tissue proliferation and vascular occlusion.

Necropsy examinations were usually made during slaughter of affected animals. The killing rate was one to two cattle per minute and complete examinations were impossible. Tongues with marked lesions were usually the only tissues condemned by veterinary meat inspectors.

Typical vesicles have not been observed in the limited number of cases investigated. However, lesions which cannot be differentiated from ruptured
vesicles (Figure 3) have been observed. The occurrence of erosive lesions indistinguishable from ruptured vesicles should be considered suggestive of a vesicular condition and laboratory specimens should be examined for evidence of vesicular stomatitis and foot-and-mouth disease. Sloughing of the tongue, deep ulcerative lesions extending into the tongue musculature and respiratory involvement should be considered in the differentiation of this disease syndrome from the classical vesicular diseases.

Further work is underway to better characterize the tissue changes. Attempts are being made to isolate the etiologic agent in cell cultures and experimental animals; however, isolation and characterization of the etiologic agent present difficulties. Tyler and Van Der Maaten\textsuperscript{2,9} were not successful in isolating an etiologic agent using cell cultures and animal inoculations. Workers at the University of Pennsylvania failed to isolate a virus in cell cultures. They also injected lesion material into the tongue of a steer. Lesions did not develop in three weeks.\textsuperscript{1}

The authors recognize dangers inherent in describing the microscopic lesions and pathogenesis associated with this condition, when the only tissues available for study are those from naturally occurring cases. When this condition can be experimentally reproduced and studied in detail, along with appropriate control animals, some of the lesions and factors contributing to elucidation of the pathogenesis will, no doubt, be evaluated differently. Perhaps this study will at least stimulate further work in this area.

**SUMMARY**

A glossitis-respiratory complex of cattle occurred in seven feedlots located in five states. Tongue lesions consisted of vesicles, erosions, ulcers and sloughing. Only the anterior one-third of the tongue was involved. Erosions and ulcers occurred on the muzzle. Mild respiratory involvement preceded tongue and muzzle lesions.

Microscopically this condition was characterized by lymphoreticular tissue hyperplasia. Blood vessels were occluded by hyperplasia of the subendothelial tissue. Muzzle ulcers appeared to be due to focal proliferations of subepithelial lymphoreticular tissue erupting through the epithelium.

**REFERENCES**

INTRODUCTION

Until 1950 the poultry industry of the United States was very regional in nature in that some problems were localized. By this date the industry had grown to where the economic impact of a problem in one area was reflected in many other areas.

This report will outline the development to the present status of control programs for *Mycoplasma gallisepticum* (MG).

ISOLATION, IDENTIFICATION AND TRANSMISSION OF THE ORGANISM

In 1948 Delaplanel described an organism which he had isolated from chickens with a chronic respiratory disease (CRD). For almost a decade this CRD terminology was used. He described an agent which could be propagated in chick embryos, producing arthritis, and when introduced into chickens and turkeys, produced a respiratory infection.

Delaplane further described the isolation techniques for the organism in 1949. The exact etiology was reported in 1952 to be a pleuropneumonia-like organism (P.P.L.O.) and was successfully cultured in artificial broth mediums up to 29 serial passages. In 1954 Chute and Cole described in detail the gross and histopathology of 13 strains of the agent from chickens and turkeys with a chronic respiratory disease or sinusitis. As late as 1961 Brion in France stated the etiology was still unresolved and included "stress factors" as important in the production of the disease. At the same time he proposed the name "Mycoplasmosis" from the species *Mycoplasma gallisepticum*. He further proposed a very impractical control program, the basis of which was slaughter and depopulation. It is fortunate that the U. S. has not had to resort to these methods or much valuable genetic progress would have been lost. Mycoplasmosis was detected in the USSR in 1959 and was attributed to an importation of Canadian hatching eggs. The most pathogenic strain of the organism was described by Zander and known as the S strain. Massachusetts workers made the important contribution that at —20°C the organism remained viable for 72 to 78 weeks in broth media and egg yolk, whereas in albumen, serum, saline, feces, feather meal, chicken muscle and on mucus the time was appreciably less. At 20°C the viability of the MG in all various media ranged from less than 1 to 7 weeks.

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Cover and Waller\(^9\) stated in 1951-52 that CRD became enzootic in the "Delmarva" area and was associated with a pronounced increase in mortality and general economic loss. They studied 70 different lots of pipped and dead embryos and found 50 positive to MG, proving the egg transmission of the organism. Two Canadian workers, Fahey and Crawley\(^10\) described in 1954 a series of outbreaks of MG and with evidence that the organism was egg transmitted. In view of the egg transmission they suggested the control must be started in the breeder hen flocks. The same workers\(^11\) in two experiments proved MG could be transmitted by air, short distances.

The etiology and detailed pathology of CRD were very well presented by Van Roekel, \textit{et al.}\(^12\) in a bulletin in 1957. They reported the complete clinical respiratory and laboratory studies on 71 field cases. Van Roekel, \textit{et al.}\(^13\) again reported on the epizootiology of the disease in 1958. They suggested that transovarian transmission was the most common mode. They found that to treat the dames with an intramuscular injection of streptomycin sulfate (125 mg.) and dihydrostreptomycin sulfate (125 mg.) was of little value. They also felt that partial flock testing may be unreliable in determining the CRD free status of a flock. European workers reported isolating MG from two dead pigeons from the sporadic death of 80.\(^14\) Some of the epizootiological factors were discussed by Serebryakov and Oskolkov.\(^16\) They found turkeys and chickens were more susceptible to respiratory mycoplasmosis than pigeons but ducks from the age of 20 days were resistant as were mice and guinea pigs. Cockerels and young fowls were more susceptible than pullets and adult birds. Hatcher and intraflock transmission of \textit{Mycoplasma meleagridis} was studied in turkeys.\(^16\) Based on the detection of agglutinins, lesions, or the organism in the dead and pipped embryos at hatching, or in pouls studied up to 10 weeks of age, the organism was not transmitted from infected to clean pouls in the hatcher, whereas, transmission did occur through continuous pen contact. Olesiuk, \textit{et al.}\(^17\) reviewed the transmission and eradication of MG in chickens over a decade of research. They stated that to eradicate the disease the logical approach was to break the cycle by establishing primary breeding and multiplier flocks free of infection. The serological methods properly employed in the testing of flocks have proved to be effective in developing MG free units when accompanied by adequate sanitary and disease preventive measures. They maintained that flocks can be kept free of infection for as long as 11 consecutive years. In 1964 thirty-eight flocks were classified as serologically negative, which represented 191,412 chickens, or approximately one-third of the total breeding birds in Massachusetts. More work on the survival of MG in the air was reported in 1967.\(^18\) \textit{Mycoplasma gallisepticum} and \textit{Mycoplasma meleagridis} were recovered in gradually decreasing amounts during a six-hour period from artificially created aerosols. One percent and 0.1\% of the original recoveries were mean values obtained for these two species after six hours in the airborne state.
SEROLOGICAL STUDIES

It was recognized early in the disease research on CRD that a serological test was extremely valuable and necessary for the ultimate control of the disease.

Adler et al.\textsuperscript{19,20,21} made excellent contributions to this area. First a slide agglutination test was described specifically for the CRD agent. At first the antigen was difficult to produce, but later studies revealed simpler production methods. Finally, a more sensitive test for MG, known as antiglobulin test, was described, using very small amounts of antibody. Roberts et al.\textsuperscript{22} stated that the most reliable MG serological test was the hemagglutination-inhibition, the next the tube agglutination, and the least reliable being the serum plate test. In spite of advancements, reliable antigens and standardized tests have not been available. As late as 1965\textsuperscript{23} experiments on positive birds showed a variation in degree of agglutination between antigens as well as a variation of the titer of an individual bird over a period of time. Roberts\textsuperscript{24} found eight different strains of Mycoplasma of avian origin as determined by serological and biochemical methods.

The era of serology has been one of the most productive because by serum tests flocks and individual birds could be segregated for MG control and eradication.

TREATMENTS—DRUGS—VACCINATION

During the period 1950 through 1965 many types of drugs and control measures were used for MG. Streptomycin, penicillin, chlortetracycline and oxytetracycline had been ruled out early in experiments as only valuable against secondary invaders associated with the disease and not for the MG organism.

In 1960, Barnes, Ose and Ellis\textsuperscript{25} reported on the effective use of tylosin (Eli Lilly & Co.) for the control of PPLO in broilers. Either an injection of 5 mg./lb. body weight or 2 mg./gal. of drinking water for 3 to 5 days, or 50 mg. dust/sq. ft. floor space was effective. Chalquest et al.\textsuperscript{26} incubated turkey eggs four hours at 37°C and then immersed them in water to which 0.1% Triton N-101 had been added. This solution was then cooled to 5°C and before the eggs were again dipped erythromycin was added at a concentration of 400 p.p.m. The dipped eggs hatched better than controls in every hatch, with an average increase of 6.8%. There were also less dead-in-shell.

The real breakthrough in the egg dipping technique began in 1959 when Chalquest and Fabricant\textsuperscript{27} reported that the number of MG isolations that could be made from embryos and chicks produced from fertile eggs dipped in antibiotic solutions and subsequently infected with MG was reduced markedly below those from undipped infected controls. In 1962, Levine and Fabricant\textsuperscript{28} and Olson et al.\textsuperscript{29} reported additional data substantiating the value of egg dippings in reducing egg transmission of MG. Hall et al.\textsuperscript{30} summarized further work by stating that the following were successful: (1) Use of the egg dipping technique in broiler hatching op-
erations, for CRD/air succulitis control, (2) Use of the technique by foundation breeders to assist in the development of MG-free breeding stock. Ose et al.\textsuperscript{31} experimentally proved that tylosin administered in the water or feed to MG embryo infected chicks during the first few days of life, and following vaccinating at 3 weeks (Newcastle and infectious bronchitis) reduced active infections in chickens and turkeys.

Inglis et al.\textsuperscript{82,83} found the antibiotic spiramycin given subcutaneously or in the drinking water to be effective in preventing air sac lesions and positive serology to MG. They did not find this antibiotic to be practical for treating hatching eggs.

Vaccination of birds prior to collecting eggs for hatching was attempted by Fabricant and Levine\textsuperscript{84} as well as by Luginbuhl.\textsuperscript{85} MG was isolated from eggs from birds which had been vaccinated in the thoracic air sac with a virulent MG strain at 5 weeks of age. Luginbuhl developed a program called “planned exposure”. A live vaccine was given intranasally at 8 to 14 weeks of age. He reported pullets remained immune through the first laying year.

**EPIZOOTIOLOGY AND CONTROL PROGRAMS**

Although scientists and the poultry industry have had essentially the answers to MG control for several years it takes a tremendous amount of education and experimentation to actually make progress in the use of this information. In fact, the table egg industry, or at least facets of the industry, are still fighting progress toward eradication.

Crawley and Fahey in 1955\textsuperscript{30} suggested a control program which consisted of antibiotic injections, serological testing, and small replacement breeding flocks of 200 to 500 birds. This certainly was the basis for control except that the antibiotic mixture of streptomycin-penicillin was of little value. Edwin L. Jungherr\textsuperscript{87} in 1958 suggested the isolation of serological negative breeding stock and to hatch from these small flocks (not to exceed 500 birds). Promising units of prospective foundation stock should be subjected to bimonthly total blood tests, beginning with a 10% sample test at 8 to 10 weeks. Coles and Cumming\textsuperscript{38} outlined methods in 1959 in South Africa which were used to eradicate PPLO. Their scheme was flock testing for PPLO, disposing of infected flocks immediately or over a 12-month period, allowing infected houses to remain empty for a month, and restocking with birds absolutely or reasonably free from all diseases transmissible through the egg.

Although the appearance of the embryo infected with MG was well known, in 1959 Beckman, Dunlop and Staples\textsuperscript{89} applied the embryo examination technique to 35,625 in the hatchery. They found that by serological testing and a cursory pipped embryo examination, negative stock could be obtained.

Moulthrop\textsuperscript{40} in 1962 reported on 100,000 MG-free broiler chicks on 9 different farms. Livability, feed conversion, and body weight were average or better than positive field control broilers. The birds had no air sacculitis.
It should be pointed out that in 1959, with the advent of compulsory poultry inspection by the USDA, that serious economic losses were being experienced by all areas of the broiler industry in the U.S.

Olson et al.\textsuperscript{41} in 1962 reported on 8 experiments of 100 birds each which were infected with MG; a low egg-transmission rate of MG occurred in all groups infected.

By 1962 large sums of money had been spent trying to control the disease. The Agricultural Research Service of the USDA issued in September a comprehensive report on CRD. This report included major aspects of the infection, diagnostic tests and basic methods for control. Sixty-eight experts in the poultry disease, genetics, husbandry and engineering fields completed the report.\textsuperscript{42}

In September 1962 a report on Specific Pathogen Free poultry by Chute and Stauffer became available.\textsuperscript{43} They showed that by adhering to strict sanitation and isolation principles it was possible to rear 423,320 broiler chickens to market time on 28 farms free of all the common poultry diseases. The condemnation on these birds was 0.06\% whereas the U.S. average for the same period was 3.09\%. A later report by Chute and O'Meara showed that it was practical to produce broilers and keep breeding hens free of MG. Strict sanitation and isolation were considered necessary. The development of an MG-free breeding flock required that each bird be serologically tested several times to assure freedom from the infection.\textsuperscript{44}

Chute\textsuperscript{45} reported that in 2\frac{1}{2} years over 2.4 million broilers in 143 flocks had been produced under the SPF system. Birds free of MG, infectious bronchitis and Newcastle disease had a lower mortality, heavier weights, better feed conversion and lower condemnation rate than birds not on the scheme.

Olesiuk et al.\textsuperscript{46} showed experimentally that tylosin was superior to chortetacycline or erythromycin for CRD control and was most effective in improving weight gains and in preventing or reducing the incidence of clinical signs, agglutination reactions, and gross pathological lesions.

Adler\textsuperscript{47} outlined the details to be followed in preventing egg transmission in reactor hens. He suggested two serologic tests conducted on the same 50 reactor birds at a monthly interval should not indicate any rising titers.

Chute et al.\textsuperscript{48} reported in 1965 that several hundred thousand MG-free broilers had been raised in Maine. The tube agglutination test for MG resulted in about 150,000 negative breeders in an 18-month period. They used several factors to obtain their negative hens; i.e., (1) hatched from old hen (moulted) eggs, (2) dipped eggs in 800 p.p.m. erythromycin with a 30°F differential, (3) injected day-old chicks with 5 mg. gallimycin, (4) reared in small lots, (5) all farms conformed to SPF 30 standard requirements.

Another ARS, USDA report appeared in November 1965.\textsuperscript{49} This second report covered all phases of MG infection in chickens and turkeys. The "planned exposure" approach as well as the egg dipping procedure was included. Sixty-eight experts prepared the report.
STATE PROGRAMS FOR MG CONTROL

Officially recognized State programs have been very slow to get underway in the United States. This has been due to a combination of factors. Even after adequate laboratory and field research was available certain factions of the poultry industry have delayed action. The broiler industry recognized early in 1959 that MG must be eradicated. The primary poultry breeders were not prepared for eradication and with an expanding market for breeding stock, both domestic and abroad, they could see nothing but problems and no immediate necessity. After some MG-clean broiler female multipliers became available many of the primary male breeders did not have clean stock available. Other problems included (1) integrators did not have housing for breeding stock separate from infected stock, (2) State laboratories were not prepared to do even a minimum of testing for the industry, (3) the USDA developed an excellent antigen but did not produce it for the whole U. S. industry and commercial laboratories were not technically ready to manufacture such a product.

In Maine the problems were different. Due to the very high cost of broiler production the integrators saw very early that they must have the cost advantage of MG negative chicks. With an SPF program starting in 1961 they were very well prepared for the requirements, such as cement floors, one age per farm, locked doors, screened windows, etc. Besides this, our laboratory had had 45 years of experience with pullorum-typhoid testing. Therefore, to add another test such as MG was almost routine. The great problem still remained a good antigen supply. It is a well-known fact that the great progress in tuberculosis and brucellosis control in the U. S. has been the direct influence the USDA has had over the production of these antigens. It is conceivable that the same approach to the production of MG antigen by the USDA could greatly assist in the eradication of this disease in poultry. Certainly the multibillion dollar poultry industry merits such attention. Obviously the poultry industry has not been able to communicate their needs to the USDA. The present status of an MG program in Maine is as follows:

1. The pullorum-typhoid testing laboratory at the Department of Animal Pathology, University of Maine, is prepared to test any flock at any time. (The cost is underwritten by the industry at ten cents per sample).
2. All primary breeders are tested in the State.
3. All broiler female multipliers may also be tested.
4. Sample testing is available to any facet of the industry at any time.
5. All integrators demand negative MG breeding male and female chicks.
6. All poultry vaccines and their use must be approved by the State Department of Agriculture.

Table I shows the MG tube agglutination testing results for the past six years. Table II shows the testing for one month.
At the present time some MG testing is conducted in every State. North Carolina and Georgia, two important broiler States, have conducted extensive field experiments and are presently testing large numbers of birds.

One impetus to MG eradication was when Ralston Purina Company demanded negative breeding stock for all their divisions. With such a large company, controlling 6% of the U. S. broiler supply, and geographically diversified, the primary breeders made very rapid progress to supply its needs.

TABLE I

MYCOPLASMA GALLISEPTICUM (PPLO) TESTING IN MAINE

<table>
<thead>
<tr>
<th>Year</th>
<th>Number Tested</th>
<th>Number Flocks</th>
<th>Percent Birds Positive</th>
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<tr>
<td>1962</td>
<td>103,257</td>
<td>105</td>
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<td>249,679</td>
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</tr>
<tr>
<td>1964</td>
<td>322,834</td>
<td>83</td>
<td>7.0</td>
</tr>
<tr>
<td>1965</td>
<td>505,869</td>
<td>290</td>
<td>19.0</td>
</tr>
<tr>
<td>1966</td>
<td>605,176</td>
<td>854</td>
<td>0.5</td>
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</table>

September 1966 through August 1967  627,542  ...  0.35

TABLE II

MYCOPLASMA GALLISEPTICUM TESTING IN MAINE
August 1967

<table>
<thead>
<tr>
<th>Chickens</th>
<th>Number birds tested</th>
<th>Number birds positive</th>
<th>Percent birds positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>77,524</td>
<td>13</td>
<td>0.17%</td>
</tr>
<tr>
<td></td>
<td>Number flocks tested</td>
<td>55</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Number flocks positive</td>
<td>15%</td>
<td></td>
</tr>
</tbody>
</table>

In June 1966 at New Orleans, Louisiana, the National Poultry Improvement Plans Conference was very progressive by inserting in their rules, steps for recognition of MG clean stock. Since that time, a special advisory committee of experts has advised the NPIP the best scientific approach to solve the problem. Due to petty jealousies within Government and between States, and different phases of the industry it will be some time before comprehensive progress will be made. The State of Main poultry industry is proud of the fact that we follow all the NPIP rules religiously and have stock MG tested and free of the disease.

The CRD complex has been described in England, Tunisia, Poland, Russia, Switzerland, Bulgaria, India, Finland, Indonesia, Sweden, Czechoslovakia, Rumania, Japan, Peru, Brazil, Greece, Australia, and, in fact, almost
all of the European countries. Most countries do not have compulsory poultry inspection. They are not forced by economics to eradicate MG as we are in the U. S. Their own incentive is the benefits of economics due to growth and feed conversion.

England has some private company MG testing, but to date there is no organized government controlled scheme.51

South Africa has had a very interesting program. A voluntary State control program was started in 1955. A rapid plate agglutination test was developed. Free stock was developed and stayed free for a number of years. A breeder was able to obtain a "negative PPLO certificate" from the State Veterinary Authorities. During 1966 many flocks were found to be positive. No official reason for these breaks has been reported. One large breeder (Rainbow Poultry Farms (Pty) Ltd.) are pursuing eradication on the basis of a 100% plate agglutination test. Eradication on a National basis in South Africa may be very difficult.52

Italy has a progressive poultry industry but to date there is no official Government MG control scheme. In northern Italy a very large company, Cip-Zoo, which produces both broilers and table eggs, has practiced "controlled exposure" in their breeders with apparently good success.53

In Holland the Poultry Health Service and a large cooperative have proceeded on an MG eradication program. Basically it consists of injecting hatching eggs with 1.6 mg of tylosine. Large egg dipping stations have been established.54

Cumming55 in Australia has been successful in obtaining negative stock for commercial purposes by testing, together with a progressive depopulation and repopulation program.

DISCUSSION AND CONCLUSIONS

Fundamentally the scientists know enough about MG to eradicate it from the poultry industry. Scientific facts do not surmount politics and it is apparent that this impasse may take several years to overcome even with the known facts.

One could conclude from the known facts that eradication of MG from chickens requires the following steps:

1. A standardized antigen (preferably the USDA) should be available in large supply to all States.
2. Each State laboratory must be prepared to test 100% of the birds.
3. All farms must upgrade hygiene and sanitation. A few simple things must be done: (a) one age per farm, (b) screen from wild birds, (c) maintain strict isolation.
4. An unbiased agency, such as the State or Federal Government, should make the final decision on an infected flock. Private testing usually means that infection is not reported.
5. Live poultry vaccines must be controlled to the extent that they are adequately tested and found to be free of MG.
6. Unequivocal cooperation and trust between States, Federal Govern-
ment and commercial companies are necessary to make progress in an area eradication program for MG.

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Differential Diagnosis of Avian Viral Respiratory Diseases: A Comparison of Fluorescent Antibody and Virus Isolation Techniques*

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The Pennsylvania State University
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INTRODUCTION

Differential diagnosis of the common avian respiratory virus infections, Newcastle disease (ND), Infectious Bronchitis (IB) and Infectious Laryngotracheitis (ILT) is not reliable when based on clinical symptoms and gross lesions alone. In advanced cases there may be characteristic lesions, such as the formation of caseous casts in the trachea during the latter stages of ILT infection. Under such conditions with a complete flock history, a relatively accurate diagnosis may be possible. However, during earlier stages of infection, symptoms and lesions of these three virus infections often are so similar that differentiation is impossible. Therefore, it has been necessary to employ indirect laboratory methods to aid in arriving at a diagnosis. The most common method employed has been virus isolation (VI). Some of the problems inherent with the use of the VI technique includes (1) the time involved in setting-up the test followed by a considerable lapse in time until results are obtained (2) the availability of embryonated eggs of the proper age and free of antibodies against the common respiratory viruses (3) the possible presence of other contaminating viruses, bacteria or Mycoplasma in the eggs employed and (4) the presence of contaminating organisms in the diagnostic specimens (usually tracheal scrapings) which cannot be eliminated by antibiotics or filtration. The first of these, time, is especially important when ILT is a factor since a rapid diagnosis would permit vaccination with blockage of the field infection. The other items, parental antibodies or contaminates in the egg source or contaminates in the specimen, may alone or in combination prevent recovery of virus and thus render the VI procedure of little or no value.

A procedure which would permit the direct identification of the causative agent, namely, the specific virus, would bypass the many difficulties encountered in VI tests. The fluorescent antibody (FA) technique provides such a procedure and has the added advantage of requiring a minimal amount of time. Utilization of the FA procedure for the detection of the viruses of ND, IB and ILT in the tissues of experimentally infected birds was demonstrated by Braune.¹ Reagents (specific anti-gamma globulins con-

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¹To be presented at the 71st Annual Meeting of the United States Livestock Sanitary Association, October 16-20, 1967, Phoenix, Arizona.
jugated with fluorescein isothiocyanate) react directly with virus particles within involved tissues. Use of this method eliminates the need for indirect procedures and greatly reduces the time necessary to arrive at an accurate diagnosis. Presented in this paper are the results of six years’ experience in the use of the FA technique for differential diagnosis of avian viral respiratory diseases.

MATERIALS AND METHODS

Diagnostic samples—Materials used for this study were from cases submitted to our poultry diagnostic laboratory. Cases having a history of suspected respiratory infection or those showing gross changes in the respiratory tract were selected. Entire tracheas were removed and placed in petri dishes containing a disc of moistened paper toweling. Samples were identified only by bird and case number. No histories or other necropsy findings were made available to the individual conducting the FA tests in order to avoid bias in the interpretation of findings.

Preparation of specimens for the FA and VI tests—Each trachea was opened longitudinally and the epithelial cell layer removed by scraping with a #15 Bard Parker scalpel blade. If two or three tracheas were involved in a single diagnostic case, the cell scrapings were pooled. If more than 3 tracheas were involved, they were divided and two separate pools of cells prepared. This procedure was followed in an attempt to have a relatively large number of cells from each bird present in the preparation. One-half of each cell pool was placed in a vial containing 3 ml of tryptose broth and frozen at -80°C for subsequent use in VI tests. No antibiotic was added in the event subsequent Mycoplasma isolation was desired. The other half of the epithelial cell pool was used to prepare smears in triplicate for FA examination. Monolayer smears were prepared with the aid of a glass rod and allowed to air dry for 5 minutes prior to beginning the FA procedure.

Virus isolation—Frozen samples were thawed, diluted to 6 ml with tryptose broth and then divided into two samples of 3 ml each. One sample was refrozen. Penicillin (3,000 units) and Streptomycin (30 mg) were then added to the other 3 ml sample. In order to liberate intracellular virus, cells were broken down by grinding for 1 minute with a teflon tissue grinder. Particulate material was sedimented by centrifugation at 1,000 X G for 10 minutes, and the supernatant used for egg inoculations. All embryonated eggs were 10 days of age at time of inoculation and a standard inoculum of 0.2 ml was employed. Artificial air cells2 were produced in six eggs and inoculated on the chorioallantoic membrane (CAM). A second group of ten eggs was inoculated into the chorioallantoic sac3. Eggs were candled daily and any found dead 48 hrs or more post inoculation were examined for evidence of lesions suggestive of virus infection. At 5 days post inoculation, CAM membrane inoculated eggs were examined for plaque formation suggestive of ILT virus infection. If no plaque formation was evident, the membranes were harvested, macerated with a teflon tissue grinder, cen-
trifuged and again inoculated onto the CAM of 6 additional eggs. When necessary, a third passage was made. If no plaque formation was then evident, the sample was considered negative for ILT. Eggs inoculated into the chorioallantoic sac were intended for the isolation of ND or IB virus. The allantoic fluid from 4 eggs was harvested aseptically at 5 days post inoculation, inoculated into 10 additional eggs and also checked for hemagglutination HA activity or embryo mortality typical of ND virus or embryo lesions inoculation and examined for stunting and curling indicative of IB virus. If no HA activity or embryo mortality typical of ND virus or embryo lesions typical of IB virus were found after the 3rd embryo passage, the sample was considered negative.

**Fluorescent antibody test procedure**—Specific immune gamma globulins were prepared against ND, IB and ILT viruses and conjugated with fluorescein isothiocyanate. Specificity or reagents was determined as previously described by Braune and Gentry.4

Smears of tracheal epithelial cells which had been prepared in triplicate were used. The slides were flooded, one each, with the three specific conjugates and allowed to react for one-half hour at room temperature in a moist chamber. Slides were then washed in PO4 buffer (pH 7.3-7.4) for one-half hour, mounted with buffered glycerol and examined under the UV microscope as previously described.4

**RESULTS**

A total of 494 cases have been subjected to FA diagnosis during the period January 1, 1962 to June 1, 1967. Both the FA and VI tests were conducted on all samples during the years 1962 thru 1965. From January 1, 1966 to June 1, 1967, a positive FA reaction was reported as the final diagnosis. However, virus isolation tests were utilized occasionally for spot check confirmation of the FA test or in those cases where FA results were negative and cellular involvement (destruction) was evident. Braunel noted that in the advanced stakes of respiratory infections, extensive lysis may prevent the observation of positive FA reactions since cellular fragments or virus aggregates sufficiently large for FA detection are no longer present.

Comparative results of the FA and VI tests for the period 1962 thru 1965 are shown in Table I. The FA test was confirmed by VI in 325 of the 391 cases submitted. Of the 325 confirmed cases, 260 were completely negative, 32 positive for ND, 27 for IB and 6 cases of ILT. Test results were in disagreement in 66 cases. The FA test was positive and VI test negative in 53 cases (ND-22, IB-19 and ILT-12) and FA negative and VI positive in 13 cases (ND-8, IB-5 and ILT-0).

During the period from January 1, 1966 to June 1, 1967, a total of 102 cases was submitted and diagnosis was based primarily on the results of FA tests. As shown in Table II, 20 FA negative cases were submitted for VI and the initial FA diagnosis was confirmed in all but one case in which IBV was subsequently isolated. Of the 5 FA positive cases sent for VI, all were confirmed.
TABLE I

COMPARATIVE DIAGNOSTIC RESULTS OF FLUORESCENT ANTIBODY (FA) AND VIRUS ISOLATION (VI) TESTS (1962-65)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Test System</th>
<th>Number of Cases Examined During</th>
<th>Diagnosis Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FA</td>
<td>VI</td>
<td>1962</td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>IB</td>
<td>+</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>ILT</td>
<td>+</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>ND</td>
<td>+</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>IB</td>
<td>+</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>ILT</td>
<td>+</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>ND</td>
<td>-</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>IB</td>
<td>-</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>ILT</td>
<td>-</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Case totals</td>
<td></td>
<td></td>
<td>76</td>
</tr>
</tbody>
</table>

TABLE 2

DIAGNOSTIC RESULTS (1966-67)

<table>
<thead>
<tr>
<th>Year</th>
<th>FA Diagnosis</th>
<th>Number of FA Diagnosis Cases Submitted and Confirmed for VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1966</td>
<td>Negative 46</td>
<td>16*</td>
</tr>
<tr>
<td></td>
<td>ND 5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>IB 19</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>ILT 2</td>
<td>1</td>
</tr>
<tr>
<td>1967</td>
<td>Negative 16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>ND 2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>IB 12</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ILT 0</td>
<td>0</td>
</tr>
</tbody>
</table>

*One case was IB positive on virus isolation.
DISCUSSION

In the results reported here, differential diagnosis was limited to the three major avian respiratory diseases of ND, IB and ILT. Known strains of the viruses were used for the preparation of specific antiserums. The specificity of the FA conjugates derived from these antiserums had previously been determined by homologous and heterologous FA reactions against known experimentally infected tissues.¹

During the five and one-half year period which the results reported here represent, a correlation of 83.7% was obtained between the FA and virus isolation tests. However, during the initial two years, a correlation of only 70% was found. Of the cases which were not in agreement, 6.5% were FA negative and VI positive, while 23.5% were FA positive and negative.

SCHEMA EFFECT ON ANTIBACTERIAL THERAPEUTIC AGENT COMBINATIONS

![Diagram of antibacterial therapeutic agent combinations]

1. Reference or citation needed.
on VI. Several factors were considered responsible for the initially lower correlation of results.

Following natural exposure to any of these respiratory viruses, only a few cells of the tracheal epithelium are initially involved. As the infection within these few cells advances, the aggregates of virus become sufficiently large for detection by the FA procedure and a positive diagnosis by this method is possible. However, the virus concentration within a relatively small number of cells at this time was apparently not sufficient to induce embryo infection and negative VI results were obtained. Virus concentration may be lowered still further during freezing and storage of samples. It was necessary to store samples until embryonated eggs of the required age were available. In addition, original VI tests were carried out using eggs from the University Poultry Plant. The flock supplying these eggs had been vaccinated against ND and IB. Interference by parental antibodies may have accounted for some negative VI results when FA tests were positive. Since 1964, only eggs from pathogen free flocks (SPAFAS, Inc., Norwich, Conn.) have been employed for VI tests.

Occasionally we were requested to conduct the FA test on flocks which had no clinical symptoms or lesions indicative of respiratory infection. In one case we returned a diagnosis of Newcastle disease much to the disbelief of the flock owner. Our test results were questioned, but we were greatly encouraged concerning the validity of the FA results when a few days later the owner again submitted birds from the same flock which were then going through an active respiratory infection. Our original diagnosis of ND was confirmed by additional FA tests.

The cases showing negative FA but positive VI tests were of major concern. Pathogenesis studies\(^1\) on experimentally infected birds had shown that in advanced cases of infection, virus could still be isolated from tracheal scrapings several days after FA test results became negative. Virus was apparently present in the materials used to prepare these smears, therefore, the FA reaction must have in fact taken place. However, due to cellular destruction, virus aggregates were too finely divided to be detected.

The FA technique for the routine diagnosis of respiratory infections is, in our opinion, the procedure of choice. It permits the direct detection of viruses in a much shorter period of time. During the early stages of infection, it is more accurate than VI tests. However, during the very late stages of infection when FA tests are negative and extensive cellular destruction is evident, follow-up VI tests should be employed. It has been repeatedly emphasized to the poultrymen that respiratory cases should be submitted during the earliest possible stages of infection in order to utilize fully our diagnostic services. Their cooperation has markedly eliminated many problem cases (usually in advanced stages of infection) originally encountered, and is reflected in the results obtained since 1964.

SUMMARY

A comparison of the FA and VI tests for detection of avian respiratory viruses, ND, IB and ILT was presented. During the period 1962-1963 there
was lack of agreement in 30 percent of the cases. This was reduced to 6 percent during 1964-1965. Principal factors responsible for this lack of agreement were (1) the status of eggs used for VI tests and (2) stage of the infection when specimens were submitted to the laboratory. Use of eggs from pathogen free flocks after mid 1963 eliminated the problem of parental antibodies affecting VI tests. Specimens submitted during the early stages of infection can be diagnosed by FA before virus can be isolated in embryonated eggs. The opposite occurs during the later stages of infection when due to cellular breakdown, FA tests are negative but VI tests are positive. A positive diagnosis by the FA test is reliable. However, when gross lesions and symptoms are indicative of respiratory infection but the FA test is negative and cellular destruction is evident, VI test may provide a diagnosis.

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GUIDE FOR RATIONAL USE OF ANTIBIOTICS AND CHEMOTHERAPEUTIC AGENTS

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The ever increasing desire for rapid control of bacterial infection has stimulated the empirical use of various combinations of chemotherapeutic agents in the hope of synergistic actions comparable to that obtained with penicillin and streptomycin, and with polymyxin B and neomycin. The fallacy in this approach is that some combinations of therapeutic agents produce other than the desired results.

Bacteria react in one of four ways in response to exposure to chemotherapeutic drugs: (1) suppression, (2) habituation, (3) dependence, (4) stimulation. The one of interest here, suppression, occurs either through direct lethal effect or through inhibition of further growth. Through this action control of the disease process is made possible.

The degree of suppression, or beneficial effect, of chemotherapeutic drugs against infectious agents is influenced by many factors that cannot be covered here. However, of special interest are the effects of these drugs on each other when they are used in combination.

Additive effect is accomplished when two drugs interfere with the same constituent cellular, or metabolic processes, giving an added effect similar to an increased dosage. These combinations are useful when increased dosage rates of an agent may produce undesirable side effects.

Synergistic effect is accomplished when the two drugs interfere with different constituent cellular, or metabolic processes, giving an effect greater than could be attributable to additive action. The organism surviving the first drug, if susceptible to the second would succumb thereby giving the synergistic action.

Antagonistic effect is produced when the action of two drugs in combination is less than that which could be achieved by the most effective member of the combination when acting alone.

No effect. No difference is observed in the disease process or bacterial growth.

Antibiotic antagonism is encountered occasionally when certain bacteriostatic agents are used in combination with certain bactericidal agents. This phenomenon is due to the mode of action of the drugs involved rather than a "neutralization effect" such as the acid-base chemical reactions.

Many factors inter into this process. Concentrations of the respective drugs and the organisms are of significance in an evaluation and due to the degrees of the antagonism it is often impossible to determine clinically—that is in the sick animal. However, the phenomenon has been noted by
numerous veterinary practitioners in my experience in diagnostic and consultation work.

A relatively up-to-date scheme of Jawetz providing data on the chemotherapeutic agents commonly available in dosage forms convenient for veterinary use has been prepared and is suggested as a guide for use of anti-

SCHEMA
EFFECT OF ANTIBACTERIAL THERAPEUTIC AGENT COMBINATIONS

No attempt has been made to arrange the agents to indicate a possible beneficial paring. Results of laboratory and research studies may indicate exceptions to this schema.

The following rules have been suggested as a guideline for therapeutic agent combinations where the circumstances necessitate therapy before sen-
sitivity determination can be made so that known antagonistic combina-
tions may be avoided.

1. Avoid combining bacteriostatic and bactericidal drugs.
2. Antagonism does not occur between members of the two bacterio-
static or between members of the two bactericidal groups.
3. Bacteriostatic antibiotics are never synergistic, but may be additive.
4. Bacteriocidal antibiotics may be synergistic.
5. Combination therapy should be avoided if possible when in vitro
testing has not been done.

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THE DIAGNOSIS OF ABORTION IN SHEEP

B. D. FIREHAMMER, M.S.*

There are many causes of abortion in sheep but I will confine this paper to a discussion of infectious agents of significance or potential significance in the United States.

VIBRIOSIS

Ovine vibriosis is caused by *Vibrio fetus* var. *intestinalis*. This organism is similar to *Vibrio fetus* var. *venerealis*, the cause of vibrionic infertility in cattle. Insofar as I know, the latter organism has not been isolated from sheep, but *V. fetus* var. *intestinalis* has been isolated from cattle.

Pregnant ewes become infected by ingestion of contaminated feed or water and may abort between middle pregnancy and lambing time. In contrast to bovine vibriosis, the disease in sheep is not spread by venereal contact and infected ewes are not infertile. It has been our experience that 10 or 15 per cent of the ewes in an infected flock abort, but we have observed outbreaks where from 3 to 70 per cent aborted.

The fetus may have a slightly enlarged liver and subcutaneous edema over the ventral surface of the body is frequently found; bloody fluid may be present in the body cavities. Light colored areas of necrosis in the liver are found in some lambs (Fig. 1). I have not yet failed to isolate *V. fetus* from a fetus showing lesions of this type. These lesions seem to be more prevalent in lambs aborted near full term. *V. fetus* is very seldom recovered from mummified fetuses.

If the placenta is available and is in good condition, impression smears can be made from the cotyledons and examined for vibrios and for the inclusion bodies of enzootic abortion of ewes (EAE). The placenta may show edema of the chorion and the cotyledons are often pale and necrotic. Smears of the cotyledon and the stomach contents are stained with safranin-O and examined by phase contrast microscopy for the detection of vibrios. If vibrios are found a gram stain is made. The presence of typical vibrios is considered presumptive evidence of vibriosis to be confirmed by isolation of *V. fetus* in cultures.

Some care in conducting the autopsy will facilitate the work of the microbiologist by avoiding unnecessary contamination. The ventral surface of the fetus is saturated with disinfectant and the skin removed with a knife. Sterile scissors are used to open the thoracic and abdominal cavities. The liver and stomach are the organs of choice for the isolation of *V. fetus* and may be immobilized by the use of large hemostats. The surface of the organ to be cultured should be briefly seared with a hot iron and a bulb-equipped Pasteur pipet used to obtain a sample of fluid or tissue.

The heart blood and the lungs are sometimes cultured although they

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are inconsistent sources of *V. fetus*. If a portion of the fetus has been destroyed by scavengers the brain should be cultured as it frequently yields *V. fetus* and is somewhat protected from damage and consequent gross contamination.

Cultures are made into semi-solid Brucella** medium and on blood agar plates. The commercially prepared thiol or thioglycollate semi-solid mediums can also be used. We add 0.5 per cent of yeast extract to each of the mediums and 0.005 per cent of soluble starch to the blood agar. We have found the addition of 10 per cent defibrinated bovine blood to the blood agar to be useful from the standpoint of nutrition and also for supplying a dark background to aid visualization of small colonies. I do not believe selective mediums containing antibiotics are either desirable or necessary for the isolation of *V. fetus* from ovine fetuses.

Incubation is carried out under an atmosphere consisting of 5 per cent oxygen, 5 per cent carbon dioxide and 90 per cent nitrogen. This atmosphere is also satisfactory for use with *V. fetus* var. *venerealis*. Ten per cent carbon dioxide with air can be used in laboratories unable to obtain custom-mixed gas but it is not quite as satisfactory for the plate cultures. The cultures are examined after 48 to 72 hours incubation, but are not discarded as negative until they have been incubated four days.

**0.15 per cent agar in Brucella broth, Albimi Laboratories, Flushing, New York.
Plates are examined at a magnification of 30x with intense illumination from the top and smears made of typical colonies. There is a point of caution to be observed in examination of smears: *V. fetus* var. *intestinalis* of serotype 1 may appear for the most part in the coccoid form but careful observation will usually reveal typical vibrio forms also.

Because we have on occasion isolated *Vibrio bubulus* from aborted sheep fetuses and because *Vibrio fecalis* is often found in the intestinal contents of mature sheep, we subject pure cultures of all isolates to a simple identification procedure. I do not think *V. bubulus* is capable of causing real outbreaks of vibriosis but on occasion I have isolated it from ovine fetuses and feel that under favorable circumstances it sometimes causes an abortion.

The two essentially non-pathogenic organisms, *V. bubulus* and *V. fecalis*, can readily be distinguished from *V. fetus* because they produce large amounts of hydrogen sulfide while *V. fetus* var. *intestinalis* produces only small amounts. Stab cultures in a semi-solid medium such as SIM† or a semi-solid medium to which 0.1 per cent of ferrous sulphate (FeSO₄·7H₂O) has been added, can be used as an insensitive test for hydrogen sulfide production (Table I). Any culture producing a detectable degree of blackening after three days incubation is not *V. fetus*. *Vibrio bubulus* does not produce detectable amounts of catalase while both *V. fetus* and *V. fecalis* do. The addition of a small amount of 3 per cent hydrogen peroxide to the cultures used for the hydrogen sulfide test will reveal the presence or absence of catalase. Before validity is placed in any test, an adequate amount of growth must be present.

Isolates presumed to be *V. fetus* on the basis of the insensitive hydrogen sulfide test and the catalase test should be examined to determine if they are *V. fetus* var. *intestinalis* or *V. fetus* var. *venerealis*. Although the latter organism has not yet been isolated from sheep, both varieties have been isolated from cattle. *Vibrio fetus* var. *intestinalis* will grow in semi-solid medium to which 1 per cent of glycine has been added but *V. fetus* var. *venerealis* will not tolerate the glycine (Table II). Incubation should be for

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<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DIFFERENTIATION OF VIBRIO SPECIES</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Insensitive H₂S Test</th>
<th>Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. bubulus</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>V. fecalis</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>V. fetus</em></td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

† Difco Laboratories, Detroit, Michigan.
5 days because some \textit{V. fetus} var. \textit{intestinalis} isolates seem to be slightly inhibited by the glycine.

Although it may be somewhat out of place in a discussion of ovine vibriosis, I would like to mention the biotypes of \textit{V. fetus} var. \textit{venerealis} as described by Bryner \textit{et al} (Table II). The sensitive hydrogen sulfide test is used to detect these types. This test is conducted by suspending lead acetate saturated strips over cultures in semi-solid agar containing 0.2 per cent of L-cysteine hydrochloride. We do not use L-cystine because of its poor solubility. Any blackening of the paper after 5 days incubation is considered a positive test. Biotype 1 does not produce hydrogen sulfide but biotypes 2 and subtype 1 do.

The tube agglutination test can be used for detecting antibody in blood serum on a flock basis but the titers are frequently low and seldom persist long. Because of problems with antigen preparation and the general availability of fetuses for cultural examination, there does not appear to be any need for this test.

\begin{table}[h]
\centering
\caption{Differentiation of \textit{Vibrio Fetus} Varieties}
\begin{tabular}{|l|c|c|}
\hline
Biotype & Sensitive Glycine & Tolerance \\
\hline
\textit{V. fetus} var. \textit{venerealis} & 1 & – & – \\
\textit{V. fetus} var. \textit{venerealis} subtype 1 & + & – \\
\textit{V. fetus} var. \textit{intestinalis} & 2 & + & + \\
\hline
\end{tabular}
\end{table}

\textbf{ENZOOTIC ABORTION OF EWES (EAE)}

Enzootic abortion of ewes, due to an agent of the psittacosis-lymphogranuloma (PL) group, was described in Scotland by Stamp \textit{et al} in 1950. Although this disease was not reported in the United States until 1958, it probably has been here for a long time. Abortions usually occur in the last month of pregnancy and seldom involve more than 5 per cent of the ewes except in herds completely without immunity to it. Second-lamb ewes seem to be most commonly involved, but the disease is not confined to any particular age group.

Fetuses often show some edema and their condition may range from fresh to mummified. Weak or dead lambs may be born at full term. There is some variation in the appearance of the placenta, but necrosis of the cotyledons is common and thickening of the chorion, especially at the base of necrotic cotyledons is often found. In some cases the chorion may be leather-like or it may be edematous.

The disease is most easily diagnosed by the detection of characteristic elementary bodies in smears from necrotic cotyledons. The heat-fixed
smears should be stained for 10 minutes with dilute (1:5) Ziehl-Neelson carbol fuchsin, differentiated with 3 per cent acetic acid and counterstained lightly with malchite green or methylene blue. The elementary bodies appear red while most bacteria stain with the counterstain. The elementary bodies are rather difficult to detect without some experience but often can be found in clusters and are frequently intracellular. There may be some difficulty in distinguishing between them and the *Rickettsiae* or the ram epididymitis organism. Smears from the fetus itself are not very useful, but fluid from the mouth might be of some value.

The complement fixation test, using heated crude yolk antigen, is of value on a herd basis for diagnosis. Stamp *et al.* considered a titer of 1:16 to be suggestive of infection and higher titers to be evidence of infection; Younger and Parker considered a titer of 1:32 or higher to signify the disease. Titers are present within 2 weeks after abortion and can be detected for at least 4 months. Paired serums may be of value in detecting titer changes. It should be remembered that the antigen is a group antigen and titers against other PL group agents might be detected with it.

The yolk sacks of 6- or 7-day chicken embryos can be inoculated with fetal organs or stomach contents and elementary bodies consequently demonstrated in the embryo. Kidney tissue seems to be a good source but all organs of the fetus may not be infected and all embryos inoculated with infected suspension may not become infected. Infected embryos may survive almost to the point of hatching. In general, a presumptive diagnosis of EAE is usually made following the failure to account for abortions on the basis of bacteriological examination and the finding of elementary bodies in cotyledon smears. Confirmation is usually attained by demonstration of titer against PL group organisms, or isolation of the organisms in chicken embryos.

**RAM EPIDIDYMYSIS (REO)**

Epididymitis of rams and abortions of ewes due to a small gram-negative cocco-bacillus was described in New Zealand in 1952 by McFarlane *et al.* The name *Brucella ovis* was suggested for the organism in 1956 by Buddle but has not received complete acceptance due to differences between its amino acid metabolism pattern and that of *Brucella*. It is informally called the "ram epididymitis organism" or REO.

Although outbreaks of abortion have been reported in New Zealand, the major problem is epididymitis and infertility in rams. This also appears to be the situation in the United States where a rather serious situation exists in rams. To date only one isolation has been made from aborting ewes in Montana.

The chorioallantois may be edematous and the chorion covered with yellow or brownish exudate. There is necrosis of the chorion, which may become thickened and wrinkled. Lambs are usually aborted late in pregnancy or born dead or weak at full term.

The organism is a small gram-negative rod which is weakly acid-fast
when stained by the modified acid fast method. It is microaerophilic on initial isolation and grows under 10 per cent carbon-dioxide but may become adapted to aerobic growth on repeated subculture. The addition of 10 per cent defibrinated bovine blood to agar will facilitate growth. It produces little or no hydrogen sulfide and does not reduce nitrates or produce urease. It is catalase positive and oxidase negative. Hemolysis of sheep or cattle blood has not been observed. Colonies on blood agar are about 0.5 mm in diameter after 48-hours incubation and are circular, convex smooth, glistening, entire and grayish-white in color. The organism is non-motile and does not have a capsule. We have not been able to detect carbohydrate fermentation of any of our isolates with conventional methods. It is basic fuchsin and thionin tolerant.

**TOXOPLASMOSIS**

Ovine abortion due to *Toxoplasma gondii* does not appear to be a problem in the United States but the possibility that outbreaks might occur should be kept in mind. A characteristic lesion is the presence of white nodules 1 to 2 mm in diameter on the cotyledons. This lesion is not always found, however. The organism may be found in sections or smears of the cotyledons and isolation can be accomplished by intraperitoneal injection of tissue preparations into mice with resulting localization in the brain.

**LISTERIOSIS**

Abortion of sheep due to *Listeria monocytogenes* is not extremely uncommon and the causative organism can readily be isolated from the fetuses. I doubt if refrigeration of the fetus for a period of time is necessary to facilitate isolation as it sometimes is with cerebral tissue. Listeriosis should be seriously considered in sheep that abort following feeding of silage. The organism is an aerobic gram-positive rod that does not reduce nitrates and is motile when grown at room temperature and non-motile when grown at 37 C.

Contribution from the Montana Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, Paper No. 854, journal series.

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EPIZOOTIC BOVINE ABORTION AS A PROBLEM
IN THE DIAGNOSTIC LABORATORY

by J. Storz

Epizootic bovine abortion (EBA) was recognized in 1956 by Howarth and coworkers\(^1\) as a specific form of abortion in California cattle. The disease was differentiated by the generalized hemorrhage, ascites and hepatopathy of the aborted fetuses. Howarth and associates\(^1\) were impressed by the epizootic nature of this disease and named it accordingly. The cause of EBA was identified in 1959 as a member of the psittacosis-lymphogranuloma (PL) group of organisms.\(^2,3\) Schoop and Kauker\(^4\) presented in 1956 serologic evidence and cytologic proof that PL agents were the cause of abortions in a dairy herd in Germany. With the recognition of the specific pathologic changes in EBA afflicted calves,\(^5\) it became almost certain that Beer and Martin\(^6\) in Germany as well as Rossi and Ghittino\(^7\) in Italy, were studying a cattle disease identical with EBA. This type of abortion has also been identified etiologically in France,\(^8\) Yugoslavia\(^9\) and Israel.\(^10\)

In the United States, EBA occurs not only in California but also in Utah, Idaho, Wyoming and Colorado, where it was identified by the author through isolation of the causative agent.\(^11\) Parker\(^12\) diagnosed EBA etiologically in Montana. The wide distribution of EBA in the western states might induce one to expect this disease also in other parts of the United States. The purpose of this presentation is to describe specific procedures and findings that might help investigators in the diagnostic laboratory to differentiate, identify and diagnose EBA.

Natural History of EBA: The disease affects both beef and dairy cattle but appears to be more widespread and severe under conditions prevailing in beef herds. In California beef herds with a high proportion of first-calf heifers, abortion rates as high as 40% have been observed. A more sporadic incidence was found in dairy herds. In such herds, cows of all ages and with various numbers of previous gestations aborted, while first-calf heifers appear to be involved more frequently in abortion epizootics in beef herds as seen in California.\(^1\) The same cows may abort EBA specifically in two consecutive years.\(^13\) In the condition which was initially recognized as EBA because of the pathologic changes in the aborted calves the fetuses usually were aborted during the last 2 months of gestation and in many instances dead calves were born near term.\(^1,5\) However, others and we\(^6,11,13,14\) found PL infected fetuses that were aborted as early as the fourth month of gestation. Howarth et al.\(^1\) observed a seasonal incidence with the highest losses in herds calving from July through October. We observed EBA in dairy herds of the intermountain region throughout the entire year while we saw EBA in beef herds only in February and March because breeding was scheduled in these herds to insure calving in early spring.\(^18\) The seasonal incidence of EBA in this area had thus a definite relationship with the breeding practices.
In naturally occurring cases of EBA, no clinical illness other than an occasional febrile reaction and the event of clinical abortion was observed. Experimental infection induced a typical febrile response irrespective of the route of inoculation. The temperatures rose to at least 105°F and as high as 108°F for 3 to 4 days. A leukopenia existed during this period after inoculation. All the pregnant cows that were given the inoculum intravenously, intramuscularly and subcutaneously aborted or gave birth to weak and infected calves. A large percentage of the experimentally exposed cows had retained placentas, sequelae to the abortion also observed in the natural disease. There are no clinical and anamnestic features that are specific for EBA.

THE CAUSE OF EBA AND ITS IDENTIFICATION

Nature of the EBA Agent: The causative agent of EBA is a member of the PL group of infectious agents that comprises a large, ever increasing number of antigenically related microorganisms which are culturally, morphologically and tinctorially similar. Among other distinguishing features of the PL agents is their size which is just above the border of visibility by the light microscope. They are obligate intracellular parasites that undergo a complicated developmental cycle which includes a noninfectious phase or a phase of extremely reduced infectivity. These agents induce interferon, and their reproduction is affected by virus-induced interferon. A recent classification proposal unified a variety of different nomenclatural attempts and established "Chlamydia" as genus name for PL agents.

Isolation of the EBA Agent: Isolation of the EBA agent from field specimens is possible only under ideal circumstances. The tissues and fluids must be fresh, and a detectable amount of viable agent must be present. In many typical EBA cases, abortions occurred after the acute infection of the fetus had subsided.

The indicator hosts of choice for detecting and isolating the EBA agent are developing chicken embryos which are used at the age of 6 to 7 days and are inoculated by the yolk sac route. We use a volume of 0.5 ml as an inoculum per chicken embryo. Samples of the internal organs of aborted fetuses are ground, prepared singly or as pools to make 10-fold suspensions. These and at least 2 additional serial 10-fold dilutions are inoculated into sets of developing chicken embryos. The suspension can be cleared by low speed centrifugation, which is necessary for the parallel inoculations of cultured bovine cells but not essential for the chicken embryo inoculation. The inoculated eggs are candled daily until the 20th day of incubation unless death occurs earlier. Eggs containing embryos that died within 3 days of inoculation are examined for bacterial contamination and then discarded. All other eggs, including those that contain embryos that did not die by the 20th day of incubation are chilled overnight and then examined. The yolk sacs are harvested and impression smears prepared with small pieces which are first washed free of yolk and slightly dried on bibulous paper. The smears are stained by the methods of Mac-
chiavello or Gimenez, the latter method is preferred by us. Yolk sacs infected with PL agents have red elementary bodies, single or in clusters, in the impression smears stained by the methods mentioned. Yolk sacs showing elementary bodies or certain other features which will be described below, are chosen for subpassages. We prefer to use single yolk sacs for subpassages. The subpassages are made by inoculating serial 10-fold dilutions of the yolk sac suspensions into other 6- to 7-day old chicken embryos. In the initial passage of inoculums containing EBA agent, a sporadic embryo mortality occurs late in incubation. A specific death pattern is established after several subpassages. Isolation of a PL agent from the organs of an aborted fetus provides ultimate proof for the diagnosis of EBA.

We usually examine the specimens in parallel on cultured bovine cells to reveal the presence of cytopathogenic viruses. The diluents in our studies are Earle's balanced salt solution, other tissue culture fluids or the phosphate sucrose buffer of Bovarnik et al. These fluids contain 500 µg of streptomycin per ml. Some investigators recommend the use of guinea pigs, receiving the inoculum intraperitoneally, or mice that are inoculated intraperitoneally or intracerebrally, as primary indicator hosts for the isolation of PL agents from specimens that are contaminated. Because of the common natural PL infections of these laboratory animals, they only should be used if one is absolutely certain that they are free of such infections.

Elimination of Bacterial Contamination: Bacterial contamination of specimens from EBA-affected fetuses has never been a serious problem for us. In attempts to isolate PL agents from highly contaminated samples such as feces we employ 3 cycles of differential centrifugation at a force of 1800 x g for 30 minutes each. With great care not to disturb the layers, 5 ml of the supernatant in the conical 15 ml centrifuge tubes are withdrawn, diluted 1:1 in Earle's balanced salt solution and centrifuged in the second cycle. After an identical treatment in the third centrifugation cycle, about 3 ml of supernatant are withdrawn, 2 additional tenfold dilutions are made and the 3 dilutions are inoculated into different sets of chicken embryos. Subpassages are made from the yolk sacs of selected chicken embryos as described above.

To control bacterial contaminants, diluents containing tyrothricin, sulfadiazine and streptomycin have been used. The suspensions are held either for 2 to 4 hours at room temperature (25°C) or overnight at 4°C and are then inoculated. We prefer the centrifugation technique for the decontamination of specimens.

Behavior of EBA agent in chicken embryos: All EBA strains which we isolated multiply optimally in the yolk sac cells. Death of the embryos occurred from 4 to 14 days after inoculation. The time of death has an inverse linear relationship with the concentration of the inoculum (Fig. 1). This is a characteristic for PL agents. The slopes of the death curves of all strains are similar and relatively flat, indicating a correspondingly slow multiplication of these organisms at the passage levels tested.
The embryos that died due to infection with EBA agent were frequently deep red in color and exhibited patchy hemorrhages. When death occurred late in incubation, the fully developed embryos presented cyanotic hyperemic legs and toes (Fig. 2). The infected yolk sacs were thin-walled and lacked the trophoblastic villi. The blood vessels of the yolk sac were heavily injected, and the smaller ones were obviously congested (Fig. 2). As compared with the yolk of non-infected embryos, the contents of the infected yolk sacs were of much lower viscosity and of brighter yellow color, particularly when the embryos died a few days before hatching.

The EBA agent also multiplies in the chorio-allantoic membrane (CAM) following proper inoculation of this organ. Marked pathologic changes consisting of edema and small pox-like lesions of different size were seen 8 to 10 days after infection (Fig. 3). Embryo death was sporadic and followed only when high concentrations of the EBA agent were used to inoculate the CAM.

**Specific Antigenic Relationship of EBA Agent:** The neutralization test of Moulder et al., employing an infectivity assay in the chicken embryo, was used to elucidate the specific antigenic relationship of EBA agents isolated from California and Utah cattle with PL agents associated with enzootic abortion of ewes (EAE), inapparent intestinal PL infection of sheep and polyarthritis of calves. Results are presented in Table I. The agents of EBA and EAE are closely related and are also neutralized by M.O. 907 hyperimmune serum. The neutralization is reciprocal. These agents differ from the PL strain LW-613 isolated from the joint of a polyarthritic calf since no significant reciprocal neutralization is evident. McKercher and
Fig. 3. Ectodermal side of chorio-al-llantoic membrane with lesion following inoculation with strain EBA-59-795. Pock lesions are confluent in center and single in the periphery. Pocks also extend along blood vessels (x4).

Fig. 4. Mucous membrane of tongue and oral cavity with marked petechiae present in a calf that was aborted near term by a cow with epizootic bovine abortion.

TABLE 1

<table>
<thead>
<tr>
<th>SPECIFIC ANTIGENIC RELATIONSHIP OF EBA AGENT TO PL AGENTS ASSOCIATED WITH OTHER DISEASES OF CATTLE AND SHEEP</th>
</tr>
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<tbody>
<tr>
<td><strong>ORIGIN OF PL STRAIN</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Aborted calf*</td>
</tr>
<tr>
<td>Aborted calf†</td>
</tr>
<tr>
<td>Aborted calf‡</td>
</tr>
<tr>
<td>Aborted lamb</td>
</tr>
<tr>
<td>Feces§</td>
</tr>
<tr>
<td>Joint of calf§</td>
</tr>
</tbody>
</table>

*From an EBA case in California.
†From EBA cases in Utah.
‡Clinically normal sheep with intestinal PL infection.
§Calf had polyarthritis.
EBA = epizootic bovine abortion.
EAE = enzootic abortion of ewes.
NT. = not tested.
coworkers postulated that the agents causing EBA and EAE are probably identical. Frazer and Berman demonstrated a close relationship between these two agents in the specific complement fixation test.

### Table I

**Distribution of the Agent Causing Epizootic Bovine Abortion in Experimentally Aborted Fetuses**

<table>
<thead>
<tr>
<th>Organ Sample</th>
<th>Infected Per Total Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine content</td>
<td>8/12</td>
</tr>
<tr>
<td>Brain</td>
<td>6/9</td>
</tr>
<tr>
<td>Liver</td>
<td>5/10</td>
</tr>
<tr>
<td>Spleen</td>
<td>6/10</td>
</tr>
<tr>
<td>Kidney</td>
<td>7/11</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>3/6</td>
</tr>
<tr>
<td>Lung</td>
<td>7/11</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>2/6</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>2/5</td>
</tr>
<tr>
<td>Meconium</td>
<td>2/7</td>
</tr>
<tr>
<td>Thymus</td>
<td>0/4</td>
</tr>
<tr>
<td>Bile</td>
<td>0/5</td>
</tr>
<tr>
<td>Stomach content</td>
<td>0/4</td>
</tr>
</tbody>
</table>

Distribution of the EBA agent in aborted fetuses: While it is not easy to isolate the EBA agent from field cases of EBA-affected calves with typical pathologic lesions, a study of experimentally aborted fetuses revealed the distribution of the EBA agent in the fetus and uterine contents (Table II). In this entire study the EBA agent was recovered from practically all organs and fluids except the thymus, the bile, and stomach contents of the fetuses. There was no single organ from which the agent could be recovered in every case. It was isolated from 8 uterine contents of 12 cows tested. The infectivity titers varied from barely detectable amounts to levels of 10⁻⁴.

Pathologic changes in the EBA fetus: Howarth and coworkers first differentiated EBA from other known forms of bovine abortion because of the pronounced hepatopathy, generalized hemorrhage and ascites of the aborted calves. Kennedy, Olander and Howarth were the first to recognize the significance of the lesions in EBA as a distinct fetal disease. They thus stimulated scientific interest to explore the tissue response of the developing fetus to specific infection in utero.

Petechial hemorrhages are regularly seen in the conjunctiva and oral mucous membranes (Fig. 4). The severity of the hemorrhages varies. Similar hemorrhages are present in the skin of the unpigmented areas and are also seen in the subcutis after skinning. Petechiae are also present in
the mucosa of the trachea, thymus, lymph nodes, salivary glands, and skeletal muscles.

The livers are swollen, have a coarsely nodular surface and a firm consistency. The color is reddish yellow, and the liver can have a mottled appearance. We observed typical hepatopathy in about 50 to 60% of EBA affected calves that were aborted during the last trimester of gestation.

The lymphoid tissues are regularly involved, with changes consisting of generalized enlargement associated with lymph stasis. Small grey foci may be irregularly distributed in all tissues but can more regularly be found in the ventricular myocardium and the renal cortex. Many of the fetuses breathed prior to death and the lungs are then outlined by the thickened edematous septa.

The changes in the aborted fetuses depend on the stage of gestation at abortion. Bovine fetuses that are aborted as a result of PL infection prior to the 7th month generally do not have EBA-specific lesions except subcutaneous, blood-tinged edema and increased amounts of clear, reddish pleural and peritoneal fluids.

The placenta could theoretically be of great diagnostic value but well preserved placentas from field cases are rarely available for study. About 50% of the cows used in experimental studies on EBA had retained placentas. However, striking lesions were seen in the placentas of the rest. The lesions differed, depending on the stage of gestation and the span of time between inoculation and abortion. Fig. 5 depicts the blind end of the placenta of a cow that aborted 104 days after inoculation at the end of the

Figure 5. Blind end of a placenta from an experimental case of epizootic bovine abortion. Cow aborted in the ninth month of gestation 104 days after inoculation. Notice opacities in intercotyledonary chorion of distal part of the placenta.
8th month of gestation. The blind end had severe lesions but the remaining parts appeared normal. The intercotyledonary tissue in the area of 12 cotyledons had a leathery, tough consistency and a reddish white, opaque color on the uterine surface. The periplacentomes were involved. The margins of the cotyledons had small, round, focal areas of necrosis. Bulging edema surrounding the cotyledons was seen on the smooth fetal side of the affected part. Placentas from experimental EBA occurring before the 7th month of gestation were edematous, and had a brownish yellow color. The cotyledons were clay colored and necrotic.\textsuperscript{11}

**Cytologic studies on EBA placentas:** Impression smears from affected parts of the placentas of 4 experimental cows had elementary bodies and their earlier developmental stages after staining by Macchiavello's or Giminez' methods.\textsuperscript{17} We succeeded once in diagnosing EBA by demonstrating elementary bodies in smears from the placenta of a field case of EBA. The method of demonstrating elementary bodies in placental impression smears which was developed by Stamp \textit{et al.},\textsuperscript{29} so helpful in the diagnosis of EAE, is not equally applicable for the diagnosis of EBA.

**Histologic changes:** Kennedy \textit{et al.}\textsuperscript{6} investigated the microscopic lesions in calves from EBA. They recognized the basic histologic change as a granulomatous inflammatory process that may involve any or all organs. Individual lesions may vary from frank focal necrosis, to primary acute pleocellular inflammatory foci, to a more chronic reticuloendothelial hyperplasia with induction of epithelioid changes and giant cells of the Langhans' type. In a series of aborted calves, one may find all changes, but a single fetus seldom has the whole spectrum of possible lesions. No single organ is invariably affected. Lesions in the brain are considered to be the most obvious with respect to origin of the inflammatory cells. The reaction consists of a pleocellular accumulation within the adventitia of meningeal and parenchymal vessels.

**Possibilities for diagnosis by serologic tests:** The complement fixation test was used to detect group specific PL antibodies. The antibody response of a cow experimentally inoculated with the EBA agent is demonstrated in Fig. 6. After inoculation the titer rose only by one factor 2 and remained at that level up to the time of parturition. A marked rise was then experienced reaching a maximal titer of 1:512 by the 30th day post partum when the titer declined again. The antibody response of a pregnant cow to inoculation with the EBA agent without ensuing abortion is given as a contrast in Fig. 7. This evidence would indicate that EBA might be diagnosed by a rising group-specific PL antibody titer within 30 days after an abortion. As a measure of precaution against false positives, the guinea pig complement employed should be tested for PL antibodies because of the demonstrated natural PL infection of guinea pigs.\textsuperscript{30}

**Conclusions:** Psittacosis-lymphogranuloma infection of pregnant cows can lead to abortion in the 4th to the 7th month of gestation and, more commonly, to expulsion of the bovine fetus during the last trimester of gestation. The disease during the last stage of gestation is recognized as epi-
zootic bovine abortion because of the more or less chronic infection of the fetus and its specific tissue response to the PL infection. Epizootic bovine abortion, the PL induced bovine abortions during the last trimester of gestation, can be diagnosed by the experienced investigator on the basis of specific gross and histopathologic changes. This diagnosis can be supported by the isolation and identification of the causative agent, a task which is rather laborious, and by the demonstration of a significant group specific PL antibody response following the abortion.

If a PL infection induces abortions in cows prior to the 7th month of gestation, a definite diagnosis can only be made by isolating the PL agent from placenta or fetus and by the demonstration of a PL specific antibody response of the cow.

The presence of elementary bodies typical for PL agents in impression smears of placental tissues permits a definite diagnosis in both forms of abortions but proper placental specimens are seldom available for study.

Application of additional diagnostic tool for the identification of this disease should be explored.

ACKNOWLEDGMENTS

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Department of Microbiology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80521.

*Tyrothricin  20 µg per ml
Sodium sulfadiazine  500 µg per ml
Streptomycin hydrochloride  250 µg per ml

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An increasing awareness that there has not been a significant reduction in bovine mastitis, in spite of the widespread use of antibiotics, has compelled a revived interest in this disease. Investigations designed to increase our knowledge of mastitis are imperative. Since most bovine mastitis is due to udder infection, acceptable methods for identifying infected mammary glands are required. Many methods of culturing milk have been described. However, the methods can be divided into two main groups, those which use fresh milk and those which incubate the milk sample prior to culturing in the hope that such enrichment will make it possible to detect a greater number of infected glands. There is no doubt that the prior incubation of milk samples will produce a much larger number that show bacterial growth, but the diagnostic value of such growth in terms of identifying actual udder infection is extremely poor. This is because the procedure of incubating milk samples in the fluid state ignores a basic bacteriological principle which is vital to the pure culture study of bacteria. This is the principle of the “multiple unknown.” Because a milk sample must pass through a teat canal which cannot be sterilized and must be drawn into a vial in an environment which favors contamination, every sample of milk must be considered to have been contaminated with at least a few bacteria. It follows that the incubation of milk samples in the fluid state would prevent an accurate diagnosis of udder infection since there is no known way to determine after incubation which bacteria gained entrance to the milk from the gland and which gained entrance from the environment. The relative numbers of bacteria present after incubation of a milk sample are not a reliable index of the relative numbers present before incubation. The popular practice of relying upon concomitant evidence of inflammation (high cell count, California Mastitis Test, etc.) is also unreliable since many mammary glands are inhabited by pathogenic bacteria which are producing insufficient inflammation at the time of examination to be detected by such tests. Therefore, all milk samples should be cultured in the fresh state using an agar medium as this maintains the most accurate bacteriological numerical relationship to the freshly collected milk sample. This numerical relationship is invaluable in making a presumptive differentiation between infecting and contaminating organisms. Most infections produce large numbers of colony forming units (CFU) of one bacterial species, while contaminating organisms are usually recognized by the presence in small numbers of a variety of bacterial species. That any organism detected on the agar plate

*Bovine Mastitis Research Unit, Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania 19348.
is derived from an actual mammary gland infection is best confirmed by repeated isolation of the same bacterial specie from successive milk samples collected over a period of time from the same gland.

The laboratory's role in detecting udder infection is to determine as simply, accurately and rapidly as possible, usually from a single milk sample, whether or not the related mammary gland is inhabited by pathogenic microorganisms which are capable of causing clinical signs of mastitis. The purpose of this report is to make readily available a method of culturing milk for the detection of udder infection that is accurate (because it recognizes the principle of the "multiple unknown," ) economical, rapid, simple and which has been exhaustively tested under both research and field conditions.

EQUIPMENT AND PROCEDURES

Vials: Cork stoppered vials are not suitable. Screw-cap vials (21 x 70 mm.) with white metal caps* are a convenient size to use. After the vials and caps have been sterilized by autoclaving, the caps are marked as follows: 1, 1, 1, 1; 2, 2, 2, 2; etc. The numerator represents the sequence order in which the cows are sampled and are similarly identified on an accompanying report form. The denominator identifies the quarters of each cow: 1—LR, 2—LF, 3—RR, 4—RF (Fig. 1). The vials are placed and transported in wire racks of 40 (10 sets of 4 each). (Fig. 2).

*Type FK18, Armstrong-Cork Co., Lancaster, Pennsylvania.
SAMPLEING PROCEDURE

Samples are collected immediately prior to a regular milking without discarding any streams of milk; i.e., strict foremilk. Foremilk is the most suitable for bacteriological culturing because it usually contains the greatest number of the infecting bacteria. Although it may not be always feasible to collect strict foremilk samples, the milk samples should be collected with as much elapsed time as possible since the previous milking. The sampling area should be quiet, free of drafts and dust-causing activity.

The cows are carefully prepared for sampling. The udders are washed with clean warm water using a soft cloth towel. Disinfectants are not required in the wash water. The entire udder surface and adjacent flank area is carefully wiped to remove obvious dirt. The udder surface should be as dry as possible at the conclusion of the washing procedure (Fig. 3).

After washing the udder, the teat end is carefully scrubbed with a cotton pledget moistened with alcohol, applying moderate pressure to evert the sphincter. Alcohol is recommended because it evaporates rapidly. By inverting the soiled layers of cotton as necessary, a 2" x 2" cotton pledget will usually suffice to scrub all 4 teats of a single cow. The final “scrub” should leave no visible soiling on the cotton. The teat end must be completely dry before collecting the milk sample. Care should be taken during
Figure 3. Washing of udder and flank area using soft cloth towel.

Figure 4. Scrubbing of teat end with cotton pledget moistened with alcohol.
preparation not to express any milk through the teat canal prior to collection (Fig. 4).

The washing and scrubbing procedure stimulates milk let-down and the collection of the first stream of milk is relatively easy. The use of a vial holder as illustrated is helpful (Fig. 5). Each vial is held singly in the right hand and the cap is removed with the thumb and middle finger of the left hand holding it with its inner surface turned downward. A pocket is made for the vial by partially closing the left hand and inserting the vial with the open end extending well above the hand in order to prevent any sprayed milk from running off the collector's hand into the vial. The hand is held in a prone position to keep the vial as horizontal as possible. The teat end should not touch the vial. If milk let-down has not occurred during preparation, care is taken not to "strip" the teat over the open vial in order to avoid adding skin debris to the sample. The vial is quickly recapped and all 4 quarters are sampled in turn as rapidly as possible (Fig. 6, 7).

Following collection, the vials are replaced in proper order in the carrying racks and the milk samples are refrigerated in portable iced chests until delivered to the laboratory. The samples are then streaked immediately or held under mechanical refrigeration until it is convenient to streak. Icing of the samples during collection may be omitted under moderate temperature conditions if the samples are to be streaked within one hour after collection. Samples may be held for 4 to 5 days under mechanical refrigera-
Figure 6. Vial held in right hand while removing cap prior to collecting foremilk sample.

Figure 7. Collection of sample. Note vial projects well above hand to prevent sprayed milk from running off hand into vial. Note also cap position and space maintained between teat end and vial.
tion (2°C.) if necessary, although the best results are obtained with fresh samples.

**PLATING PROCEDURES:**

Refrigerated milk samples are warmed for approximately 30 minutes at room temperature (21°C) prior to streaking. The vials are thoroughly shaken in order to obtain a homogenous mixture of the milk. The previously prepared blood agar plates* are removed from the refrigerator, warmed and divided into 4 quadrants by marking with a wax pencil. Identification is made on the reverse side of the quadrant which will be streaked with the milk sample from the No. 1 quarter (LR).

It is helpful to use a large paper filter disc divided and marked into equal quadrants under the petri dish as a guide and to turn the petri dish to the proper streaking position (between "6 and 9 o'clock" for right-handed and "3 and 6 o'clock" for left-handed technicians). Two calibrated loops (0.01 ml.) are used alternately. A continuous streak of the milk film is made using a fixed pattern (Fig. 8). Avoid "scrubbing" in streaking. The loop is then flamed and allowed to cool while the second loop is used for streaking the next milk sample. The process is repeated turning the plate counter-clockwise until all 4 samples of one cow have been placed on a single blood agar plate (Fig. 9). At the completion of streaking, the plates are inverted and incubated at 37°C.

If leukocyte counts are to be made, a second 0.01 ml. loopful of the milk sample may be transferred to a properly identified microscopic slide using the method described by Prescott and Breed. The milk samples are carefully recapped and stored under refrigeration until all examinations of the incubated plates have been completed.

*See appendix.
After the milk samples have been streaked, the blood agar plate is examined twice; once after 24 and again after 48 hours of incubation at 37°C. The lid of the petri dish is carefully lifted and the surface of the agar is examined for CFU by strong reflected light. Light transmitted through the agar is also used to detect zones of hemolysis. It is not difficult for skilled personnel to determine accurately whether bacterial growth has occurred and whether the growth is streptococci, staphylococci, etc. In case of doubt, a colony can be picked to broth or special media for further study.

When properly collected milk is cultured on an agar medium, it soon becomes obvious that milk samples do not ordinarily contain a miscellaneous assortment of bacteria. Many quarter samples will show no bacterial growth. This is usually a reliable indication that the related gland is not infected. Other samples will show a significant number of CFU. Aside from the commonly occurring non-hemolytic corynebacteria that appear to be harmless, it is usual for only one bacterial specie to inhabit a gland at a time. For general diagnostic purposes, where it is often difficult to sample a cow repeatedly, the presence of three or more colonies of the same type is considered as significant. However, most true infections usually show hundreds of CFU (Fig. 10). Contaminating organisms sufficient to be detected by this method usually appear as small numbers of several different species of bacterial (Fig. 11). The ultimate criterion of udder infection, as already indicated, is the ability to isolate repeatedly the same bacterial specie from successive samples from the same mammary gland.

Most bovine udder infections are caused by the streptococci and staphylococci. Other forms of infection are found much more infrequently and account for only a very small fraction of the total udder infection detected when the culturing of complete herds is routinely practiced.4
Figure 10. Photograph, using reflected light, of typical heavy growth of streptocoecici from an udder infected in all quarters.

Figure 11. Photograph, using reflected light, of bacterial growth from contaminated milk samples resulting from poor sampling technique. Note variety of species.

STREPTOCOCCI

If streptococcic colonies are present (Fig. 10), regardless of number, a representative colony is transferred to a CAMP-esculin plate. If Streptococ-
**STUDIES OF BOVINE MASTITIS**

*Streptococcus agalactiae* is detected, even in small numbers, it is considered presumptive evidence of infection since this organism seldom enters the milk sample as a contaminant.

**CAMP-ESCULIN TEST FOR STR. AGALACTIAE**

The streptococci are presumptively classified in routine laboratory work as *Str. agalactiae* or "not agalactiae" according to the phenomenon discovered by Christie, Atkins and Munch-Peterson\(^1\) and on the inability of *Str. agalactiae* to split esculin.\(^1\) The CAMP\(^*\) phenomenon depends on the ability of Lancefield Group B streptococci (to which group *Str. agalactiae* belongs) to hemolyze bovine or ovine erythrocytes which have been acted upon by staphylococcic beta hemolysin that ordinarily does not destroy the integrity of the erythrocyte in 24 hours at 37°C.

The test is made on previously incubated (24 hours, 37°C.) uninoculated blood agar plates containing 5% bovine blood, known to have a low anti-staphylococcic beta hemolysin content, and 0.1% esculin. The level of anti-beta hemolysin content is determined by culturing an arbitrarily selected standard strain of a beta hemolysin producing staphylococcus** on blood agar plates prepared from the blood of different cows. Blood donors are selected on the ability of their blood to support a stipulated zone (4 to 6 mm.) of hemolysis after 18 hours of incubation.

A 1 mm. loopful of a stock culture of the beta hemolysin producing hemolytic staphylococcus maintained on blood agar is streaked across the diameter of the CAMP-esculin plate. One colony of the unidentified streptococcus from the agar plate milk culture, is then streaked perpendicular to the staphylococcic streak beginning as close as it is possible without touching and extending outward on one side for approximately 1 inch. Ten unidentified streptococci (5 on each side of the staphylococcus) can be accommodated on a single CAMP-esculin plate. The plate is then incubated at 37°C. for 18 hours and the reaction is noted (Fig. 12).

The staphylococcus and the streptococci grow simultaneously and except where a "CAMP positive" streptococcus grows within the beta hemolytic zone of the staphylococcus, the various forms of hemolysis are the same as those seen oranginarily. Where a "CAMP positive" streptococcus and the beta hemolysis of the staphylococcus occur together, there is a fan-shaped zone of clear hemolysis which is larger than is ever produced by such streptococci on plain blood agar. The size of this zone of reaction hemolysis varies, but since the streptococcus also grows outside the staphylococci hemolytic zone a distinction can be made between the natural streptococcic hemolysis and the reaction hemolysis. When a reaction does not occur, the natural streptococcic hemolysis is the same both inside and outside the hemolytic zone of the staphylococcus.

As some streptococci other than *Str. agalactiae* can produce the CAMP phenomenon, it is important to observe whether or not there is evidence of

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*From the first letters of the names of the discoverers.
**Available on request from the author.
esculin splitting as shown by a green or brown discoloration of the media along the length of the streptococcic streak. The presumptive identification is as follows:

<table>
<thead>
<tr>
<th>Camp Zone</th>
<th>Esculin Splitting</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>−</td>
<td>( \text{Str. agalactiae} )</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>( \text{Str. &quot;not agalactiae&quot;} )</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>( \text{Str. &quot;not agalactiae&quot;} )</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>Unknown, Serology recommended for definitive identification.</td>
</tr>
</tbody>
</table>

Certain of the streptococci that are CAMP negative and esculin negative have a characteristic green appearance of the streptococcic streak itself. Many of these streptococci have been subjected to Lancefield’s serology and have been identified so uniformly as not belonging to Lancefield’s Group B streptococci, that the green appearance is considered sufficient to arbitrarily categorize these streptococci as “not agalactiae.”

Murphy found that approximately 99% of Lancefield’s Group B streptococci were positive and approximately 95% of those not of Group B were negative. When considering both the positive and negative reactions, the CAMP-esculin test has an accuracy of approximately 97%.

The ability to differentiate \( \text{Str. agalactiae} \) rapidly and simply from other streptococci is of importance because this organism is an obligatory udder pathogen and it can be eliminated from a herd. The definitive identification of the other streptococci is of little practical importance at the present time because of their constant presence in the cow’s environment and because of our inability to prevent udder infections with these forms.
It is important to be able to distinguish between the pathogenic and non-pathogenic staphylococci since both forms infect the bovine mammary gland. Studies on staphylococcal pathogenicity have shown that such characteristics as coagulase production, ability to ferment mannite and ability to hemolyze bovine or ovine erythrocytes are associated with pathogenicity. The ability to hemolyze the standardized bovine blood correlates with pathogenicity as well if not better than the others and is the characteristic most readily employed in the fresh milk-blood agar cultural method (Fig. 13). Therefore, all blood used in the agar plates should be of bovine origin and should be tested for staphylococcal anti-beta hematoxin content using the method already described.

OTHER INFECTIONS

Although other infections occur comparatively infrequently, most are readily recognized on blood agar cultures. Coliforms, pseudomonas, yeast and fungi usually grow satisfactorily (Fig. 14). In unusual cases of clinical mastitis where the standard aerobic incubation does not produce bacterial growth, other more specialized conditions of culturing may be required. It should be kept in mind that altered milk secretion will frequently persist for sometime after udder infections have been removed by antibiotic therapy. However, in general, most udder infections will be readily detected using the standard procedures as described.

Three broad bacteriological results will be obtained with the fresh milk-blood agar method: 1) Some 0.01 ml. quantities of milk will yield no bacteria colonies, 2) Some will yield small numbers of 1 or more bacteria types, and 3) Some will yield significant numbers of bacterial colonies, perhaps from 10 to several hundred colonies of only one or possibly two bacterial types. If the sampling has been performed on 10 representative
cows and has been reasonably good, 4 or 5 of them should show little or no bacterial growth in any quarter, that is, those samples which would fall in categories 1 and 2. The plates in this group should compare favorably with those shown in any of the 5 horizontal lines of plates in Fig. 15. The re-

![Figure 14. Photograph, using reflected light, of coliform organisms. Note the tiny colonies of corynebacteria in adjacent quadrants.](image)

Figure 14. Photograph, using reflected light, of coliform organisms. Note the tiny colonies of corynebacteria in adjacent quadrants.

![Figure 15. Consecutive daily samples of 5 uninfected cows. The numerical notations represent the number of quarter cultures with contaminating CFU.](image)

Figure 15. Consecutive daily samples of 5 uninfected cows. The numerical notations represent the number of quarter cultures with contaminating CFU.

sults from collecting 3 consecutive samples at one time from 5 cows is shown in Fig. 16. Although these cows were known to have uninfected udders as the result of twice-daily cultural examinations made over a long period of time, 17 of the 100 quarters (17%) in Fig. 15 and 9 of the 60 quarter cultures (15%) in Fig. 16 did show one or more CFU's. This is an indication of the degree of contamination to be expected even when the
Figure 16. Consecutive samples collected at one time from 5 uninfected cows. The numerical notations represent the number of quarter cultures with contaminating CFU.

Figure 17. Cultural results from the twice-daily sampling of an uninfected cow for 5 consecutive days.
Figure 18. Prevalence of infection forms based on the cultural examination of complete herds (New York State data).

Figure 19. Frequency of infection forms in 11,654 culture positive quarters based on the quarter culturing of complete herds (New York State data).
sampling has been carefully performed. The results of the twice-daily culturing of milk from an uninfected udder is shown in Fig. 17.

It is clear that the fresh milk-blood agar cultural method makes it possible to recognize the uninfected state of a given gland. It is also equally efficient in detecting the infected state. Its successful and extensive use by the New York State program made possible the data shown in Figs. 18, 19. Although the sampling errors intrinsic to a method which bases diagnosis on a single examination undoubtedly inflates the infection data as shown to a small extent, the numbers involved would preclude that this would have any significant effect on the overall prevalence as shown.

We can conclude from these data that the two forms of bacteria most commonly associated with udder infection are the streptococci and the staphylococci. How successful the fresh milk-blood agar method is in demonstrating the presence of these two forms of infection in essentially pure culture in both AM and PM samples for 5 consecutive days is shown in Figs. 20, 21. The streptococci cultures shown were the result of an experimental exposure and it can be noted that one gland failed to become infected. The staphylococci infection was produced as the result of a teat injury. This particular strain produces both the alpha and beta hemolysis which can be clearly seen in the photographs.
Although additional tests of the accuracy of the method have been made and will be reported in a later paper, the data presented show that the presence or absence of udder infection can be accurately determined by the fresh milk-blood agar method.

Supplementary media for specialized purposes may be used in conjunction with this method as desired, but the fresh milk-blood agar method is ideally suited for the primary culturing of milk for the detection of udder infection. It can be applied uniformly to all samples including dry

secretion. It is accurate, simple and economical. It detects a broad spectrum of organisms and makes possible definitive identification in the shortest period of time. It does not require microscopic examination and is simple enough that technicians can master its use with a minimum of training. The final interpretation as to whether any cultural growth detected represents infection or contamination should be determined by skilled personnel with an extensive background and experience gained from the intensive study of udder infections as related to bovine mastitis.

Although the most desirable method of instruction in the use of a laboratory method is by actual demonstration under laboratory conditions, it is hoped that the procedures are described in sufficient detail to facilitate their use by experienced laboratory personnel.
STUDIES OF BOVINE MASTITIS

APPENDIX

MEDIA PREPARATION

### Blood Agar Media

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>1,000 ml.</th>
<th>200 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypticase soy agar* (agar 1.5%)</td>
<td>40 g.</td>
<td>8 g.</td>
</tr>
<tr>
<td>Agar (to make 2% final concentration)</td>
<td>5 g.</td>
<td>1 g.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1,000 ml.</td>
<td>200 ml.</td>
</tr>
<tr>
<td>Bovine blood (low anti-beta hemotoxin content)</td>
<td>50 ml.</td>
<td>10 ml.</td>
</tr>
</tbody>
</table>

If the larger quantity is prepared as one unit, part of the water is added to a 2,000 ml. flask, then the dry ingredients are added and sides of the flask are washed with the remainder of the water. The agar is melted by gently heating to 37.8°C. for 20 minutes with care taken not to overheat. When the media is completely melted, it is dispensed in 200 ml. quantities into 300 ml. flasks. When smaller quantities are required the ingredients may be measured directly into 300 ml. flasks and are mixed well by swirling and the melting is completed during autoclaving. The media is autoclaved at 15 lbs. pressure for 15 minutes and then cooled in a water bath to 45°C. Sterile bovine blood (10 ml.) is added to each 200 ml. of media. The blood is mixed into the media thoroughly by swirling gently and the media is poured into petri dishes (10 to 12 plates per flask). Each plate is gently swirled as it is poured in order to remove bubbles.

There should be a minimum of activity in the room when the plates are prepared for at least 2 hours prior to pouring to minimize dust contamination. After the plates have solidified, they are inverted and incubated overnight (24 hours) to test for sterility and to dehydrate. Sterility is checked by examining under the bright light of a gooseneck lamp and any that show bacterial growth are discarded. The satisfactory plates are placed in cans or paper bags, marked with date of preparation, and stored under refrigeration. The dehydration is a necessary part of the method as it permits the fluid part of the milk to be rapidly absorbed by the medium. Plates should not be used if they have been prepared for more than two weeks unless representative plates are checked each day before use for ability to support the growth of streptococci and staphylococci. Additional media may be prepared in advance without adding the blood and stored in flasks until needed.

### CAMP-Esculin Media

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>200 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypticase soy agar* (agar 1.5%)</td>
<td>8.0 g.</td>
</tr>
<tr>
<td>Agar (to make final concentration of 2%)</td>
<td>1.0 g.</td>
</tr>
<tr>
<td>Esculin</td>
<td>0.2 g.</td>
</tr>
<tr>
<td>1% Ferric Citrate Solution</td>
<td>2.0 ml.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>200.0 ml.</td>
</tr>
</tbody>
</table>

*Baltimore Biological Laboratory, Baltimore, Maryland.*
The media is dissolved by gently heating to 37.8°C for 20 minutes in a 300 ml. flask or melted by flowing steam. Care should be taken not to overheat this media at any time during its preparation. It is then autoclaved at 15 lbs. pressure for 20 minutes and cooled in a water bath to 45°C. Fresh bovine blood (10 ml.) is added just prior to pouring and the plates are poured as described for the blood agar media.

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