PROCEEDINGS
SIXTY-SIXTH
ANNUAL MEETING
of the
UNITED STATES LIVESTOCK
SANITARY ASSOCIATION

MAYFLOWER HOTEL
Washington, D. C.
October 29-30-31, November 1-2, 1962
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Western Region
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O. H. Timm, Dixon, California
# RECORD OF PREVIOUS MEETINGS

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<th>Secretary</th>
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<td>1. Sept. 23-24, 1897**</td>
<td>Fort Worth, Texas</td>
<td>Mr. C. P. Johnson, Springfield, Ill.</td>
<td>Mr. D. O. Lively, Forth Worth, Texas</td>
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<td>2. Oct. 2-3, 1898</td>
<td>Chicago, Ill.</td>
<td>Mr. C. P. Johnson, Springfield, Ill.</td>
<td>Mr. Taylor Niles, Kansas</td>
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<td>4. Oct. 2-3, 1900</td>
<td>Dayton, Ohio</td>
<td>Mr. C. P. Johnson, Springfield, Ill.</td>
<td>Mr. F. T. Eisenman, Louisville, Ky.</td>
</tr>
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<td>5. Oct. 8-9, 1901</td>
<td>Chicago, Ill.</td>
<td>Mr. C. P. Johnson, Springfield, Ill.</td>
<td>Mr. F. T. Eisenman, Louisville, Ky.</td>
</tr>
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<td>6. Sept. 23-24, 1897**</td>
<td>Wichita, Kansas</td>
<td>Mr. W. E. Dunn, Tennessee</td>
<td>Mr. P. Smith, Monticello, Illinois</td>
</tr>
<tr>
<td>7. Aug. 23-24, 1902</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. M. Hankins, Quanah, Texas</td>
</tr>
<tr>
<td>8. Sept. 22-23, 1903</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
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<tr>
<td>9. Aug. 15-16, 1904</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
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<tr>
<td>10. Aug. 15-16, 1905</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
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<tr>
<td>11. Aug. 15-16, 1906</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
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<tr>
<td>12. Aug. 15-16, 1907</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
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<td>13. Aug. 15-16, 1908***</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
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<tr>
<td>15. Aug. 15-16, 1910</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
</tr>
<tr>
<td>16. Aug. 15-16, 1911</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
</tr>
<tr>
<td>17. Aug. 15-16, 1912</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
</tr>
<tr>
<td>18. Aug. 15-16, 1913</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
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<td>19. Aug. 15-16, 1914</td>
<td>Chicago, Ill.</td>
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</tr>
<tr>
<td>22. Aug. 15-16, 1917</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
</tr>
<tr>
<td>23. Aug. 15-16, 1918</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
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<td>26. Aug. 15-16, 1921</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
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<td>27. Aug. 15-16, 1922</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
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<td>28. Aug. 15-16, 1923</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
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<td>29. Aug. 15-16, 1924</td>
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<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
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<td>30. Aug. 15-16, 1925</td>
<td>Chicago, Ill.</td>
<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
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<td>31. Aug. 15-16, 1926</td>
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<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
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<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
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<td>33. Aug. 15-16, 1928</td>
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<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
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<td>34. Aug. 15-16, 1929</td>
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<td>Mr. W. M. Hankins, Kansas City, Mo.</td>
<td>Mr. W. E. Dunn, Tennessee</td>
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*Dr. Charles G. Lamb, Denver, Colo.*

**Dr. C. A. Cary, Auburn, Alabama.

***Dr. M. M. Hanks, St. Paul, Minn.***
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<th>Date</th>
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<td>Dec. 3-4-5, 1930</td>
<td>Chicago, Ill.</td>
<td>*Dr. A. E. Wight, Washington, D.C.</td>
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<td>Dec. 2-3-4, 1931</td>
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<td>*Dr. J. W. Conaway, Columbia, D.C.</td>
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<td>Nov. 30 - Dec. 1-2, 1932</td>
<td>Chicago, Ill.</td>
<td>*Dr. Peter Malcolm, Des Moines, Mo.</td>
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<td>Chicago, Ill.</td>
<td>*Dr. E. T. Faulder, Albany, N.Y.</td>
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<td>Chicago, Ill.</td>
<td>*Dr. T. E. Robinson, Providence, R.I.</td>
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<td>*Dr. Edward Records, Reno, Nevada</td>
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<td>Dr. Walter Wisnicky, Madison, Wis.</td>
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<td>*Dr. J. L. Axby, Indianapolis, Ind.</td>
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<td>*Dr. H. D. Port, Cheyenne, Wyoming</td>
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<td>*Dr. E. A. Crossman, Boston, Mass.</td>
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<td>*Dr. I. S. McAdory, Auburn, Alabama</td>
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<td>Dr. W. H. Hendricks, Salt Lake City, Utah</td>
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<td>Dr. J. M. Sutton, Atlanta, Ga.</td>
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<td>Dr. C. U. Duckworth, Sacramento, Calif.</td>
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<td>*Dr. William Moore, Raleigh, N.C.</td>
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<td>Chicago, Ill.</td>
<td>*Mr. Will J. Miller, Topeka, Kansas</td>
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<td>Oct. 12-13-14, 1949</td>
<td>Columbus, Ohio</td>
<td>*Dr. T. O. Brandenburg, Bismarck, N.D.</td>
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<td>Sept. 23-24-25, 1953</td>
<td>Atlantic City, N.J.</td>
<td>*Dr. T. Childs, Ottawa, Canada</td>
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<td>Nov. 10-11-12, 1954</td>
<td>Omaha, Neb.</td>
<td>Dr. T. C. Green, Charleston, W. Va.</td>
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<td>Nov. 16-17-18, 1955</td>
<td>New Orleans, La.</td>
<td>Dr. H. F. Wilkins, Helena, Montana</td>
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<td>Nov. 28-29-30, 1956</td>
<td>Chicago, Ill.</td>
<td>Dr. A. L. Brueckner, Baltimore, Md.</td>
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<td>Nov. 13-14-15, 1957</td>
<td>St. Louis, Mo.</td>
<td>Dr. G. H. Good, Cheyenne, Wyoming</td>
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<td>Nov. 4-5-6, 1958</td>
<td>Miami Beach, Fla.</td>
<td>Dr. John G. Mulligan, Montgomery, Alabama</td>
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<td>Dec. 15-16-17-18, 1959</td>
<td>San Francisco, Cal.</td>
<td>Mr. F. G. Buzzell, Augusta, Me.</td>
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<td>Oct. 3 - Nov. 1-2-3, 1961</td>
<td>Minneapolis, Minn.</td>
<td>Dr. A. P. Schneider, Boise, Idaho</td>
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*Deceased.
***This was the last meeting of the Interstate Association of Livestock Sanitary Boards.
INVOCATION

R. A. Hendershott

Almighty God we give thee thanks for permitting us to be able to convene this meeting in this time of increased world tension, considered so grave that one Governor canceled the attendance of members of his staff.

We humbly ask thy blessing upon all in attendance here and trust you will grant us wisdom and foresight to provide out of our conferences those recommendations and programs that will lead to the development and maintenance of a more healthful and productive livestock and poultry industries.

We implore thee to grant wisdom and understanding to our President, his Cabinet, to Congress and to those charged with responsibility at local, state and federal level and to all in attendance at this our 66th annual meeting, to the end that what we do will be found pleasing in thy sight.

In all of our endeavors we ask thy Blessing.

Amen
WELCOME TO WASHINGTON
Dr. M. R. Clarkson*
Washington, D. C.

It's a pleasure, indeed, to welcome to Washington so many of our partners in the vital mission of safeguarding the livestock of the United States.

I know you are disappointed that Secretary of Agriculture Orville L. Freeman was unable to address this session. It is my privilege to bring you the following message from Secretary Freeman. He says:

"I am sorry I cannot be with you this afternoon to extend a personal welcome to the Nation's Capital as you open this Sixty-sixth Annual Meeting of the United States Livestock Sanitary Association.

"Let me assure you of my warm regard for your long and distinguished service in the protection of animal health. I know that you have worked side by side with the Department to help make this country probably the safest place in the world to raise livestock.

"And the world marvels at the results: Our people enjoy an unmatched abundance of wholesome meat, dairy, and poultry products that are high in quality, wide in variety, and reasonable in price.

"All of us recognize, of course, that there is still a great deal to be done in reducing the losses from animal diseases and increasing the efficiency of production. I am sure this challenge will be met as all of us concerned with the health of the Nation's livestock continue our work together to move forward toward these goals.

"You have my best wishes for a most successful meeting.

"Orville L. Freeman."

I'm sure this will be one of many successful meetings in the long history of this Association.

You last met here in Washington in 1908. It is fitting that you have returned to Washington in 1962, while our Nation is celebrating the centennial of two momentous events: Just a century ago this year, Abraham Lincoln signed the bills that created the United States Department of Agriculture and opened the way for the States to establish Land-Grant colleges.

Who is in better position than you are to appreciate what happened? The "people's colleges"—as Land-Grant institutions came to be called—began to make higher education available to Americans like ourselves. And the "people's department"—as President Lincoln referred to United States Department of Agriculture—began to acquire and diffuse new knowledge of agriculture.

*Associate Administrator, Agricultural Research Service, United States Department of Agriculture.
These two institutions of the people joined hands . . . over the century that followed . . . to foment the revolution that swept American farms.

Some of this revolution's most significant advances have come in the area of animal health. I know you share our pride in these accomplishments, because many of them have also been your accomplishments.

It was cattle tick fever that brought together the little group of State regulatory officials who formed this organization in 1897. The State-Federal eradication effort begun in 1906 eventually wiped out tick fever and saved the livestock industry of the South.

This principle of State-Federal cooperation goes back to 1884, when Congress created the old Bureau of Animal Industry as a result of cattle losses from contagious pleuropneumonia. This disease was completely eliminated from the United States by 1892.

Several other costly diseases have since been eradicated: dourine, glanders, foot-and-mouth disease, and vesicular exanthema. These were explosive diseases that could quickly reach epidemic proportions.

Together, we have also succeeded in bringing under control some of the most serious chronic diseases that threaten our livestock. Tuberculosis has been reduced to a low point. And the campaign against brucellosis is steadily advancing.

It will be difficult to surpass some of the accomplishments of this last century.

I believe we have been able to do so well because we give the livestock industry a united effort. We have come a long way in this respect.

This Association has made invaluable contributions in promoting the adoption of improved control and eradication procedures.

We identify our problems scientifically and call on cooperative research to find the answers. This Association has contributed by helping direct attention to the areas where further research is needed.

The result of this united effort is that few countries even begin to approach our level of livestock health. We have been able to keep many of the worst diseases from getting into the United States at all. And the safety and wholesomeness of our meat and poultry food products set the standard for the world.

And yet, as Secretary Freeman pointed out, our work is far from done.

We realize just how big the job ahead is when we remember that diseases still cost livestock growers of this country a staggering $2 billion a year. It has been estimated that an average farm of 160 to 200 acres loses around $1,500 a year from livestock diseases.

We realize how hard the job ahead will be when we remember that there's still no fully effective way of getting rid of some diseases. In other cases, it's increasingly difficult to separate the reactions to our diagnostic tests. Then, too, the rising concentration of livestock is intensifying the threat of serious disease outbreaks. And air transportation has brought many new diseases within a few hours of our shores.

So the challenge is great . . . and still growing.
But so is our ability to deal with this challenge. Our cooperative procedures for fighting animal diseases are getting better every year, and our techniques are constantly being improved. New laboratories are bolstering our research capacity. We know that some of the most difficult questions in this area still remain to be answered, so our goal must be a scientific effort of the highest quality.

It's not just our ability to deal with animal diseases that we need to be concerned about today. It's also necessary that there be a strong national determination to meet this challenge with all the vigor that you and I know it takes to win the fight.

Look at some of our major problems:

We've been living with tuberculosis in this country since colonial days. We set out to eradicate it 45 years ago. We did fine for the first quarter of a century and came very close to complete eradication. But now, the scattered cases that remain are not only harder to search out but also harder to identify with certainty. There has been no real progress against tuberculosis in nearly two decades.

We've been living with brucellosis for at least 57 years. It's true that substantial gains have been made since the cooperative eradication program was started in 1934. But here, again, we find it difficult to identify and root out some of the more atypical cases that remain. We have some hard work ahead to meet our goal of complete eradication of brucellosis by 1975.

We've been living with hog cholera for 129 years. We've talked for a long time of trying to eradicate this virulent disease. I am happy—as I know you are—that we are finally ready to tackle this job. Present thinking is that hog cholera can be eradicated in about 10 years... for the price of just two years' losses.

Hog cholera, brucellosis, and tuberculosis are important threats today. But we are also living with other diseases that could spread rapidly throughout the country with serious consequences.

This challenge must be faced. The United States can no longer afford to live with such diseases indefinitely. Even now, there's a critical need to improve our production efficiency. And in the future, we will have to feed a population that's expected to double within 40 or 50 years.

Now—as this centennial year comes to a close—this country should resolve to focus on animal diseases the full power of our cooperative research and regulatory resources.

At the same time, let us who carry on this work resolve to reinforce the traditions of leadership that distinguish our cooperative endeavors: alert recognition of new developments... sober evaluation of changing conditions... consistency in our official actions... persistence in carrying out our duties... constant exercise of sound, cool judgment under fire. These traditions are the key to success in regulatory operations.

We in the Department take the greatest pride in our fine working relationships with State regulatory officials, with private practitioners, with the livestock industry, and with the Land-Grant colleges. Together, we will meet the challenge of protecting the Nation's livestock.
In closing, I urge you to find time, while you are in Washington to visit the Department offices here as well as our installations at Beltsville. Please accept this as your personal invitation to drop by. We hope your stay in Washington will be both pleasant and profitable.
RESPONSE TO WELCOME

R. W. Smith
Concord, New Hampshire

Thank you, Mr. President. I don't know if I can qualify as an expert or not; they say that an expert is a fellow you go out on the street and bring in and he is foreign to your organization—they call him an expert. Well, I am not foreign to this organization for I can proudly state that this is the forty-second consecutive year that I have attended its meeting. Probably I should not have told you that, but the first meeting that I attended was in Chicago forty-one years ago.

In listening to the address of welcome of Doctor Clarkson, it was a little bit different than that which we have been accustomed to in the past. He gave us a wonderful welcome here to the Nation's Capitol. It is different because we recognize that while Doctor Clarkson is one of us, he holds the unique position in the cooperative effort, of the United States Livestock Sanitary Association and the Agricultural Research Service, to eradicate all of the contagious and infectious diseases of domestic animals and poultry.

As far as I am able to recall and determine the United States Department of Agriculture and its Animal Disease Eradication Division has given us their cooperation and assistance, furnished us with the knowledge whereby we could go out and tackle these many problems and conditions that confront us. I do not need to repeat what Doctor Clarkson has told you that the United States is on top of the world insofar as the eradication of disease in domestic animals is concerned. They have furnished us who are out in the field with the facts that we have needed to put into operation and carry on the many disease programs that history tells us have been taken care of here in these states for a long while. You are proud and I am proud that we are a part of that organization.

I had an occasion some while ago to consult the statistics of the United States—I believe it was the 1959 issue;—I consulted them because I heard someone make a remark that I doubted very much and I am proud to say that I was wrong. If you will check up on that you will find that the Agricultural industry of the United States represents three-fifths or more than one-half the value of all the industries of the United States. You and I, Doctor Clarkson, his Associates, the United States Department of Agriculture and the several States Department of Agriculture and all that go with them have taken a very, very integral part in keeping this mammoth industry on the road and on a true course.

Now, I did not get in here on time but I did hear you state that Doctor Clarkson for part of the time was a citizen of Virginia. We had a man working for us a few years ago who had lived in Virginia and he made it very plain, Doctor Bendix, that you had to live in the State of Virginia two hundred years before you became a citizen and that you had to be there four hundred years before you could call yourself a native. I do not know
if this is true or not. I do know this, that I have a young nephew, a lawyer for the past four or five years in the State Department, who lives in Fairfax, Virginia; last Sunday Mrs. Smith and I went out to pay him a visit. We intended to stop in Falls Church, Virginia to visit a physician friend in practice there, formerly a resident of New Hampshire, and we came to a place where it said "seven corners." Well I spent two hours; I never did see the good doctor—I always got back to that "seven corners."

Doctor Clarkson and Doctor Anderson and others, I think before we go as much as we like our National Capitol this organization should pass a Resolution and present it to the Manager of the City of Washington and ask him to straighten out a few of the circles here in the City. I think we would appreciate it if we could get that done in the next fifty-four years. I asked Doctor Hendershott when I arrived here if this Association had ever met here before and he informed me not since 1908. I think we should not stay away from our National Capitol for such a long period again. Also, Doctor Hendershott spoke of the publication "What Is Known About Brucellosis;" there were five men on that Commission—two are gone but we spent three or four days out of every month here during the summer. The Department of Agriculture furnished us with every facility they had to dig out the information that I believe, and you believe, has had a great deal to do with the furtherance of the eradication of this disease that confronted us—namely, Brucellosis.

It also has come to my attention that in researching for my remarks on this occasion of the Hundredth Anniversary of the Creation of the Department of Agriculture that hog cholera was one of the very first diseases that was reported by the public outside to the United States Department of Agriculture. It is entirely fitting and proper that we should go on down through the years making our country a safer and better place to live. One of the best things we can do is to make our agriculture sound, safe and productive, which we are doing at the present time.

Now without any further remarks from me, Doctor Clarkson on behalf of this Association we want to thank you for coming here today and giving us this warm welcome, and we appreciate the conveniences that we are having here in this fine city which belongs to all of us and let us come back a little more often.

Thank you.
I appreciate this opportunity to talk with you about the Agricultural Research Service and what it does. Ours is a scientific organization dealing with both research and regulatory activities. In the next half hour I hope to show you how we are staffed, the jobs that we perform, what we hope to accomplish in the future and some of the problems that we face.

First, I want you to look with me for just a moment at the whole broad field of agricultural research, its significance, its breadth and depth, its cooperative spirit. This, I believe, will make my discussion of the Agriculture Research Service more meaningful. I have a short film that will give us a glimpse of scientific farming in America and remind us what agriculture research has contributed to this country, not only to farmers but to all of our people. Science and technology have transformed American agriculture in the space of a lifetime. Agriculture has made more progress here in the last 75 years than it made anywhere else in the previous 75 centuries. Here in the United States you see farmers producing food and fiber on the world's most efficient farms, farms where new machinery and equipment have been put to work to take the drudgery out of farming and do a better, faster job. Farms where one worker can produce twice as much as he could twenty years ago; enough today to feed himself and 26 others. You see farms where good breeding, feeding and management practices have helped build a livestock industry unmatched anywhere else in the world. You see farms that grow a wide variety of crops which have been specially bred to improve their quality, heartiness and resistance to insects and diseases; farms where more effective conservation techniques are helping save our soil and water and trees. America's farm people recognize what agricultural research can do and is doing on our farms. But agriculture research goes far beyond the farms—it can affect the whole community. Higher incomes from more efficient farms can mean better homes, better schools, and better community lives. And the nation as a whole benefits from a more efficient marketing and distribution system, from a safe food supply, from a greater variety of high quality farm products which are available all year round, and from new and more nutritious ways to use our food, the result means better diet for all our people.

And agriculture research means still more; it has helped raise our whole standard of living in this country. As agriculture's efficiency has increased, labor has been released from our farms to produce other goods and services, and this has opened the way for the development and growth of our industrial economy, and even that's not all. More and more, agriculture research provides breakthroughs that advance all science and reveal the secrets of life itself. That's agriculture research—at work on
the farm, in the community, and for the nation. Such research is conducted by the United States Department of Agriculture, the State Agriculture Experiment Stations, and industry; they work together to advance agricultural science. Our country leads the world in agriculture largely because of this cooperative research structure. Our relationship began on a nationwide scale a century ago. At first, it was mainly a partnership of the Federal and State Government—a partnership created in 1862 when Congress established the Department of Agriculture and the State Land Grant Colleges. Later legislation established the State Experiment Stations and provided them with Federal grant funds, extended the scope of the Department's scientific work and strengthened the bonds between research and education. In the last few decades, industry has become a vital force in agricultural research, because of the machinery, chemicals and biologicals that industry supplies to agriculture and because of the raw materials that agriculture provides to industry. Today these public and private research agencies have joined hands in advancing our agricultural revolution. Let's look now at the money and manpower being put into this work. Agricultural research funds are divided about evenly between public and private sources. The public, Federal-State portion, amounts to $278,000,000. We don't know exactly what private industry spends, but the best estimate we can get is roughly $286,000,000. Industrial research is concentrated largely in the processing and marketing fields. However, substantial work is also being done on chemicals and on machinery. Total funds for agriculture research amount to approximately $564,000,000 a year.

Now let's examine the public funds for agriculture research a little more closely. As we look on this chart, under the source of funds, you see that Federal funds amount to $156,000,000. This includes $36,000,000 in Federal grant funds administered by the Department. An additional $122,000,000 is provided by the States. Let us see who spends this money. USDA spends $120,000,000. The $36,000,000 in Federal grant funds is included in this total of $158,000,000 which the State Experiment Stations will spend. These figures are for the fiscal year 1962. There has been a gradual increase in Federal funds for agriculture research since World War II. Expenditures climbed from $29,000,000 in 1940 to the high of $156,000,000 in 1962. Although this has been a five-fold increase in funds, you can see that these funds actually buy only a little more than twice as much research as we had in 1940. Right here, in passing, I want to call your attention to another significant point. While Federal support for research in agriculture has grown since World War II, it hasn't grown nearly so fast as Federal support for research in other areas. Let me illustrate: agriculture's share of the total Federal funds for research over the past 20 years looked like this—in 1940 agriculture received about 40 percent of all Federal funds for research. By 1950, although Federal funds for research had grown considerably, agriculture's share had shrunk to five percent of the total—and today agriculture receives less than two percent of the Federal funds for research. As for manpower, USDA employs about 5,000 scientists, including specialists in practically all of the biological sciences, most of the physical sciences and many of
the social sciences. The States have about 9,700 scientists, more than half of whom divide their time between research and teaching, and a few also do extension work. State scientists work side by side with Department people at most of the Stations. The Department directs most of its effort towards problems of regional or national significance. The State Experiment Stations are free to investigate any problems of interest to people of their state, but on matters of regional or national interest they usually work in cooperation with other states and with the Department. Through many years of working together, we have developed a productive partnership. ARS scientists work in every state and in Puerto Rico and the Virgin Islands, more than 230 locations in all. The facilities at two-thirds of these locations are State-owned and the remainder are Federal establishments. ARS research is world-wide in scope. These foreign research projects are helpful to the countries involved, but the most important consideration in setting up a project overseas is the contribution that it can make to American agriculture. For example, we are studying foreign insects and diseases so that we will be ready to combat any outbreak that might threaten the crops, livestock, or forests of this country. We are seeking out new plant material that may be valuable to our commercial and industrial usage. We are developing processing and marketing methods that will help sell United States farm products abroad. These are just a few of the many lines of work now going forward. This foreign research is financed primarily by local currency from the sale abroad of the farm commodities that we grow in abundance.

So far I have talked largely about funds and facilities for research. Now I'd like to introduce you to the scientists themselves and tell you something about what they do. In our farm research group, our agricultural engineers are concerned with putting materials, energy and men together in the most efficient possible combination. For example, here at our tillage Auburn machinery laboratory at Auburn, Alabama, agricultural engineers are determining the best possible shapes for tillage tools under varying farm conditions. As the result of the mechanization of fruit harvesting equipment, we are able to harvest plums, apples and other tree fruits at the rate of from 30 to 50 trees per hour. Our plant breeders are tailoring plants to fit special needs. For example, adapting them to fit mechanical harvesters as was the case with the dwarf castor bean. Breeding plants for resistance to the ravages of insects, diseases and weather. Field tests like this are an important part of the work. In livestock production, our desire is to develop the animals that are able to convert the least feed into the most and best use in the quickest possible time. The broiler industry is an outstanding example of what can be done. In 1940, it took 13 weeks to produce a three pound broiler. Today, it takes only nine weeks to produce the same size bird. What's more, it can be done on half the feed. Such efficient conversion of feed into meat is of course an important way of helping livestock growers cut production costs. Scientists are making fair progress in dealing with animal diseases and parasites. Scientists are working with the highly contagious foot-and-mouth virus at our Plum Island, New York laboratory where we study livestock diseases. Now that our new national animal disease laboratory
at Ames, Iowa is in operation, our research on domestic diseases will be stepped up considerably. Our parasite work will continue to be done at Beltsville. Anaplasmosis is one of the diseases receiving major attention from our scientists. This disease costs livestock growers millions of dollars every year. The healthy looking test-herd constitutes one of our major problems—the difficulty of diagnosing the disease in the carrier state. However, our scientists have now developed a highly accurate test for detecting carrier animals and this has helped us expand our research considerably. Entomologists develop new methods to use in our battle with insects. They work on both chemical and biological control. The principle of applying insecticides as aerosols was developed by our entomologists. Aerosols are now used for everything from paints to shaving creams. Today, one of our big problems is the growing resistance of insects to insecticides. Another problem is pesticide residue. So the entomologist is trying many new ideas; for example, radioactivity is being used to sterilize insects. This technique was highly successful against a Screwworm fly in the southeast. Large numbers of Screwworm flies were raised in our plant at Seabring, Florida. As many as 70,000,000 flies a week were sterilized by exposure to radioactive cobalt and packaged into boxes. Then the boxes were loaded into planes and when they were dropped from the air, the sterile flies escaped to mate with normal flies. Since no new flies could be produced from such mating, the fly population was gradually reduced and finally eliminated from the southeastern part of our country. We are now using this same technique to wipe out the Screwworm in the southwest.

Soil and water are the basis of all agriculture, indeed of life itself and the need for wise use of our soil and water resources is growing more urgent every day. The scientists seek a better understanding of the fundamental relationship of soil, plants and water so that we can make the best possible use of these resources. In the Home Economics group, our nutritionists are helping develop more exact knowledge of the kinds and amounts of food necessary for optimum growth and health throughout the span of life. For example, one recent study with laboratory animals shows the type of carbohydrates in the diet has an important effect on fat metabolism and the control of cholesterol levels in the blood. Home economists develop information to help families choose a well-balanced diet. This information is published in both popular and technical forms, and it is widely used by families, teachers, social workers, physicians and by other researchers. Our studies on the strengths of new fabrics and other work on clothing and housing makes further contributions to the well-being of American families. ARS is also stressing the use of science and technology to increase the present uses for our farm products and to discover and develop new uses for them. These are just a few examples. Nearly all of our utilization research is done at four regional laboratories. At our southern laboratory in New Orleans, cotton receives major attention. Our chemists took a leading part in making cotton flameproof, making it resistant to soiling, rotting, mildewing, making it water-repellant and wash-and-wearable. As a result, cotton is meeting the tremendous challenge of the synthetic fibers—not only in wearing apparel but for
industrial uses too. A recent development from our northern laboratory at Peoria, Illinois has been in the field of starch chemistry. Scientists there have developed the first practical process for making a whole new family of industrial chemicals called Dialdehyde Starches. They are made from corn and wheat. The chemicals have exceptionally good prospects for use in two big industries—leather and paper. A few companies are already in semi-commercial production making these new chemicals. One of the most significant projects at our eastern laboratory near Philadelphia is the development of dry whole milk. It's not ready yet by any means, because making whole milk in powder form is a great deal more complicated than making non-fat dry milk. But dry whole milk could make a tremendous contribution to the nutrition of people throughout the world. Many of them do not have adequate supplies of fresh whole milk or the necessary refrigeration facilities if they did. Dried whole milk would be the answer. Another project at the Eastern Laboratory has to do with inedible animal fat, and the scientists are finding new uses for these fats. Such things as plastic, waterbase paints, fertilizers, and livestock feed. At the Western Laboratory in Albany, California, our scientists pioneered in the research on frozen foods. An outstanding example is converting perishable foods into storable commodities. Our scientists developed the technique of dehydrofreezing, which combines the advantages of both freezing and drying. Other new products include powdered fruit juices such as tomato juice that will be made into a powder. These powders do not require refrigeration, and take only limited shipping and storage space. They are a step beyond our earlier work with frozen concentrated juices.

That will give you an idea of how ARS scientists help our farmers, processors, and consumers through research. But there is another side to the ARS story. We are concerned not only with research but also with regulatory work, activities that help protect the nation's crops and livestock from diseases and pests and insure the wholesomeness of our meat supplies. It was 75 years ago that Congress first combined agriculture research and regulatory work, and they have proved to be an ideal team. Each serves the other. For example, ARS research is often directed towards specific pest control problems. The entire pest control field operations provides factual tests for research and points the way to new investigations. Our regulatory workers are often the first to use research results. Similar regulatory activities in the States are largely handled by the State Departments of Agriculture and we maintain close working relationships with these departments. In some regulatory activities the States are not in a position to act and the Congress has given us full responsibility. For instance port and border inspection is a Federal job. The inspection of meat and meat products that move across state lines can be done more uniformly by the Federal Government.

Let's look now at the money and manpower for regulatory work. This regulatory job is a big one. Of the total ARS funds, regulatory activities will account for $80,000,000 in the fiscal year 1962. This is more than half the total budget spent directly by ARS. The job requires the efforts of more than half our ARS employees. We have about 8,400
regulatory workers in all. About 2,700 of these provide the leadership. They are professionally trained people—mostly pathologists and entomologists and veterinarians. ARS regulatory activities are headquartered at nearly 700 locations in all of the States, and in Puerto Rico and the Virgin Islands. In addition, we cooperate with neighboring countries for our mutual protection, and our workers constantly gather information on pests and diseases throughout the world. Our regulatory work is guided by three firm convictions: first, it is better to keep out diseases and pests than to fight them in this country; second, if they slip past our doors, it is better to eradicate than to live with them; third, if these fail, then controls have to be set up. Quarantine inspectors stand guard at our Mexican and Canadian borders and every air and sea port of entry to intercept any foreign pest that might otherwise get into the United States and destroy our crops and livestock. Agricultural pests are found in passengers' baggage, mail, cargo and ships stores. Customs and postal authorities help us in our detection work. Last year, we stopped an inbound plant pest on an average of every seventeen minutes around the clock. To give you an idea of the size of this job, there were more than 168,000,000 inspections of travelers entering the United States last year. Such record-breaking travel has greatly increased the risk of invasion by foreign insects and diseases and the growing number of planes, ships and motor vehicles entering our country also adds to the job. Inspectors check them all and treat when necessary. Sometimes a single spray is all that is required. In other cases, the treatment may be much more complicated. Poultry and animals are also checked and this is no small task. Nearly 1,250,000 of them were inspected last year and over 65,000 were denied entry.

While these quarantine inspectors are on guard to keep foreign pests out of this country, other State and Federal workers have the job of dealing with diseases and insects already here, as well as those few that occasionally get past our guard. In spite of our best efforts, livestock diseases and pests are costing agriculture about two billion dollars every year. They reduce our meat supply by claiming one animal out of every five. Insects alone take a big chunk out of the economy. Their kinds and numbers stagger the imagination. They are at work everywhere—they bite, suck, and chew up millions of dollars worth of our crops. As I mentioned earlier, it is our joint Federal-State policy to eradicate, to wipe out completely our most destructive diseases and insects rather than try to live with them. Where this is not feasible, we try to contain the pest until research can work out tactical methods of eradication. Where we can neither eradicate nor contain them, research also helps us to find ways for farmers to live with these pests and still keep on producing. Plant pest control workers use the safest and most modern methods that scientists devised for large-scale warfare against insects. When the Mediterranean fruitfly invaded Florida some several years ago, control workers loaded planes with a special spray and took to the air to fight the "Med fly." Valuable fruit and vegetable crops were saved by this all-out attack and we were free of the "Med fly" for five years. But just last June a new infestation was discovered, so spray operations are under way again. The Khapra beetle poses a different problem. This tiny insect is of
vital concern to us because it threatens the billions of bushels of grain, feed and seed stored in this country each year. If the Khapra beetle ever invaded our Midwest grain stores, it could cause untold damage. So control workers literally surround the Khapra beetle by wrapping up the infected buildings and then fumigating them with methylbromide gas. So far, we have confined the pest to four states in the west and southwest, and a cooperative program in these states is aimed at eliminating the Khapra beetle altogether.

Other specialists have the same responsibility for protecting our livestock. The value of eradicating disease is clearly illustrated in the case of Vesicular Exanthema or VE as it is commonly called. This disease broke out at California in 1952; it spread rapidly throughout the country. In one month alone, 150,000 animals were infected or had been exposed to the disease. From research, we learned that VE is spread chiefly by feeding raw garbage to hogs. Action began immediately in the states to obtain laws requiring the cooking of garbage fed to hogs. Quarantines were established. Infected and exposed swine were slaughtered and all contaminated facilities were thoroughly cleaned and disinfected. During the peak year, VE cost the livestock industry an estimated 20 billion dollars, but we are now free of this disease. A number of other animal scourges have also been eradicated. Among them, foot and mouth disease and contagious pleuropneumonia. They no longer exist in this country because of the work of regulatory veterinarians backed up by research.

Our disease-detection system is further strengthened by the inspectors of meat. They are stationed at stockyards and meat-inspection plants throughout the country. They often find the first clue to a disease situation, and we are then able to trace the infected animal back to the herd of origin and wipe out pockets of the disease before they can spread further. The best known activity of the meat inspector, however, is the protection that he provides the public by watching over our meat supply. An average of one-million pounds of meat was condemned by Federal meat inspectors each working day last year. Only meat and meat products that are clean, wholesome and free from adulteration receive this stamp of approval. This, then, is a brief look at the regulatory side of ARS.

We have seen how agriculture benefits from the joint efforts of research and regulatory work. Underlying these efforts—in fact, supporting all of our major advances in agriculture, is basic research; and by this I mean probing the unknown, striving to find out the why of everyday happenings. Basic research gives us the new scientific laws and principles. It is a starting point for the imaginative processes that lead to new things and new ways of doing things. In agriculture research, we place strong emphasis on finding practical answers to farmers' problems, but increasingly the solutions to these problems call for more fundamental knowledge. So more and more in ARS we are concentrating on basic research. The proportion of our total efforts devoted to such work has risen in a dozen years from seven percent to about 35 percent. We look forward to putting at least 50 cents of every research dollar into this quest for new knowledge. I feel that this is the surest way to get the breakthroughs that we need to help agriculture move ahead. Agriculture
research in this country has paid for itself many times over. It has ac-
complished much. But I can't end on the thought that everything is won-
derful. We only need to remember such matters as pesticide residue, the
toll taken by livestock diseases, the importance of conserving our soil and
water, the lack of exact knowledge in many areas of human nutrition, the
search for new industrial uses for farm products. We have some big
problems ahead of us, but I am confident that we can meet them if as in-
dividuals and as a nation we are sufficiently determined to do so and our
opportunities were never greater.

Thank you.
Let us begin with a review of the livestock disease situation a hundred years ago. Those were troublous times. Thinking people knew diseases of domesticated animals were threatening the food supply and well-being of the human race. But neither knowledge nor personnel to cope with them successfully was available. In Europe and North Africa history had repeated itself during and following the wars of Napoleon; diseases had been spread far and wide. Congregation of large numbers of army horses had resulted in the assembling of many of their diseases. These horses carried their diseases with them as the army moved and, at the end of the wars, took them back to the farms, villages, towns, and cities. Cattle, sheep and swine, brought together to feed the army, spread their diseases to each other and to the livestock in the areas through which the army moved. And when replacement animals moved into the ravished areas their diseases moved in too.

Glanders in horses was rampant in Europe during the first half of the century. Contagious pleuropneumonia of cattle which was already enzootic in parts of Switzerland and France, moved slowly across France and into adjacent areas. It was recognized as a transmissible disease in regions in which it was intrenched but when it appeared in a new territory many people, including both veterinarians and physicians, argued it was brought on by unfavorable climatic conditions. Thus the French proved by experiments that it could be spread by contact but Englishmen refused to accept the evidence when it invaded their country about 1841. In this connection it is interesting to note a British veterinarian who was a firm believer in the spontaneous generation of disease had recently come into power in Great Britain and the ports of the country had been opened to cattle importations from Europe in 1838. Foot and mouth disease invaded the country in 1893, contagious pleuropneumonia in 1841 and rinderpest in 1865.

Contagious pleuropneumonia appeared in both South Africa and Australia in the 1850-60 decade.

Foot and mouth disease spread more rapidly and more widely than did pleuropneumonia but because of its low mortality it did not cause so much excitement.

Rinderpest, which was enzootic along the European-Asian border, moved across Europe in this same era.

The destructiveness of these three diseases during these sweeps is almost unbelievable. France is said to have lost 10,000,000 cattle from pleuropneumonia during an 80 year period in the 18th century. Official reports say this disease and foot and mouth disease combined killed more than 1,000,000 cattle in Great Britain in the six years preceding 1862. Hutyra and Marek state that rinderpest killed half a million cattle in Great
Britain in the two years after it appeared in that country in 1865. Law reports that a single extension of rinderpest across Europe killed 30,000,000 cattle.

What about our country? Fortunately for our farm animals the Atlantic was wide and sailing ships moved slowly. So when the founding fathers brought livestock to America some few of their diseases, specially the acute ones, did not land with them. Fortunately our Eastern seaboard had no native wild species of the horse family, no wild hogs, and only one wild ruminant, the deer, that was widespread. So when our first farm animals arrived they were not met by a reception committee of indigenous transmissible diseases. Fortunately, too, during our first two hundred years or so as the population expanded into new territory their livestock walked rather than rode to their new homes. Most of the sick ones either did not start or died along the way.

But significant changes were already occurring a hundred years ago. We had passed the peak of annual per capita production and consumption of meat about 1840. In the next 95 years it dropped from well over 200 pounds to about 115 pounds. Steamships shrank the Atlantic to such an extent that both pleuropneumonia and foot and mouth disease invaded our country twice in the four decades 1841-1880. Steamboats on our rivers and along our coasts and railroads across our lands were moving animals long distances quickly. Pleuropneumonia was spreading slowly from the two foci of infection which had been established by importation of diseased animals. Furthermore, we had two indigenous diseases which were becoming increasingly menacing. Tick fever or piroplasmosis had been with us since early days. North Carolina had tried to limit its progress by establishing a quarantine as early as 1795. But the cows that harbored the blood parasites, the tick that transmitted them, and the parasites themselves were all illiterate. They couldn't read and paid no attention to the posted quarantines. The disease moved across the state at the rate of from two to four miles per year for nearly a hundred years after this first quarantine was established.

The civil war was beginning to move full speed ahead and the resulting spread of glanders was to plague our country for the next two generations.

Hog cholera, an apparently new disease, had appeared in the Ohio Valley about 1832 and had spread rather slowly. But now it was ready to move to the center of the stage and assume the rule of the captain of the hosts of death. It was to hold this title without challenge for well over 50 years.

A hundred years ago we had neither personnel nor legal authority to wage a successful war against transmissible diseases. We had very few veterinarians and no prospects of increasing their number significantly in the near future. Only three veterinary colleges, all private institutions, had been organized in the United States. The oldest of them had been operating for only 10 years and was to close in eight more. The second had already closed and the third was to graduate an average of about seven students per year during the 42 years of its existence.
The American Veterinary Medical Association had not yet been organized.

Neither the Federal Government nor the States had laws and regulations which were adequate for controlling and eradicating transmissible diseases. Massachusetts had begun a "too little and too late" effort to stamp out contagious pleuropneumonia in 1860. Even two of the three commissioners appointed to conduct the campaign did not at first believe it was infectious. But in 1862 they stated "The conclusion is irresistible that if any disease be infectious this one is." Due to continued opposition from interested people and the failure of the legislature to appropriate additional funds the members of the commission resigned. Finally, though, it was reorganized and under the leadership of Doctor Thayer the last case of this disease in Massachusetts was killed in 1865. Incidentally, Doctor Thayer was one of the early presidents of the AVMA.

Yes, these were troublous times. But these were also yeasty times. Fortunately the French Government had established veterinary colleges in the last years of the preceding century and other European countries had followed this example. So Western Europe had a fair number of trained veterinarians. Although it had been accepted as far back as history goes that some diseases are transmissible just how was still a moot question. Now the mystery was beginning to unfold. Rayer and Davaine had transmitted anthrax to a sheep in 1850 by injecting blood from one dead of this disease and had reported seeing "small filaments" in the blood. The German Polender had reported the results of his examinations of the blood of animals dying of anthrax and another French veterinarian Delafond incubated anthrax blood under a watch glass and saw the rods grow into filaments. The great Pasteur had turned his attention from chemistry to biology and was already the recognized leader of the students of the "infinitely small" living things. During this period veterinarians were in the forefront. Galtier vaccinated against rabies before Pasteur did and Tois-saint vaccinated against anthrax before Pasteur put on his world famous demonstration of the potency of anthrax vaccine. Doctors Arloing, Cornevin and Thomas, three French veterinarians, developed a vaccine that protected against blackleg, using heat to attenuate the infectious material before Pasteur grew his anthrax organisms at high temperatures to produce his vaccine.

During the first two decades after the Department of Agriculture was organized diseases of farm animals were attracting increasing attention in our country. In the late 1860's southern cattle moved up the Mississippi by boat and spread piroplasmosis with devastating results. Texas cattle were spreading this disease throughout the Plains States as they were trailed north. In the late 1870's Great Britain issued an order that cattle imported from the United States must be slaughtered on the docks of the port of entrance because of contagious pleuropneumonia. As early as 1869 the Commissioner of Agriculture "strongly" recommended "the establishment of a Division of Veterinary Surgery in the Department."

In 1878 the Congress appropriated $10,000 to be used in the study of diseases of swine. Two years later $15,000 was appropriated to ascertain as accurately as possible the facts concerning contagious pleuropneumonia
in our country. Using these funds the Department employed some of the few trained veterinarians in the country. Among these was Dr. D. E. Salmon from North Carolina who had graduated at Cornell under Doctor Law. Later Doctor Salmon was called to Washington in 1883 to organize a Veterinary Division in the Department.

Luckily a physician, Doctor Loring, was Commissioner of Agriculture at this time. He recognized the value of well trained men. So when the act establishing the Bureau of Animal Industry was signed May 29, 1884, it stated the chief must be a "competent veterinary surgeon." It further provided that the different states and territories be invited to cooperate in the "execution and enforcement" of the act. As you know, Dr. Salmon was appointed chief. He was certainly one of the best trained men in the country having studied in France after graduating at Cornell.

Twenty two years after the Department of Agriculture was organized, then, our country recognized the importance of diseases of farm animals and gave the "go" signal to the Department to do something about them. Incidentally, the Bureau of Animal Industry was the first Bureau to be set up in the Department, was the only one which never changed its name, and grew to be the largest of the bureaus. What progress had our country made in these 22 years since the Department came into being? Personnel was still an acute problem. But we now had seven veterinary colleges in the country and two in Canada. Four of ours and one of the two in Canada were private institutions. Two of ours were connected with State institutions and a third with an endowed university, Harvard.

Many of our States were organizing livestock disease control units. At least 10 had enacted laws by 1885 authorizing the appointment of a State veterinarian and two others had authority to appoint a veterinarian on a temporary basis. Fifteen had such laws with either the State board of health or the Commissioner of Agriculture as the enforcement agency. Six had laws but no very clearcut provisions for their enforcement. The pattern which has been followed ever since was already in evidence. Those States which exported large numbers of cattle with piroplasmosis had no laws and State veterinarians but the importing States were very much concerned. Nearly all of them mentioned specifically the movement of southern or Cherokee cattle as being prohibited. I am sure you remember how eager the States without Vesicular Exanthema were to control the movement of swine and swine products. Immediately after BAI was set up the Treasury Department turned over to it the quarantine stations it had been operating for handling imported animals.

The new bureau started a campaign against pluropneumonia immediately. By the end of the year it had inspected over 4,000 herds in New York, New Jersey and Washington, D. C., finding 249 of them infected. It had also set up two experiments to test the infectiousness of the disease. Both showed it was.

In his first annual report Doctor Salmon recommended that funds be provided for paying indemnities for cattle slaughtered during the campaign. Congress appropriated funds for this purpose which became available July 1, 1886. In August of that year the Department submitted a proposed cooperative eradication program to the States harboring the disease. It included, among others, the following provisions:
1. The necessary inspectors will be furnished by BAI.
2. BAI inspectors assigned to the different states for duty are to be authorized by the State authorities to make inspections under laws of the State; they are to be given such protection and assistance as would be given to State officials.
3. They shall be permitted to examine quarantined herds whenever they are so directed.

Thus, almost as soon as it was organized the Bureau established the basic principles which are still in effect.

Some states objected to the provisions of the agreement. Two were practicing what they thought were protective innoculations and were not willing to forego them. But differences were gradually ironed out and the program moved forward with increasing momentum. The last case known to exist in our country was destroyed in March 1892 and the following September the Secretary declared the United States to be free of contagious bovine pleuropneumonia. Its eradication had cost the Federal Government about $1,500,000. As you know, this disease has not invaded our country since then.

What about the other two very serious diseases, tick or Texas fever and hog cholera? Here conditions were entirely different. The State and Federal officials did not have enough knowledge to eradicate either of these. So BAI immediately inaugurated active research on both of these. Within less than 10 years the Bureau had (1) proved Texas fever was spread by the tick *Boophilus annulatus*, (2) worked out the life cycle of this tick, and (3) identified the causative agent as the blood parasited *Babesia bigemina*. Every veterinarian knows this was the first evidence that an external parasite can be a vector of disease.

Right away the workers realized the disease could be eradicated if they could find a way to get rid of the ticks. BAI employees, workers at several State experiment stations and veterinarians and parasitologists in other countries in which piroplasmosis was a problem concentrated their research on methods of eradicating ticks. Good progress was made. In 1902 North Carolina, under the leadership of State Veterinarian, Tate Butler, began an eradication program. Within four years they had freed about 20 counties of ticks. Other States followed North Carolina's lead with encouraging results. The Bureau, sensing that Congress would soon provide authority and funds for tick eradication, began a survey of the laws and regulations of the States concerned to find out which States would be able to cooperate in the program when Congress acted.

When funds became available in 1906 the Bureau was ready to begin cooperative programs in seven States. The trail had already been blazed in the fight against contagious pleuropneumonia. The principles of cooperation had been worked out and applied successfully. But this was an infinitely bigger job. It involved nearly all the cattle in about a fourth of the country. Up to 35 dippings of every animal were required in many areas. And, as an old pro said, "Just good work won't eradicate ticks; it must be perfect."

The task was made infinitely harder by lack of confidence in, and in some instances, active opposition and resistance to, the program. It was
bitterly fought by some politicians who owned no cattle but who seemed to think here was a way to win favor with the people. But as the work progressed the results were so dramatic that the opponents became increasingly less effective. At long last, tick eradication became a fact rather than just a theory. In my opinion this is still the greatest single accomplishment in controlling and eradicating disease that has occurred in our country; perhaps even in the whole world. We had to start from scratch, doing first the research and then carrying the research results to the field.

Research with hog cholera was equally as successful. After many disappointments the 1901-10 decade brought the discoveries that (1) the causative agent is a filterable virus, (2) this virus is present in the blood and practically all the other tissues of infected animals, (3) a protective serum can be produced by injecting large amounts of virulent blood into recovered or immunized hogs, (4) injection of this serum along with virulent blood into susceptible swine resulted in immunity and (5) this method of immunization was practicable and economical. By 1910 a little serum was being produced and practicing veterinarians were beginning to immunize hogs.

During these years research was adding immeasurably to our knowledge of transmissible diseases. BAI was studying or had studied, tuberculosis, brucellosis, blackleg, anthrax, actinomycosis, equine pernicious anemia, surra, dourine, glands, blackhead in turkeys, coccidiosis in poultry, ox warbles, trichinosis, nodular disease in sheep, gastro-intestinal parasites of ruminants and many other problems. The studies of gastro-intestinal parasites led to the discovery of a new hookworm *Necator americanus* in our human population. Investigations by the Bureau's Doctor Stiles proved hookworm disease was very widespread and serious among the human population of many of the Southern states.

During this same period the United States Livestock Sanitary Association was organized. It was to become an ever-increasing factor in the organization and execution of programs for controlling, eradicating and preventing transmissible diseases of farm animals.

Another notable event was the setting up in the Bureau of what has come to be generally recognized as the best meat inspection organization in the world.

Control of sheep and cattle scab was inaugurated as a cooperative project in the two decades 1891-1910.

Foot and mouth disease invaded our country twice in the 1901-1910 decade, appearing in New England in 1902 and in Pennsylvania, New York, Michigan and Maryland in 1908. The virus causing both these outbreaks was probably brought into the country in 1902 in smallpox vaccine. Both were eradicated by the quarantine and slaughter method. As compared to later outbreaks these could be called small. A total of about 8,000 farm animals sacrificed in the two eradication campaigns.

What was the situation in 1912, the middle of this one hundred year period? On the world-wide front the more advanced countries were either free of rinderpest or nearly so. But it had lost none of its deadliness as evidenced by the fact that a few years previously an outbreak in Rhodesia
had, according to an official report, killed about 500,000 cattle, leaving about 25,000 living. This disease continued to be very severe in some parts of the world. Bovine contagious pleuropneumonia had also well-nigh disappeared from the more advanced countries but was a serious problem in parts of Africa and Asia. Foot and mouth disease continued to be a scourge although our country was free from it.

Research had come to be recognized as a definite part of any animal disease control program and many countries had active units for this work.

By 1912 most of our states had reasonably satisfactory laws and regulations for handling transmissible diseases of domesticated animals. It was still true in a good many states, though, that a new governor usually appointed a new state veterinarian.

At this half century point we had about 20 veterinary colleges in the United States and Canada. These institutions were still definitely horse-minded. Since most of the work of both state and Bureau veterinarians was concerned with meat or milk animals graduates of these colleges were not very well trained for public service. But the administrations of the colleges did not concern themselves because of this.

The American Veterinary Medical Association now had just over 1,400 members. The United States Livestock Sanitary Association was growing in both membership and responsibilities.

Bovine tuberculosis was becoming a more serious problem and plans for a control and eradication program were being discussed. The development of the intradermal test for this disease made it possible to test large numbers of animals with a relatively small staff. It seemed probable that a successful campaign against the disease could be launched. As far back as 1906 Doctor Salmon had said "When public sentiment favors the eradication of tuberculosis in animals, the task will not be found an impossible one." Funds for this work became available in 1917 and the work got under way. An important proviso in the "Uniform Methods and Rules for Tuberculosis-Free Accredited Herds" which were drawn up by a committee appointed at the 1917 annual meeting of the United States Livestock Sanitary Association and approved by United States Bureau of Animal Industry granted authority for practicing veterinarians to be testing in connection with this program. In my judgment this was a big step forward. Since all of us are acquainted with the tuberculosis eradication program I shall not take your time for a discussion of its accomplishments.

A very important addition to our armament for the fight against transmissible animal diseases came in 1913 when Congress passed the Virus-Serum-Toxin Act. This required that all biologics shipped interstate must be prepared in establishments licensed by the Secretary of Agriculture. It made it unlawful to move interstate any worthless, contaminated, dangerous or harmful biological product intended for use in treating any domesticated animals.

During the two decades 1911-30 Bureau and state veterinarians stamped out six different outbreaks of foreign diseases; four of foot and mouth disease and two of European fowl pest. Again the familiar pattern with changes and improvements justified by additional knowledge and experience was followed.
A significant development beginning about the time of the first world war was the decline in the importance of glanders. With the coming of the automobile and the truck most horses stayed home. There was little opportunity for this disease to spread and, with not too much assistance from our profession, it has almost, if not completely disappeared from our country.

During this period, too, we saw very clear evidence that "Eradication of disease spreads disease." As soon as ticks were eradicated from an area movement of better breeding stock into it began. Both tuberculosis and brucelosis were introduced with these cattle. When ticks were out of the picture the quarantines against southern cattle were removed and many thousands of them moved into the different Corn Belt States. They didn't go alone. Along with them went parasites of many species some of which were not established in the area. Paratuberculosis was introduced into many dairy herds along with replacements brought to fill the stanchions vacated by animals reacting to the tuberculin test. Time after time we saw evidence that the most scrupulous compliance with laws and regulations does not give complete protection.

Along with the depression and the drought came a cattle reduction program which was centered on diseased cattle; and a brucellosis eradication program was born. Good rains fell in the South and Southeast during the years of little or no rain in the range states. It was smart to move hungry cattle to good grass; but not so smart to move screwworms in with them.

In the last three decades scrapie, blue tongue, and Newcastle disease have invaded our country and are still with us. We celebrated the hundredth anniversary of the appearance of hog cholera with another new disease of swine; Vesicula Exanthema. This one is gone but what and where will the next one be? No one knows.

Two very spectacular accomplishments of the recent past were the cooperative eradication of foot and mouth disease from Mexico and the eradication of screwworms from the Southeast.

In my opinion we haven't done a good job in controlling and preventing losses from internal parasites. Evidence is clearcut that they cause tremendous losses. Research has shown us how we can minimize these. Such a program will not be easy. But if we were looking for easy jobs we should not have become veterinarians.

Now a brief word of warning. More and more of the men in our Congress have spent their lives on concrete. They know nothing about livestock or problems of the livestock producer. The so-called "farm" vote is becoming increasingly smaller. It may be increasingly more difficult to get legislation supporting animal disease programs. Perhaps we got the laboratories on Plum Island and at Ames just in time. I am suggesting that we give serious thought to developing information which will show the consumer the necessity of protecting our livestock from disease.

May I close with this statement? I believe on the whole we can be proud of what we have done but we must be humble that we have so many tasks partly done or undone. If we have worked with our ears ever listening for the plaudits of the multitude we have probably been disappointed.
But if cattle on a thousand hills mean more to us than a monument of marble, if a well nourished and healthy population is more important than a complimentary resolution carrying myriads of names, if the laughter of happy children is music to our ears we can enjoy the long hours and hard days of our tasks; because we have helped bring these to our country.
PRESIDENT'S ADDRESS

W. L. Bendix

Richmond, Virginia

We are met here in the capital of our country, in the sixty-sixth year of our Association's history, to honor the United States Department of Agriculture on its one hundredth birthday. Our heartiest congratulations to the Department for a century of vital service to the nation! We have heard something here this afternoon of its accomplishments of the past century—accomplishments, incidentally, in which we all may take great pride. Because we are who and what we are, we appreciate better than many what these achievements have meant to our own country and to mankind.

This year is something of a duel centennial insofar as its relation to the work of this Association is concerned. It also represents the one hundredth anniversary of the establishment of the land-grant college system in the United States. I am sure that to most of you this fact is not news, but I wonder how many realize the immense socio-economic impact of this legislation and its implementation upon the world. In this country, there are now sixty-eight institutions of higher learning in the land-grant college system. I learned recently that the first one was established at what is now Michigan State University at East Lansing within a matter of months after the passage of the Land Grant College Act and its signature by President Lincoln. Being a native Virginian, I take some pride in the fact that our own land-grant college, the Virginia Polytechnic Institute, is this year celebrating its nintieth anniversary.

At Michigan State University, about a month ago, I had the privilege of hearing its President, Dr. John Hannah, outline the far-reaching effects of these actions of a century ago upon this country and the ensuing consequences of their implementation around the globe.

Until a century ago, the world's concept of higher education was of a privilege belonging to the sons of the upper classes. The sons of the working man, and particularly the sons of the farmer, had no place in such things. Farming was back-breaking, menial work, involving excessively long hours, and the sons of farmers had nothing to look forward to but more of the same. The land-grant college concept was, and is, a direct denial of this philosophy.

If you want to see what a miracle this has wrought in a century, look around you. The tiller of the soil stands on his own feet, proud and independent, his head held high. In great stretches of this earth, the struggle for food still is the largest single effort among countless millions of human beings. Here, one of the problems of the movement is what to do with our surplus. In many nations, fifty percent or more of the total labor force is needed to produce the bare essentials of an adequate food supply. Here, less than eight percent of our total labor is needed. This has freed millions to make for us, in great abundance, the other things we enjoy.
and has achieved for us the highest standard of living yet possessed by mankind.

I spoke of troublesome farm surpluses. This may yet prove a transient problem, and simply mean that for a period of productivity of our farms has outstripped our population growth and needs. But population is catching up, and fast, for within this generation, at the present rate of growth, there will be need for more—not less production if we are to feed our people. The work of the Department of Agriculture and of the land-grant-colleges, of this Association and of others, must go on at an increased rate to provide and to make the best use of new knowledge, new tools, and new techniques, if we are to meet the needs of what is almost the immediate future.

Experience in the past century has clearly shown that we can leave the providing of the new knowledge we will need and the new tools and new techniques we will use in the hands of the Department and of the land-grant colleges. They have the vigor and the enthusiasm for the tasks ahead of us. They also will provide a continuing supply of trained people to pick up the reins as they are put down by those now engaged in these pursuits, and this will continue also in an unbroken procession. It is our job in this Association to support these efforts with every means at our command. It is also our job to see that this new knowledge is properly applied to the problems at hand in a consistent and uniform manner and that all who are concerned with these matters are kept informed as to our aims and purposes. The importance of an informed profession and of informed professional opinion cannot be overemphasized, and this is without question the key to successful implementation of any program.

I am indebted to Dr. Grant S. Kaley, Director of the Division of Animal Industry in New York State, for a copy of an address made by the Honorable John H. Stone, Assistant Commissioner, New York State Department of Agriculture and Markets, at the annual meeting of the New York State Veterinary Medical Society during September just passed. On the subject of informed professional opinion, among other things, Commissioner Stone had the following to say, "Insofar as informed opinion on the current approach to infectious disease is concerned, I know of no better source than the annual Proceedings of the United States Livestock Sanitary Association. This organization merits the support of every practicing veterinarian, and its annual report belongs in every veterinarian's library."

I would like to take this opportunity to thank Commissioner Stone for his comments regarding our efforts. Our place in the formulation of procedures that determine the final approach to our handling of infectious diseases is firmly established. I would like also to call this comment specifically to the attention of our incoming officers and to our Executive Committee, in the hope that it will provide them some food for thought. The distribution of the annual Proceedings of this Association is far too meagre, and it is up to us to do something about it.
It is agreeable to be able to report to you that almost without exception 1962 has been a year of progress in one degree or another. Nothing startling or of a potentially calamitous nature has occurred, here at home at least, in the field of livestock diseases. Steady progress is being made in those areas in which we are currently engaged.

The problem of the increased incidence of tuberculosis in cattle continues to disturb us all, but extensive research is giving us new insight into this problem. If we can transmit our own sense of urgency and concern to those who actually are conducting the tuberculin test in the field, particularly the practicing veterinarian and the cattle owner, we can without doubt achieve our goal of total eradication and will not be forced to settle for a lesser compromise. Our Committee on Tuberculosis had a special meeting called in Chicago this spring, which as your President I attended. That meeting resulted in a rather substantial change in the Committee's recommendation for both initial accreditation and reaccreditation of counties. This seeks to require testing where experience had indicated tuberculosis was most likely to be found and to make this a more or less continuing procedure, rather than a periodic last-minute attempt to meet a deadline. The Executive Committee approved this recommendation, which was then transmitted to the Department of Agriculture, where it was also promptly accepted. At this meeting, no doubt some further refinements will be suggested and recommended in this area, but I am sure the basic concept will not be changed. I am also encouraged by the fact that, again at our suggestion, the Department of Agriculture is in the process of studying a new technique for the administration of tuberculin, which, if successful, will at long last guarantee us what has been sorely lacking up to the present: a guaranteed exact dosage of tuberculin in every test, identically administered.

Satisfactory progress has been made in the National Brucellosis Eradication Program, and there is substantial evidence to indicate that the goals that have been established for the eradication of this disease will be met. This, of course, depends upon continued vigorous support for the program by all concerned. This Association has played a key role in this effort for many years, and we cannot allow a spirit of complacency to replace the vigor with which we have pursued this work.

The Fourth Annual Conference on Anaplasmosis was held this spring at the University of Nevada. I attended this conference as your President and, while a great deal yet remains to be learned about this extremely baffling disease, I was much heartened by the scope of the information currently available. Anaplasmosis is rapidly becoming of national concern, not only to the livestock sanitary officials at both State and Federal levels, but to the livestock owner. Pressure is already being applied by industry for local official action and for the application of interstate restrictions. I know full well how insistent these local pressures can be, and I sincerely hope that this Association can, at this meeting, at least begin to recommend a uniform national approach and that this will tend to offset multiple unilateral action by the States. The responsibility for such action, of
course, rests with our extremely competent Committee on Anaplasmosis. I have no fear that, within the scope of the knowledge we now possess, they will be found wanting in the proper discharge of their responsibilities. The eyes of the nation are turned to us for leadership in this matter, and we must provide it.

I had the privilege of representing this Association at the annual meeting of the National Association of State Departments of Agriculture held in Grand Rapids, Michigan, during September. It is a pleasure to report that two other members of our Executive Committee were also present: Mr. Francis Buzzell of Maine and Dr. John F. Quinn of Michigan. Opportunity was given us to sit with their Animal Industry Committee at their meetings and to discuss with them freely and fully our Association's position on many disease problems, with particular reference to our relationships with the United States Department of Agriculture.

These meetings and discussions resulted in the adoption of resolutions at that Association's final session which put our two organizations in agreement on the following matters: the remodeling of the Beltsville facility into a parasite research laboratory, the eradication of screwworm from the Southwest, the development of regulations permitting depopulation procedures in certain tuberculosis-infected herds with the payment of full indemnities, scabies eradication, and poultry diseases. Their Executive Committee was directed to attempt to implement these resolutions, both in the Department of Agriculture and in the Congress, and I am sure our Committee on Federal Programs and Policy is looking forward to working with their Executive Committee on these matters at the next congressional session.

Several matters came up in the discussions which resulted in resolutions on subjects about which this Association has yet to take action. I present them to you briefly in the hope that they will be considered by the proper committees of this Association and that some recommendations will be forthcoming:

1. A resolution was adopted requesting the Department of Agriculture to provide expanded reference and consultation services and training in diagnostic methods to employees of State diagnostic laboratories and to otherwise assist such state laboratories in the identification and control of animal diseases for which there are no established programs.

2. A resolution was adopted favoring a more equitable and realistic approach to the importation of foreign beef under the Trade Agreements Act and resolving that all meats imported into this country be required to meet the same high standards as are now required for meats moving in interstate commerce.

3. A resolution was adopted urging the Department of Agriculture to refrain from importing meat animals, either on foot or in carcass form, from countries with foot-and-mouth and other contagious and infectious diseases.

4. A resolution was adopted directing the Executive Committee of the Association to express to pertinent congressional committees the Association's opposition to the proposed legislation extending the coverage of Federal meat inspection (H. R. 12383 and S. 3513) and to advise the proper
Department of Agriculture officials of the Association's desire to consult with them on arrangements for cooperation and assistance in the matter of red-meat inspection.

5. A resolution was adopted strongly supporting the requirement that backtags officially adopted in any State for the identification of cattle shall become a part of such cattle's identification while in commerce and shall not be removed except on the authority of regulatory officials, and further resolving that the Association urge the Department of Agriculture and the States to adopt regulations to implement this resolution.

6. A resolution was adopted expressing the Association's vigorous opposition to the sale of dangerous biological materials to unqualified persons and called upon the Secretary of Agriculture and the Secretary of Health, Education and Welfare to more stringently enforce existing laws regulating such practices and to promote the passage of any legislation necessary to control this operation. By way of explanation, this resolution originated with the Western Association of State Departments of Agriculture. It appears that these materials are being furnished by certain supply houses to high school students and others of the general public in whose hands they may be dangerous.

7. A resolution was adopted calling upon the Secretary of Agriculture, the Customs Service, and the Department of Defense to urge continuing maximum cooperation of the military services to make sure that the movement of military aircraft into the United States will not be a means of introducing new animal diseases.

HOG CHOLERA

We are on the threshold of a program to eradicate hog cholera from the United States. Let us not forget that the first voice raised in support of a national program of this kind was the voice of this Association. Let us also remember that the outline of an eradication program, including the nine points necessary to accomplish this purpose, originated with this Association. Our recommendation has been approved by the National Advisory Committee and by the Department of Agriculture. An interstate regulation has been proclaimed by the Secretary and will become effective November 5. I urge the continuing support of all the States and of this Association for this program, and specifically urge the Department of Agriculture and the States when implementing the program as it develops to stay within the accepted outline that has been agreed upon, and that any changes that may be desirable or suggested or urged on any of us by any group or groups not be considered unless and until they have gone through our Committee for the Nationwide Eradication of Hog Cholera and have been approved by our Executive Committee.

WAYS AND MEANS

Considerable thought has been given during the past year to the internal affairs of this Association. In any organization of this size and
scope, once a successful pattern of operation has been achieved, there seems to develop an inherent opposition to any form of change. Certainly, change merely for the sake of change is wrong and tends to destroy stability and lower confidence. On the other hand, the necessary change created by an expanding, growing, vital nation, with its attendant problems which seem ever to grow both in number and complexity, must be achieved in order to insure stability and effectiveness.

Previous Presidents have given thought to these matters and certain recommendations have been made by each in turn. Some of these changes have been accomplished, but it is my opinion that much yet remains to be done. The recommendation of President Hay of two years ago regarding the disposition of the capital assets of this Association was achieved last year under the very able leadership of President Schneider. The same is true of his recommendations regarding modification of our annual programs and our method of developing them. We have attempted this year to implement further both the recommendations of President Hay and of President Schneider in this regard. President Hay's recommendations, concurred in by his immediate successor, President Schneider, relative to budget preparation by our Secretary for submission to the Executive Committee, also have been carried out, but not yet, I think, in sufficient detail to inform the membership fully.

On two occasions this year, your officers have met and discussed these matters at some length. The need for additional membership is obvious, but just what kind and type of membership is yet to be decided. The need for additional revenue is urgent. For four of the last ten years the Association has operated on an annual deficit. It has been our policy not to let our capital assets go below approximately $25,000, but as of now we have fallen approximately $2,600 below this amount. At last year's meeting, the dues for official membership were increased from $50 to $100; the dues for individual membership were left unchanged at $5. The Secretary was authorized to increase the registration fee at our annual meetings from $5 to $10. As this is the first meeting this new fee has been in operation, its value is yet to be determined. These steps were taken in an attempt to at least balance our annual budget, so that the Association's affairs could continue at approximately the present level of operation. We have not achieved this for this year, but must absorb a $1,600 deficit from our capital.

The need for increased activity in our Association is both obvious, I think, and certainly in large measure overdue. We have been not only imposing on our Secretary-Treasurer in the work that he is required to perform, but he is spending a large part of his time doing merely clerical work, thus denying the Association his services in more important fields. I submit that his knowledge and experience are far too valuable to continue this type of operation. He should operate in the area of public relations, contacts, assistance to committees, informing the membership, increasing the membership, liaison with other national groups, and the like.

The work of the key committees of this Association is important and far-reaching. The recommendations of our committees, once they are
accepted, frequently represent the type and amount of regulation that is imposed upon various industry groups in this country in the ensuing year or years. In large measure, these steps are taken by our committees and gain our approval without sufficient direct contact between this Association and whatever industry groups may be directly affected by such action. This not only results in considerable delay in implementation, but also results in misunderstanding, lack of cooperation, and in some instances direct opposition on the part of industry. These same recommendations also represent frequently a bone of contention within this organization and in particular in its Executive Committee, because we ourselves are all too frequently called upon to vote for recommendations and policies about which we have been inadequately informed.

Some years ago, a proposal was made and accepted involving a change in the membership of our Executive Committee. This involved the nomination by our Nominating Committee and the election by the general membership of eight industry representatives to the Executive Committee, two from each of the four regions of the country. This procedure has not, in my opinion, been an unqualified success. One or two of industry's representatives on our Executive Committee who have been selected by us for membership have been fairly consistent in their attendance, but it is my impression that the rest of them do not bother even to attend meetings, and they do not certainly pay the official membership dues that are required of most of the rest of us for this privilege. I can see no reason why official membership cannot be offered to certain national organizations whose aims and purposes are consistent with those of this organization and who are willing to pay the official membership dues for the privilege. The suggestion has been made that contributing memberships be established. In fact, I am told that some organizations have requested this. With the proper safeguards, I can see little objection to this.

It is not my purpose to suggest the remedy for the problems that I have mentioned and the others we currently have or that may develop in the future. During the year, I have canvassed either directly in conversation or by mail the members of the Executive Committee with regard to some of these. I find no disagreement that the problems exist and should be corrected, as I find no general agreement as to the ways and means. This brings me to a single recommendation for putting our own house in better order and keeping it that way, and for more nearly fulfilling our obligations to our membership and to industry. This recommendation represents my own opinion and in large measure incorporates the feeling of our previous Presidents, as is reflected in that part of the recommendations they have made to us that as yet remain to be acted upon.

I recommend that the Executive Committee direct the incoming President to appoint a Committee on Ways and Means of not over five members, one of whom shall be the Secretary-Treasurer. I recommend that this committee be directed to address itself to what might be called the housekeeping affairs of this Association in the area of membership, budget and finance, expenditures, income, travel, and other matters related to the effective operation of this organization in the proper discharge of its responsibilities. I recommend that this committee be directed to present
its recommendations to the Executive Committee prior to and at our next meeting, including whatever recommendation for change it may feel necessary in our Constitution and By-Laws, including if necessary one for its continued official existence, and that all matters pertaining to the internal operation of this Association be channeled through the Committee on Ways and Means and be subject to the approval of the Executive Committee.

In closing, I want to thank the Association for the many kindnesses and courtesies it has extended me over the years. This past year, during which I have had the honor of serving as your President, has been a most rewarding one for me. The cooperation that I have received and the consideration shown me by the other officers, the members of the Executive Committee, and the chairmen of our standing committees have been of the finest. I wish to assure the Association and, in particular, the incoming officers that I stand ready at all times to contribute to the work of the Association in any manner that I may be of assistance. Thank you very much.
PRESENTATION OF KEY TO RETIRING PRESIDENT BENDIX

R. A. Hendershott

In accord with established custom, it is now my duty to represent you in honoring our retiring President. This custom was begun at the meeting in '50 at Phoenix, Arizona when keys were struck and presented to all living past Presidents and it has become an annual affair since that time. It is my pleasure this year to present to Doctor Bendix this token of appreciation of his service to the Association on many of our committees, and as our President for this, our sixty-sixth Year of recorded existence.

Our thanks to you, Doctor, for your interest and labor in behalf of the Association. I trust you will wear this tie holder, made with the replica of the key of the United States Livestock Sanitary Association, as a memento of our appreciation of your service.

RESPONSE OF PRESIDENT W. L. BENDIX TO PRESENTATION OF KEY

Well thank you very much Ralph and Gentlemen. I will wear this tie holder with great pride and I want to take this brief moment to specifically thank our fine Secretary-Treasurer for all the cooperation, assistance and understanding that he has shown me not only this past year but all of the years we have been associated.

We have not always agreed, I want you to understand, but we have always been friends and I hope and pray that it so continues.

Thank you.

...Applause...
MEMORIAL SERVICE
M.N. Riemenschneider
Oklahoma City, Oklahoma

President Bendix, Distinguished Guests, Ladies and Gentlemen: We have come to that part of the program where we pause to pay our respect to our departed colleagues. To the best of my information, the following members have passed away since our last meeting.

HARRY P. ARONSON
(COR '27) 58
Roosevelt, Long Island, New York

Harry P. Aronson died March 14, 1962. He was named public health veterinarian of Nassau County in New York in 1938 and held this position until his death. He was a member of the New York State V.M.S. and of the A.V.M.A.

CLARENCE L. CAMPBELL
(OSU '26) 67
Escondido, California

Dr. Campbell died February 10, 1962. After many years of association with the Lederle Laboratories Division of the American Cyanamid Co. of New York, Dr. Campbell retired about two years ago, moving at that time from St. Louis to the West Coast.

THOMAS T. CHRISTIAN
(KCV '10) 80
Waco, Texas

Dr. Christian died June 14, 1962. Dr. Christian was one of Texas' first licensed veterinarians. He was instrumental in getting the veterinary practice act of 1911 passed in that state. He was a past president of the Texas V.M.A.

T. B. CLOWER
(GA '33) 52
Douglasville, Ga.

Dr. Clower died of a heart attack August 23, 1961. Dr. Clower entered general practice in Valdosta, Ga., immediately after graduation. In 1941 he became chief veterinarian of the State Department of Agriculture. In 1954 he returned to general practice in Douglasville.
Dr. Curry passed away April 19, 1962. Dr. Curry was associated with the Bureau of Animal Industry as a lay inspector in the Western States. He subsequently received his degree from the Kansas City Veterinary College and served as an instructor or associate professor in the College for sometime thereafter. He later became President of the United Serum Co., Wichita, Kansas, which subsequently was merged with Pitman-Moor and others to form Allied Laboratories.

Dr. Curry served as State Veterinarian in Missouri 1932 thru 1942 and again from 1947 to 1953.

Dr. Curry was an honor roll member of the AVMA and a life member of Missouri V.M.A.

R. R. DYKSTRA
(ISU '05) 82
Manhattan, Kansas

R. R. Dykstra died May 8, 1962. He was the first Dean of the School of Veterinary Medicine, Kansas State University. He was fully retired in 1953, having served on the Kansas State University faculty for 42 years. In 1932 he became the first Kansan to serve as President of the AVMA. He also had been president of the Kansas V.M.A. and of the Association of Deans of American Colleges of Veterinary Medicine. He was an honorary member of the Iowa State, the Nebraska, and the Eastern Iowa V.M.A.'s, and an honor roll member of the AVMA.

CLARENCE W. LAWSON
(PUR '16) 65
Boswell, Indiana

Mr. Lawson passed away in September, 1961. He was Vice-Chairman of the Indiana Livestock Sanitary Board. He was very active in most phases of agriculture.

He was a graduate of Purdue University in 1916 and was very active in farming, community, church and service organizations.

R. E. LUBBEHUSEN
(OSU '18) 64
St. Louis, Missouri

Dr. Lubbehusen died April 17, 1962. As manager of the Disease Control Department of the Ralston Purina Company, he was planning to retire in August. He joined Purina’s Research Department in 1938. Prior to that he was engaged in experiment station research for 18 years including work at North Dakota State University, the Pennsylvania State Department of Agriculture, the University of Minnesota, and the University of California.
Dr. Mays passed away in April of 1962. Dr. Mays was employed by the state of South Carolina since 1919. He retired in July of 1961. The Doctor was State Veterinarian of South Carolina for eight years.

BERT STRICKLER
(KCV '18) 71
Skidmore, Missouri

Dr. Strickler passed away on February 7, 1962. After receiving his degree, Dr. Strickler entered practice in Skidmore, Missouri and was so engaged throughout his lifetime.

Dr. Skidmore passed away while making a professional call.

RAYMOND C. SURFACE
(KCV '15) 72
Oklahoma City, Oklahoma

Dr. Surface died January 2, 1962. Dr. Surface worked at the Oklahoma City, Oklahoma, stockyards at a USDA inspector for many years. Before joining the USDA in 1947 he was a pharmaceutical representative for a veterinary pharmaceutical company in New Jersey.

CLARENCE A. WOODHOUSE
(OSU '35) 54
Wilmington, Delaware

Clarence A. Woodhouse died April 2, 1962. Dr. Woodhouse was a station veterinarian for the E.I. DuPont deNemours Company's stine Laboratories. He served as a delegate from Delaware to the AVMA House of Delegates for many years.

Believing in the Fatherhood of God and the Brotherhood of man, I humbly request all present to arise and remain standing for a moment of silent prayer for the peaceful repose of the souls of our departed colleagues.

SILENT PRAYER

Thank you for your respectful participation.

Your speaker feels very inadequate and humble on this occasion in endeavoring to properly memorialize these men. I am certain that no words may be said here today to adequately express the keen personal loss we feel. It is comforting to recall what Jesus taught when he said, "Blessed are those that mourn for they shall be comforted."
As we bid farewell to these colleagues who have been called on before, let us be thankful that these men have had the opportunity to so ably contribute to their respective field and we to be thankful that we have had the privilege of knowing them, working with them and for the ideals that they have passed on to us. Thus we memorialize these men this day and let us dedicate ourselves to those ideals and principles so well emulated by their lives.
PROPOSED AMENDMENT TO THE CONSTITUTION

R. A. Hendershott
Trenton, New Jersey

President Bendix, at this time I will ask Doctor Hendershott to explain the action of the Executive Committee with respect to the proposed Amendment. R. A. Hendershott.

R. A. Hendershott: Mr. President and members in attendance. A year ago I presented a proposal to amend the Constitution through the following addition starting with line 89 as follows—"That with the exception of a change in the name of this Association, upon the dissolution of this corporation or the termination of activities thereof, all remaining net assets thereof shall be contributed for utilization in the advancement and research of diseases of animals, and no part of the net assets shall inure to any person or group of persons for private gain."

In accord with our Constitution and By-Laws, proposals to amend must be presented in writing and published in the Proceedings where one may have an opportunity to study it from one meeting to the next at which time if approved by the Executive Committee it is then presented for final action of acceptance or refusal by the Members in session at the Annual Meeting. This proposed Amendment was presented and published on page 25 of the 65th Annual Proceedings. At the Executive Committee Meeting Wednesday, October 31, 1962 this proposal was approved and is now being presented to this Assembly for its considered action.

Chairman Bendix: Gentlemen, what is your pleasure.

Voice: I move the adoption of the Amendment.

Voices: I second it.

Chairman Bendix: Any discussion? If not, all in favor express by your voice vote—aye's. Contrary—no response. I declare the Amendment adopted.
REPORT OF THE COMMITTEE ON LAWS AND REGULATIONS


INTERSTATE LIVESTOCK HEALTH REQUIREMENTS

Last year this Committee presented the areas of greatest variation between the states' livestock health requirements and made recommendations directed to accomplish more uniformity in livestock health requirements. Your Committee urges continued study of areas of variation presented in last year's report and urges increased effort of the states, on a regional basis, to obtain greater uniformity of livestock health requirements within the limits of assuring each state maximum protection against the introduction of a costly livestock or poultry disease.

The Committee recommends that the states and Federal agencies reverse a trend that seems apparent and is causing undue resentment among livestock and poultry owners. That trend is the ever-increasing requirement of more and more Federal and State documents that must be obtained to transport healthy and unexposed livestock and poultry in intrastate and interstate commerce. The trend of the increase of unnecessary documents on livestock and poultry results not only in an unwarranted expenditure of public funds but encourages concentrated attempts to evade health requirements. The final result makes it more difficult to enforce the control of the movement of livestock and poultry actually infected with or exposed to a communicable and infectious disease. The Committee recommends that the United States Livestock Sanitary Association take note of this trend and do everything possible to encourage maximum freedom of movement of healthy and unexposed livestock and poultry in intrastate and interstate commerce.

The Committee urges that the United States Livestock Sanitary Association recommend more concentrated enforcement of existing State and Federal laws governing the movement of animals and poultry actually infected with or exposed to a communicable or infectious disease.

PROPOSED FEDERAL-STATE HEALTH CERTIFICATE

Your Committee recommends that the United States Livestock Sanitary Association request the states, again, to adopt the uniform health certificate form recommended by this Committee and adopted by the United States Livestock Sanitary Association in 1954.

Your Committee recommends that the United States Department of Agriculture, Animal Disease Eradication Division, adopt and use the uniform health certificate it has developed to replace Federal Form 48.
FOREIGN DISEASE IMPORT REGULATIONS

One of the most important responsibilities assigned to the Secretary of the United States Department of Agriculture is to prevent the introduction or dissemination of any contagious, infectious, or communicable diseases of animals from a foreign country into the United States. This is solely and properly a function of the Federal government which requires full support of all facilities and manpower of every single state. We dare not take the slightest risk with such an essential and basic resource as food provided by the livestock and poultry industry. History repeatedly has demonstrated that the dissemination of livestock diseases has so weakened nations that those nations had to succumb to forces surrounding them.

Modern transportation facilities have magnified the threat and intensified the problem. The challenge cannot be met with present facilities and personnel. This Association has repeatedly discussed the problem with appropriate budget subcommittees with seemingly little success. Your Committee, therefore, urges the United States Secretary of Agriculture and the Congress to review the existing situation and provide at least realistic appropriations to secure needed additional qualified personnel and facilities to meet this constant threat.

PROPOSED MEAT INSPECTION LAWS

It is recommended that the United States Livestock Sanitary Association adopt the following policy:

1. Urge all states to adopt adequate meat inspection laws that will provide a system of meat inspection that will assure safe, wholesome meat and meat products for all people within their respective states.

2. Approve Federal meat inspection laws that will tend to encourage the establishment of economical State-administered meat inspection.

3. Approve Federal meat inspection laws that will recognize State-inspected meat and meat products in interstate commerce provided the meat inspection service of the State is equal to that of Federal meat inspection and, provided further, that such laws will provide Federal grants to the states to assist in the cost of the meat inspection of that portion of State-inspected meat and meat products shipped in interstate commerce.

4. Approve Federal meat inspection laws that will utilize to the maximum State Administration of meat inspection and avoid all Federal duplication of the administration of meat inspection within the states.

5. It is recommended by the Committee that in all instances meat inspection laws and/or regulations be promulgated or amended to incorporate principles that may be utilized to facilitate efficiency in the animal disease control or eradication programs.
REPORT OF THE COMMITTEE ON NOMINATIONS

J. G. Milligan, Montgomery, Alabama, Chairman; A. L. Brueckner, Hyattsville, Maryland; F. G. Buzzell, Augusta, Maine and A. P. Schneider, Boise, Idaho.

The Committee feels that the Association had made a wise choice in its selection of Dr. T. J. Grennan, Jr. as President for the coming year by electing him President-elect last year. It is our desire that Doctor Grennan have the best possible group of officers to work with during the coming year so that he can lead this Association through another successful year. Careful and thoughtful consideration has been given to this task.

We would like to present to this Association for its approval the names of a group of officers that we feel can do the job ahead. These nominees are for:

- President-Elect: Dr. L. A. Rosner, Jefferson City, Missouri
- 1st Vice-President: Dr. J. W. Safford, Helena, Montana
- 2nd Vice-President: Dr. C. L. Campbell, Tallahassee, Florida

This Committee is also charged with the responsibility of recommending a slate of nominees of industry members from the four geographic areas of the United States to the Executive Committee as follows:

Nominees from the Southern Region
- Mr. J. B. Finley, Ecinal, Texas
- Mr. James Nance, Alamo, Tennessee

North Central Region
- Mr. R. Hogue, Lafayette, Indiana
- Mr. M. Steddom, Granger, Iowa

Western Region
- Mr. O. H. Timm, Dixon, California
- Mr. A. O. Wilson, St. Xavior, Montana

Eastern Region
- Mr. J. McKenny Willis, Easton, Maryland
- Mr. Paul R. Anthony, Strausstown, Pennsylvania

It was moved by Dr. H. G. Geyer and seconded by Dr. Janawicz that the Secretary should cast the unanimous vote of the Association for the election of the slate proposed.

Secretary Hendershott: In accord with your expressed command I hereby cast the unanimous vote of this Assemblage for Dr. L. A. Rosner of Jefferson City, Missouri as President-Elect; for Dr. J. W. Safford of Helena, Montana as 1st Vice-President; for Dr. C. L. Campbell of Tallahassee, Florida as 2nd Vice-President and the following industry representatives to the Executive Committee:

- Dr. L. A. Rosner, Jefferson City, Missouri
- Dr. J. W. Safford, Helena, Montana
- Dr. C. L. Campbell, Tallahassee, Florida

- Mr. J. B. Finley, Ecinal, Texas
- Mr. James Nance, Alamo, Tennessee
- Mr. R. Hogue, Lafayette, Indiana
- Mr. M. Steddom, Granger, Iowa
- Mr. O. H. Timm, Dixon, California
- Mr. A. O. Wilson, St. Xavior, Montana
- Mr. J. McKenny Willis, Easton, Maryland
- Mr. Paul R. Anthony, Strausstown, Pennsylvania

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Messers - J. B. Finley, Encinal, Texas
James Nance, Alamo, Tennessee
R. Hogue, Lafayette, Indiana
M. Steddom, Granger, Iowa
O. H. Timm, Dixon, California
A. O. Wilson, St. Xavier, Montana
J. McKenny, Willis, Easton, Maryland
Paul R. Anthony, Strausstown, Pennsylvania

Dr. W. L. Bendix introduced the new officers and Industry representatives to the Assembly. All pledged their best effort to carry on the high traditions of the office to which they were elected.
REPORT OF THE COMMITTEE ON PROGRAM AND POLICY


The Committee on Program and Policy of the United States Livestock Sanitary Association met in Washington, D. C. in February and June, 1962; a third meeting was held in Miami Beach, Florida in August.

This Committee wishes to again recognize those agencies and organizations that financially support the travel and the time required on the part of each member in order to discharge the responsibilities designated by this Association.

As Chairman, I wish to express the appreciation of each member, for the time and effort given by the various staff members of the Agricultural Research Service during the customary annual meetings in Washington. This Committee further wishes to publicize to this entire assembly the high degree of cooperation shown by these members of the Agricultural Research Service. A sense of cooperation which has become more apparent year-by-year, and is entirely necessary if our common aims are to be achieved, which by their very nature demand a combined effort.

Your Committee has attempted to keep the Executive membership of this Association informed. It has further attempted to secure the application of many recommendations proposed by standing Committees of this Association and to echo these recommendations. When requested, it has recognized the wishes of the regional subdivisions of this Association. A recent example was the incorporation of a resolution originating in the Association of Western States Livestock Sanitary Officials as a part of the recorded testimony presented to the Congressional Sub-committee on Appropriations.

ANIMAL DISEASE ERADICATION DIVISION

Tuberculosis and Brucellosis:

Funds for the eradication of these two diseases have recently been incorporated into a single appropriation item.

Monies to be used in the cooperative Brucellosis eradication program must, by Congressional action, be matched by the states on at least a 60 - 40 basis. Most states will exceed these minimum requirements. While Brucellosis presents an encouraging picture, some signs of relaxation are becoming evident. This Committee is deeply concerned that our obligation in this field be met, and that our target date be arrived at on schedule.

Tuberculosis continues to present a problem and a burden to our livestock industry. Changes and revisions to the Uniform Methods and Rules, recently adopted, will probably prove their worth in the over-all eradication program. There is one vital phase of such a program, however, that
is badly in need of revision. This phase is money. Money to provide for
the necessary and recommended initial program testing; money to provide
for the recommended field studies, and to develop a more effective trace-
back system; money to provide for the requested increase in the annual
rate of testing, and to allow for herd depopulation procedures, when and
where recommended by the proper authorities. At the present time, the
states are contributing approximately four dollars for each one dollar ap-
propriated for use by the Agricultural Research Service in this effort.
The members of Congress were impressed when your Committee explained
to them the change in attitude toward an eradication effort, and the aban-
donment of a qualifying or a hold-the-line effort. This impression was not
long-lasting as is apparent by their action in appropriating funds for an
eradication effort.

Scabies Eradication:

Scabies, and in particular Sheep Scab, continues to exact its toll, and
continues to show an alarming increase. During the last eight years, we
have witnessed a 100 percent increase in number of infected counties, and
in the number of infected flocks. This disease has been completely eradi-
cated in many of our Western states, but these same states are daily faced
with the problem of reinfection being introduced. Such an introduction
could virtually destroy the economy of some states. Your Committee has
given its full support to the requests of this Association and the Western
States Livestock Sanitary Officials to initiate an all-out eradication pro-
gram. This support should be continued, to assure the complete elimina-
tion of this disease through a cooperative federal-state effort.

Poultry Diseases:

The Animal Disease Eradication Division was again asked to take a
more active part in the field of poultry diseases. The time has arrived to
recognize the distinct need for direction. An industry that has assumed
such economic importance in a relatively short period certainly is worthy
of our consideration and recognition. The studies made by Animal Dis-
ease Eradication epidemiologists have provided excellent help in some
areas. Such an effort, however, has barely scratched the surface. It is
believed that if and when the problems of this industry are to be under-
taken, work should be done systematically rather than on a crash pro-
gram, for maximum results.

ANIMAL DISEASE AND PARASITE RESEARCH

The present level of expansion and the results of the capabilities of
both the Plum Island laboratory and the National Animal Disease Labora-
tory should provide a degree of satisfaction to all concerned. This highly
important phase of disease investigation must be continued and enlarged.
Significant progress has been achieved in obtaining research facilities
during the past few years, but this is merely the beginning. We must con-
tinue to point up the needs of research, and to direct our attention and
interest to this field. The concern of the Department toward the training of personnel, their outspoken objective to obtain only the finest available, is to be complimented. The importance of those persons that steer our course now, and will operate present and future facilities cannot be overlooked. Your Committee has no recommendations to make at this time other than those relating to Tuberculosis, and the request that the old animal disease station be converted into a parasite disease station be done as soon as possible.

ANIMAL INSPECTION AND QUARANTINE DIVISION

Your Committee wishes to take this opportunity to express a deep feeling of gratitude, which should come from the entire nation, to this division for a job well done. A job that has been done under the most adverse conditions and trying circumstances.

There is no need to outline the duties and the contributions of this Division to our Agricultural strength, and to our national economy. To point out its importance as the first line of defense of our livestock industries. To elaborate on the inadequacies of the facilities provided for carrying out their assignments. Facilities that have at times been described as completely lacking, and at times as an absolute disgrace.

There is no need to repeat the requests made by your Committee asking for financial and statutory support to fulfill these assignments.

It is not the intent of this Committee to point out to this Association that these facts have been presented to the proper appropriating authorities by the Department, by this Committee, and by this Association year after year after year.

It is our intent, however, to point the finger of Responsibility directly at these appropriating authorities for the failure on the part of the Congress to provide the necessary funds to completely insure our livestock industries against the introduction of disease. An insurance that these industries not only deserve, but one that we rightfully owe to them.

It does not make sense to adopt the attitude that because over the past decades we have been spared the feared introduction of disease—accidently or otherwise—our protection service is adequate. We are here today—not in the past.

The failure to fully provide insurance not only jeopardizes our livestock industries, but may very well jeopardize the well-being and the public health of the nation.

On the day that decimating livestock diseases appear in this country. On the day that foreign or exotic diseases are introduced, this responsibility will that day come into full focus. This responsibility will then have to be borne.

Years of explaining. Years of calling the attention of those responsible to our inadequacies have failed to bring about the desired aims of this Association and of this Committee. We cannot afford at this time, however, to relax our efforts; but rather, they should be increased by each and every member of this Association at every given opportunity.
Each member of the Committee is grateful for having the opportunity to serve you during this past year. I would personally like to express my appreciation to the individual members for their time and effort; and to the members of the Agricultural Research Service with whom we have worked. We sincerely hope that we have followed a course which will be beneficial, and that we have earned your endorsement.
This Committee has heard many complaints against the unrestricted and continuous interstate movement of untested cattle of unknown origin to Approved Markets and from market to market within the States. There is increasing evidence of the extent to which this practice is retarding the eradication programs of the various States. It is now evident that the Federal Interstate Regulation has permitted such movement especially by dealers and market operators who have neither the interest nor the desire to cooperate in the eradication effort.

It should be noted that "Specific Approval" as provided for in the regulation is permissive and was primarily intended to facilitate the interstate movement of cattle by the producer to the market serving the area and until the area could be Certified. It is now evident that dealers and market operators alike have taken advantage of an exemption originally intended for the producer. The continuous movement of unidentified cattle from market to market has favored if not encouraged diversion; has made it impossible for the States to determine the origin or status of such animal, or, to regulate their movement within the State. Furthermore, the status of cattle from the qualified herd in the Modified Certified Area and its intended advantages to the producer of such cattle is lost.

The members of this Committee believe that the Federal Interstate Regulation in its present form has served its original intended purpose and that it is now favoring the dissemination of the disease within Certified Areas and retarding the progress of the eradication program in noncertified areas. Since this is now becoming a documented fact, this Committee recommends:

(1) That the Animal Disease Eradication Division of the United States Department of Agriculture restrict the privileges extended to Specifically Approved Markets under the Regulation: and,

(2) That the Secretary of the United States Department of Agriculture immediately amend the Regulation to provide restrictions on cattle moved interstate as follows: "Cattle moved interstate from a market, dealers premise or noncertified area to a Specifically Approved Market shall be tested and marked negative for brucellosis and released: (a) under permit when consigned to a farm for breeding or grazing purposes, subject to quarantine and retest in not less than thirty (30) nor more than sixty (60) days; or, (b) under permit for direct movement to a second "Specifically Approved Market" one time only; declared as to origin, and, provided that movement thereafter is
to a farm under the permit, quarantine and retest provisions of (a); or, under permit to a feed-lot; or, under permit to an approved slaughtering establishment; or, under permit to a stockyards market operating under full-time Federal inspection and are slaughtered.

(3) That the Secretary of Agriculture amend the Regulation to eliminate the "Specifically Approved" status of all markets effective January 1, 1964.

RESOLUTION NO. 2
Survey and Control of Diseases of Poultry, Agency in ARS

(1) In cooperation with the several states involved survey the disease problems of the nation's poultry industry, and

(2) Encourage and support within the Agricultural Research Service the necessary research to provide the nation with the essentials, of both knowledge and tools, to begin an organized cooperative attack on the most serious poultry diseases, and

(3) Set up and establish within the Animal Disease Eradication Division of Agricultural Research Service a section specifically designed to combat such diseases as Newcastle, bronchitis, airsacculitis, and PPLO infection, and others as they may appear, in cooperation with several states, and

(4) To provide specific financing for this function annually. The first year of operation it is requested that an amount of not less than $200,000 be provided, and that steps be taken to provide sufficient standardized antigen for flock testing for PPLO infection, and that the development of a cooperative program for such testing be speedily implemented.

RESOLUTION NO. 3
Exotic Ticks, Scabies. Congressional Appropriation

"In view of the finding of exotic ticks in Florida and New York, and in other States, be it resolved that the United States Livestock Sanitary Association urge that the Secretary of Agriculture take necessary action to provide that the importation of all wild and domestic animals, regardless of species, be included in the regulations administered by the United States Department of Agriculture. All animals offered for importation, regardless of species, be held in the country of origin and an import permit be withheld until such animals are freed of ectoparasites and certified as such. All animals, regardless of species or place of origin, be inspected at the port of embarkation, found free from ectoparasites, and again treated with approved pesticides and by approved methods for the eradication of ectoparasites. Be it further resolved that insofar as possible, anthelmintics be administered for the control of endoparasites and that feed and bedding and crates unloaded at the port of debarkation, or used during transportation of animals from port of debarkation, be immediately destroyed or appropriately treated."
REPORT OF THE

Be it further resolved that the United States Livestock Sanitary Association urge that the Secretary of Agriculture request, and that the Congress appropriate adequate funds in the amount of $1,450,000 for Fiscal Years 1964, 1965, and 1966 to provide for the final drive to eradicate psoroptic sheep and cattle scabies from the United States.

RESOLUTION NO. 4
Request Meat Inspection Service Report Diseases of Animals

WHEREAS, Tuberculosis, Atrophic Rhinitis and Cervical Abscesses are responsible for an ever increasing economic loss to both the swine industry and pork processor and these diseases result in increased condemnations thereby increasing the cost to the consumer of pork products; NOW THEREFORE, be it resolved that the Federal Meat Inspection Service and all its personnel intensify their reporting to proper Animal Disease Control Officials of the several States in an effort to reduce these losses.

RESOLUTION NO. 5
Request United States Department of Agriculture Furnish Copies of Report of Harrar Mission, etc.

WHEREAS the introduction of foot and mouth disease into the United States could cause untold damage to the livestock industry and the economy of this country and
WHEREAS foot and mouth disease does exist in Argentina and a mission known as the Harrar Mission has been sent to that country to study the feasibility of the importation of livestock, meat and animal products into this country from the Argentine, and
WHEREAS there is a possibility that existing restrictions designed to prevent the introduction of foot and mouth disease may be modified as a result of the studies and recommendations made by the Harrar Mission:

It is hereby resolved by the United States Livestock Sanitary Association in session in Washington, D. C., November 2, 1962 that the Agricultural Research Service of the United States Department of Agriculture furnish each state livestock regulatory official with all information available or that will be made available covering the activities, findings and recommendations of the Harrar Mission and subsequent missions to study this matter."

RESOLUTION NO. 6

WHEREAS, bovine mastitis is accompanied by general confusion and lack of well organized and scientifically logical attempts toward control; possibly more than any other dairy cattle anomaly; and,
WHEREAS, it is one of the most prevalent and costly diseases of dairy cattle, and in accordance with Agricultural Research Service statistics, represents an annual loss of more than one-half billion dollars in
milk and animals alone, and which estimates do not encompass the additional cost of the several therapeutic agents, notwithstanding the problems created for the regulatory agencies, as well as the public health implications; and,

WHEREAS, the presence of antibiotics in milk as a result of treatment is also considered a public health problem; and,

WHEREAS, the newly formed National Mastitis Council, Inc., has accepted the challenge to determine what is being done in the fields of research, education and control programs, and has set forth principles upon which a well organized control program should be established; and

WHEREAS, the principles of this program may be adopted in whole, or in part, by the regulatory agencies and affiliated interests of the several states; and,

WHEREAS, the results of the application of these principles in the several states may be appropriately evaluated and the information relative thereto be disseminated to all interested groups and agencies;

NOW, THEREFORE, BE IT RESOLVED: That the Executive Committee of the United States Livestock Sanitary Association concur in directing that the Mastitis Committee of this Association expend its efforts in collaborating with the National Mastitis Council, Inc., and concur in having the Chairman of the Mastitis Committee represent the United States Livestock Sanitary Association, as a director on the National Mastitis Council, Inc. Board, and lend such other assistance as the Executive Committee of this Association deems appropriate for the expeditious resolution of the problem.
Mr. President, Honored Guests, Members of the United States Livestock Sanitary Association, Ladies and Gentlemen: Welcome to the 66th Annual Meeting of this Association; I am indeed very pleased to see so many registrants at both this meeting and that of the Conference of Veterinary Laboratory Diagnosticians and as well in attendance at the various Committees that held open meetings here during the past three days.

As you know, we are meeting in the Nation's Capitol this year in order to assist in the One Hundredth Anniversary Celebration of the creation of the United States Department of Agriculture. Twenty-two years later, the Bureau of Animal Industry was created. Evidence exists that the forerunner of this Association called the Interstate Association of Livestock Sanitary Boards prior to 1897 actually informally existed as a group of chief state disease control officials who frequently conferred with one another and discussed such conditions as Texas fever, anthrax, tuberculosis, contagious pleuropneumonia and swine fever.

Texas fever apparently was responsible for the first interstate health requirement when in 1795 the Legislature of North Carolina passed a law limiting the trailing of cattle from an area where the long leaf pine grew, into or through that part of the state where the growth of timber was of a different kind, between the first day of April and the first day of November under a penalty of four dollars per head. In 1814 Virginia refused to allow the trailing of cattle through the state from certain areas of South Carolina. Between 1883 and 1895 the tick quarantine line was established principally along the 38th parallel from the Atlantic to the Pacific Ocean.

Thereafter the livestock sanitary officials met to discuss the location of the quarantine line and the dates of the open season. They then requested the Federal Government to promulgate the regulation on "the line and open season for the year.

As a result of the restriction of the movement of southern cattle north of the quarantine line to the cold months of the year, Southern ranch owners sought a way of eliminating the ticks. Mr. R. J. Kleeberg of the King Ranch constructed an experimental dipping vat on his ranch. Subsequently, when the results of their experiment looked promising a similar vat was constructed at Fort Worth, Texas and the group of State Livestock Sanitary Officials, about to meet on the establishment of the quarantine line and determine the free time for movement of cattle north across the line, were invited by Texas people to hold their meeting at Fort Worth and to observe the dipping and freeing cattle of ticks.

This meeting then became the first printed report of the Interstate Association of Livestock Sanitary Boards, the forerunner of the United States Livestock Sanitary Association. It is also the first recorded evidence of the States looking to the Federal Government to promulgate the
location of the quarantine line and to set the dates of the open season. Here we see the cooperation of State and Federal Agencies working together in the interest of disease control. Such cooperation has continued throughout sixty-six years.

On our committees on animal infectious diseases are representatives from the livestock industry, from research, from Veterinary Medicine, from State and Federal Departments of Health and Agriculture. In addition, most of our committees hold open meetings to which any person is welcome to sit in and request permission to talk on the subject under discussion and express his view. The report of these committees are presented at our annual meetings and are, when advisable, recommended to the United States Department of Agriculture for their adoption and activation nationwide.

I believe that we should always keep in mind the fact that each state has the authority and legal responsibility to carry out their program of animal disease control to protect the industry within the confines of their geographic boundaries.

That we recognize the fact that the United States Department of Agriculture is supreme in that nebulous area of interstate movement and in those areas where Congress has directly charged them with the responsibility such as meat inspection and control over drugs and biologics.

We also appreciate the fact that the United States Department of Agriculture serves to head up the effort to control and eradicate infectious diseases. That they are supreme in the area of interstate commerce where they promulgate regulations relative to animals and things moving into the country and between the respective states. Among "things" I include biologic products.

Since 1954 we have been privileged through our Committee on Program and Policy to appear before the hierarchy of agriculture and in some instances praise their activity, in other areas criticize the department for its failure to carry out some of the operations we feel are necessary to strengthen animal disease research, prevention or control and eradication of infectious diseases as well as control over the licensed production of biologics for use in animals so that those permitted have merit, are safe, and are free from contaminants.

I trust I shall not be thought of as sounding a discordant note at this day of celebration on the occasion of the One Hundredth Anniversary of the creation of the Department of Agriculture but to many of us it seems quite incongruous that the department should engage in the promulgation of health regulations governing the interstate movement of livestock, prescribing that animals originate from disease-free herds and at the same time exercise no control over the interstate movement of the infectious agent responsible for the infection in the animal.

At a time when we are about to engage in an all-out effort to eradicate hog cholera and are still endeavoring to eradicate brucellosis I suggest that we review Amendment 15 to Bureau of Animal Industry order number 276 and take action to exercise control over the interstate movement and unauthorized or unreported use of certain products. The original amendment would have provided information to the State of destination of the
name and address of recipients of any of the following products: Ovine Ecthyma vaccine, Tuberculin, products made from Brucella Organisms, Hog Cholera Virus, Erysipelas Culture Vaccine, Newcastle Vaccines, Laryngotracheitis Vaccine and Anthrax Spore Vaccine.

In closing this portion of my remarks I wish to state that a perusal of the printed proceedings of this Association gives evidence of the following facts—

That for the past sixty-six years this Association has provided a meeting place and rostrum for the discussion of infectious diseases of livestock.

That from its committees' activities has evolved the sound programs of disease control.

That our recommended programs are sound is in a large measure due to the fact that we have had such excellent support of those engaged in research and from the assistance provided by industry representatives sitting in and expressing their judgement relative to the practical application of suggested procedures.

The politically minded state veterinarians have provided an excellent entree to ground root support and I am sure are regarded by those in charge of work at Federal level as a very important right arm in their effort to advance animal disease control nationwide. Our efforts, influence, guidance and accomplishment in the field of animal disease control of such diseases as contagious pleuro-pneumonia, Tuberculosis, Anthrax, Foot and Mouth Disease, Vesicular Exanthema, Glanders, Dourine, Spanish Splenic Fever, Scabies, Pullorum and Typhoid are too voluminous to report in detail. Let us, as an example, review the history of one, namely Brucellosis, which while not eradicated as yet is well on the road to that goal.

It was from this forum that Doctor Miller Barnes in 1922 reported his success in the suppression of Brucellosis (Contagious Abortion) through the application of the agglutination test for the detection of infected animals and the employment of sanitary procedures.

It was at meetings of this Association that the silver-tongued orator, Dr. C. P. Fitch of Minnesota, reported on the work of Fitch and C. R. Donham that led to a dilution of the antigen in the early thirties, which program in 1934 became the standard adopted by the then Bureau of Animal Industry. It was one of our members, I. Forrest Huddleson in 1926 in Lexington, Kentucky first introduced the plate method of testing for brucellosis. Later in the mid-thirties he advanced the theory of avirulent Brucella Vaccine and still later in the forties the so-called Mucoid Culture or M Vaccine.

It was also from this rostrum that Doctors Jacob Traum and Bruce Edgington made their report of research results with M Vaccine.

In our Proceedings will be found a report by Doctors Cotton and Schroeder on Strain 19 Brucella vaccine and the subsequent reports Dr. C. A. Manthei on the important facts relative to the optimum age of calves for vaccination, the duration of resistance and the Bureau's activity in testing and standardizing the antigen and vaccine.

It was from a subcommittee composed of regulatory officials and research men that the pamphlet "What is Known About Brucellosis" was
developed and some one hundred thousand copies provided for distribution throughout the nation.

It has been stated by some opposed to disease control and eradication methods advocated and advanced by this Association that our major reason for these endeavors is to provide job opportunities for Veterinarians and I submit to you that our objectives have always been disease eradication and not control and living with disease. Livestock farming is a more prosperous way of life in this country because of the work of men in this Association. True, the programs conceived in the Committees of this Association over the past 66 years have been adopted by the United States Department of Agriculture and advanced by them in cooperation with the State Regulatory Veterinary Medical Departments of the respective states with important assists from Extension, Veterinary Medicine, Health Departments, farm people, transportation, marketing and packing industries.

As we meet in the Nation’s Capitol to help celebrate the first hundred years of the United States Department of Agriculture service to the Nation, we in the United States Livestock Sanitary Association can be justly proud of the part we have played in making the United States the best place on the globe for the production of healthy and profitable livestock.

The philosophy of the United States Livestock Sanitary Association has and shall continue to be, that any eradicable disease constitutes an unwarranted tax upon both the producer and consumer of livestock and poultry, and their products.

I am certain we can look ahead with confidence to continued cooperation of all interested parties and greater accomplishment in the years ahead.

In a local newspaper I noted an article relative to a United States Department of Agriculture advice to newlyweds. I would like to suggest an article entitled "Advice to Livestock Owners" as follows: When embarking on a program of livestock production be certain to provide an Isolation Barn. Clean and disinfect the barn twice a year or oftener when needed. Inquire into the health history of herds from which replacements are contemplated and isolate newly added stock for a period of at least 15 days and treat the added animals as infected until you are satisfied they are not carrying some infectious disease into your farm. Be a good neighbor and consign exposed or unwanted animals direct to slaughter or a licensed rendering plant.

It has indeed been a pleasure for me to serve you this year and I am particularly pleased to see the launching of the program to eradicate Hog Cholera, a movement which I envisioned some twelve years ago when I selected the Committee on the Nationwide Eradication of Hog Cholera. I foresee the day when outbreaks of this disease will be handled as is Foot and Mouth and Vesicular Exanthema. In this endeavor I wish you God-speed.

Following is the report of the Treasury by the Certified Public Accountant, C. Bergen Groendyke.
Dr. Ralph Hendershott  
Secretary-Treasurer  
United States Livestock Sanitary Association  
Trenton, New Jersey

Dear Sir:

From the books and records of the United States Livestock Sanitary Association, I have prepared a statement of cash receipts and disbursements for the period from October 17, 1961 to October 9, 1962.

The cash balance at the end of the period was reconciled to the actual balance in the First Trenton National Bank, Trenton, New Jersey, as confirmed to me directly by the bank. Confirmation was also received direct from the Trevose Saving and Loan Association, Morrisville, Pennsylvania, for the balance on deposit in their saving account, which is pledged as collateral for the temporary loan of $4,000.00.

On separate pages are shown a summarized statement of operations for the period, and a statement of Net worth as prepared from the figures found in your cash books and from other information furnished by you.

The U. S. Treasury Bonds were examined by me on October 20, 1961. You informed me that these bonds are kept in a safe deposit box of the Broad Street National Bank, Hermitage Branch, Trenton, New Jersey, in the name of the United States Livestock Sanitary Association.

Respectfully submitted,

C. Bergen Groendyke  
Certified Public Accountant
REPORT OF THE SECRETARY-TREASURER

UNITED STATES LIVESTOCK SANITARY ASSOCIATION

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE PERIOD FROM OCTOBER 17, 1961 TO OCTOBER 9, 1962

Cash Balance, October 17, 1961:
- First Trenton National Bank, Trenton, J. J. $ 737.21
- United Savings and Loan Association, Trenton, N. J. 2,641.65
  Total $ 3,378.86

Increased by Cash Receipts:
- Individual Dues 5,592.50
- Official Dues 5,300.00
- Proceedings 2,117.15
- Foreign Annual Disease Handbook 175.44
- Hog Cholera Pamphlet ("What One Should Know About Cholera") 38.00
- Brucellosis Facts ("What is Known About Brucellosis") 126.50
- Reprints 3,792.60
- Registration Fees 1,605.00
- Interest on U.S. Treasury Bonds 800.00
- Interest on United Savings and Loan Account 93.24
- Proceeds of Share Loan from Trevose Savings and Loan Association 4,000.00
- Interest on Trevose Savings and Loan Account 92.63

Total Cash Receipts 23,733.06

Decreased by Cash Expenditures:
- Meeting Expenses 1,262.13
- Printing and Stationery 10,382.83
- Salary 6,500.00
- Travel 1,818.74
- Communications 514.95
- Rent 360.00
- Insurance 153.36
- Electric 94.89
- Miscellaneous 253.45

Total Cash Expenditures 21,340.35

Cash Balance, October 9, 1962:
- First Trenton National Bank, Trenton, N. J. 219.20
- Trevose Savings and Loan Association, Morrisville, Pa. 5,552.37
  Total $ 5,771.57
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

SUMMARY OF OPERATIONS FOR THE PERIOD
FROM OCTOBER 17, 1961 TO OCTOBER 9, 1962

Revenue:
Total Cash Receipts $23,733.06
Less: Proceeds from Share Loan 4,000.00
$19,733.06

Expenditures:
Total Cash Expenditures 21,340.35
Deficit from Operations for fiscal period 1,607.29

NET WORTH - OCTOBER 9, 1962

Balance, First Trenton National Bank, Trenton, N.J. $219.20
Balance, Trevose Savings and Loan Association, Morrisville, Pa. 5,552.37
U. S. Treasury Bonds, 4%, Due February 15, 1980 20,000.00
Furniture and Fixtures 640.00
26,411.57

Less: Loan Payable to Trevose Savings and Loan Association 4,000.00
Net Worth at October 9, 1962 $22,411.57

ANALYSIS OF CHANGE IN NET WORTH

NET WORTH, October 17, 1961 $24,018.86
Reduced by:
Deficit from Operations for Fiscal Period Ended October 9, 1962 1,607.29

NET WORTH, October 9, 1962 $22,411.57
REPORT OF THE COMMITTEE ON STOCKYARDS,
MARKETS AND TRANSPORTATION


The livestock disease control problems that have grown out of present day marketing practices and the rapid transportation of livestock are numerous and complex. Because these problems involve all segments of the livestock industry, disease control officials cannot solve them alone.

It is absolutely essential to the economy of the livestock industry to maintain relatively free movement of animals to and from markets. However, it is also recognized that the continual movement of livestock from auction to auction and state to state is responsible for the spread of many domestic diseases—and could possibly be the unwilling disseminator of foreign diseases which may make their appearance in the United States, either by accident or design. In view of this it is apparent that control of livestock movements is necessary, but not to the extent that the restrictions would strangle the industry we are pledged to protect.

Since over 90 percent of the livestock marketed in the United States is through federally approved auctions and stockyards, it is essential that free movement be maintained to these markets. At present the federal brucellosis regulation, and the future hog cholera regulation, will prohibit cattle and swine moving in interstate commerce without meeting specific health requirements but, at the same time, will allow these animals to move to federally approved auctions and stockyards without meeting any health requirements. Because there is no restriction on the admittance of livestock to these markets, there must be definite control over the animals that leave them. In most cases this control must be by regulations adopted by the individual states in which the federally approved auctions and stockyards are located. Therefore, it becomes apparent that state and federal regulations must complement each other, and this calls for closer cooperation between officials of both agencies.

The recent development of the Cattle Market Testing Program is destined to save the state and federal governments, as well as livestock owners, a great deal of expense and inconvenience in the brucellosis and tuberculosis eradication efforts. Since auctions and stockyards bring together livestock from all localities, why not identify, test and vaccinate at these points and use the market records to determine the origin of healthy as well as diseased animals. In this way the need for "down-the-road" testing is practically eliminated and the only on-the-farm testing required is in known infected or exposed herds. No doubt other specific disease eradication programs will make use of the opportunities and facilities that are available at livestock markets. However, these programs must be
designed to cause as little inconvenience as possible to the operation of the markets.

The movement of livestock across state lines to points other than federally approved establishments has its problems also. Since most of these movements require a health certificate, it is necessary that the practicing veterinarians know the requirements of the states of destination. At the present time it is virtually impossible for the practicing veterinarians—and, for that matter the regulatory veterinarians—to keep abreast of the almost daily changing state regulations. It sometimes appears that the only reason for these numerous changes is merely to demonstrate the right and ability of each state to be different. Unfortunately this lack of uniformity in interstate health requirements discourages rather than encourages compliance. For this reason another all out attempt should be made to develop uniform regulations governing the interstate movement of livestock, and since the federal government has under its control practically all other phases of interstate commerce, it is only logical that the interstate movement of livestock should also be under federal jurisdiction. The United States Department of Agriculture, however, should not attempt to formulate these regulations until it has consulted with all aspects of the livestock industry. Then it would be hoped that each state, in turn, would adopt in toto the industry developed federal regulations as their own.

This report cites only a few of the most complex problems that involve transportation and marketing but from these examples it is apparent that one group alone cannot formulate the answers. Since transportation and marketing are here to stay, and the problems will become even more numerous and complex in the future, NOW is the time for representatives from all segments of the livestock industry to sit down—perhaps at the invitation of the Secretary of Agriculture—and begin to develop regulations, policies and programs that will produce the most effective disease control with as little interference as possible with the industry's modern, rapid marketing procedures.
FACTORS THAT INFLUENCE THE NEUTRALIZATION TEST*
L. E. Carmichael
Ithaca, New York

The present lack of confidence in quantitative neutralization tests for measuring the immune status of animals seems largely justified, for the prerequisite standardization of tests to a common level of accuracy has not yet been accomplished for most animal virus neutralization systems. Reasons for the lack of standardized procedures are probably diverse, among them being inadequate characterization of the biologic conditions that affects the various neutralization reactions, individual preferences for apparently satisfactory empirical procedures that have been developed in various laboratories to serve particular needs, and the general preference for traditional animal protection tests as the final criteria for immunity, despite certain inadequacies. Arguments for employing serologic criteria as indicators of immunity for certain animal virus infections have been presented in previous reports and, though the method appears sound and efficient in the use of animals, it has not supplanted animal protection tests in actual practice.

The most essential requirement for neutralization test procedures to be used as a substitute for the challenge test for immunity (or vaccine evaluation) is naturally a high level of accuracy and reliability in the test. After suitable test procedures are established, antibody levels then can be related meaningfully to protection against a standard dose with virulent virus. After this has been achieved for individual viruses, the neutralization test can be used to distinguish between immune and susceptible animals—with certain noteworthy exceptions. For example, animals in a resistant state of secondary response following a single inoculation with killed virus would not be so identified by serological tests, nor would animals during the period of decline of passive antibody, where the transition from the susceptible to the immune state is unclear. A state of resistance not measured by specific neutralizing antibody may also be induced by different, but antigenically related viruses. Nevertheless, under usual circumstances where seroconversion from a negative or low antibody level to a higher level can be measured, as following vaccination or infection, serological techniques will distinguish immune from susceptible animals. With standardized tests a firmer immunological basis than currently exists can be established for vaccine standards and allow comparisons of quantitative immunological data.

Because a system as complex as that composed of virus, antibody, and host cells is capable of showing many variations in any one of its components, standardized neutralization test procedures should be based on

*This investigation was supported in part by the American Kennel Club and the Co-op Grange League Exchange, Inc.

Veterinary Virus Research Institute, Cornell University, Ithaca, New York.
experimental data that define the interactions between a particular virus and its homologous antibody and between residual virus (following neutralization) and the selected test host, e.g. tissue culture, chick embryos, or laboratory animals. In the present discussion some of the general principles and factors which seem likely to contribute most to neutralization test variation will be considered. The infectious canine hepatitis (ICH) virus-antibody system* will be used for certain illustrations, however, it should be realized the conclusions drawn from experience with one virus system are not necessarily translatable to other viruses, even ones within the same general group.

In general, the neutralizing capacity of a serum is assessed by mixing graded serum dilutions with a concentration of virus that causes regular infection in a susceptible host. After a suitable incubation, the serum-virus mixtures are inoculated into the host to determine the ability of the serum to protect against the infective capacity of the virus. The test, then consists essentially of two systems, the serum-virus neutralization system and the assay system for residual virus. While the techniques employed for virus neutralization vary widely, there are two basic procedures for assaying serum antibody. Only the constant-virus-varying-serum technique will be considered here, for this method appears the more satisfactory for measuring differences in serum antibody content. Factors thought essential to consider in devising individual neutralization tests are as follows:

1. The growth and harvest of stock virus for tests.
2. Conditions influencing the virus assay system.
3. Rates of viral inactivation by immune serum under various conditions.
4. Relationships between the amount of virus used in the test and the amount of antibody required for complete neutralization ("neutralization slope").
5. Technical variations introduced by dilution schemes, endpoint criteria, etc.

Growth and harvest of virus for stock. Because the neutralization test is based upon the assay for residual infective virus following a suitable incubation with serum dilutions, it is essential to minimize the amount of dead or inactive virus in the stock virus pool. This may be accomplished by harvesting virus for stock at, or shortly before, the peak if infectivity is reached. The appropriate time for harvest will vary according to the individual virus growth characteristics. For ICH virus in dog kidney cells (DKC) the peak of the growth curve is reached 36-40 hours after superinfection with a multiplicity of approximately five. At this time cytopathic changes (CPC) are first evident in unstained cultures. With ICH, virus is mostly cell-associated, however, in other systems virus may predominate in the fluid phase.

*The author expresses thanks to the Editor of the Cornell Veterinarian for permission to use data submitted for publication.
In order to obtain a uniform suspension, the stock virus pool should be clarified by centrifugation or other appropriate procedures that do not inactivate virus, but eliminate cells and other debris with which virus aggregates may be associated. Since most virus suspensions lose a portion of the original infectivity following lyophilization, this procedure should be used for stock virus preservation only with those agents that are shown to be stable. A generally more suitable method to preserve stock virus is to store working aliquots (one or two ml) in sealed vials at -50°C or below. Mechanical freezers afford certain advantages over dry ice boxes, since carbon dioxide (acid) inactivation and temperature fluxations are reduced. Some viruses may require a stabilizing fluid such as "SPGA"9, or other non-inhibitory buffered protein solutions. Virus thus prepared and stored must naturally be tested as a portion of each neutralization test. If a decrease in infectivity is observed, an additional vial should be examined and, if similar results are obtained, the stock should be discarded and replaced.

**Virus assay.** For standardized tests, if would seem essential that a single host type be used for each test system. The host should be the one that is uniformly susceptible and shows minimal endpoint variation. If laboratory animals are used, the strain and age of the animal, as well as the route and method of inoculation is important.

Virus dilutions should be made in media that will not adversely affect the virus or the assay host. With tissue culture, the media must be such that the cells will remain in a satisfactory nutritive state throughout the observation period. Frequently substances in cell culture media will inhibit virus nonspecifically. The most frequent offender is fresh or heated serum, although certain batches of lactalbumin hydrolysate may also be inhibitory. A globulin inhibitor for the adenovirus group, which includes ICH virus, has been found in cattle serums and sera from other species10,11. Certain sera may contain ICH virus inhibitor levels that exceed usual immune serum titers; therefore all sera to be incorporated in cell culture media or virus diluents must be tested for inhibitory activity. With the hog cholera system, for example, only serum from certain sheep could be incorporated into tissue culture media because of inhibitory properties12. Similar experience has been encountered with a variety of viruses.

Once satisfactory growth conditions are established, the sensitivity of the test host to the infecting virus should be established by dose-response studies. By measuring the dose range over which the responses to virus vary from 100 to zero percent infection, the dilution rate can be established that will not exceed the inherent sensitivity of the host to the test virus and, by the following formula: \( \frac{4D^2}{(2E - D + R)^2} \) where \( D \) is the log dilution rate, \( R \) the log range from 100 to zero percent infection, and \( E \) the log tolerable error, the number of animals (culture tubes, eggs, etc.) required per dilution can be estimated so that the variation will not exceed the desired tolerance. An allowable error of 0.5 (three-fold) has been suggested. Figure 1 shows the dose-response pattern of ICH virus in dog
DOSE RESPONSE PATTERN OF ICH VIRUS IN DOG KIDNEY CELL CULTURES

Figure 1

kidney cells. Here, the dilution range over which 100 to zero percent infection occurs is 60-fold \( \log_{10} 1.8 \). Accordingly, the dilution rate to be used for virus assay should not exceed 60-fold. With 10-fold dilutions, two tubes per dilution are required for assurance that errors in log titer will not exceed three-fold. Similar results have been obtained for virus diarrhea, canine distemper, and hog cholera assay systems\(^{13,14}\).

Because differences in rates of virus growth occur in various host cell systems, the time at which endpoints may be read will vary. For instance, canine distemper virus assay by the plaque technique in chick embryo cell cultures could not be concluded with confidence until the 11th day\(^{15}\). ICH virus titrations in DKC, on the other hand, can be read on the seventh day following inoculation and hog cholera virus diarrhea tests can be finally read by the fifth day\(^{10,14}\). The time at which individual tests should be read must naturally be determined for each virus in the host system used. With some adenoviruses, titration poses great difficulty since dilutions of a limiting nature may not produce recognizable changes (CPC) for as long as 20 to 30 days\(^{16}\) and endpoints often may be judged as positive only after a subsequent passage. Growth characteristics of certain tissue cultured strains of distemper virus, especially those propagated solely in mammalian cells, essentially precludes these strains from serologic usefulness since endpoints are estimated only after tedious staining procedures or in certain instances, back-passage in eggs\(^{15}\). Experience with ICH virus, as well as other adenoviruses\(^{16}\), has amply demonstrated that the yield of infectious virus is dependent upon the constituents of the media, for inhibitors such as ones found in ox, horse, and
calf serums may greatly delay the appearance of CPC. Possibly as the culture media becomes acid there is dissociation of the virus-inhibitor complex, as with poliovirus-antibody complexes\textsuperscript{17}. The inhibitor, on the other hand, may be metabolized by the cells and slowly release virus. Such a mechanism could account for delayed appearances of endpoints in certain titrations where no other obvious explanations are evident such as "resistant" animals or cell types.

**Kinetics of viral inactivation by immune serum.** From the practical point of view, information on the rate of viral inactivation under different conditions of temperature and serum concentrations allows the experimental determination of optimal incubation periods for serum-virus dilutions. In such experiments, control titrations of virus in the diluent without serum must be performed in order that any temperature effects may be observed. This is of considerable importance with such viruses as canine distemper and herpes simplex viruses, where the half-lives are short. For example, the estimated half-life of distemper virus was two hours at 21°C, one hour at 37°C and 10 minutes at 45°C\textsuperscript{15}. With such viruses, it is essential to know the time at which neutralization is complete at temperatures that do not cause thermal inactivation.

A typical first-order inactivation curve is shown for ICH virus and its homologous antiserum in Figure 2. Here, neutralization proceeds instantaneously and is virtually complete in 20 minutes. With viruses that are relatively stable at usual incubation temperatures, such as ICHV, a wide range of safety attends the incubation period. In practice, then it would
seem appropriate to use that incubation time and temperature which is compatible with the inactivation rate and stability of the virus. For ICH virus, any period greater than 20 minutes and less than 24 hours at 21 C or 37 C is satisfactory and would contribute little error to the test. It would be helpful to know if rates of neutralization differ between strains of a particular virus type. Such phenomena are indicated with ICH virus (Figure 3), for the initial rate of inactivation of a fox strain of ICH virus (FV7233) was slower with a serum prepared in dogs against our usual laboratory ICH strain (Cornell-1) than with a homologous serum of approximately the same titer. With other viruses these differences may be magnified and assume greater significance, however data is limited.

Neutralization slope. The slope of the line that relates graded increments of virus neutralized to the amount of antibody (represented by titers) required for complete neutralization is frequently termed the "neutralization slope." This inverse linear slope which relates the antibody titer to the amount of virus in the test has been determined for several virus-antibody systems, and it has become clear that the relationships are subject to great variation. The complex interactions between virus, antibody, and host cells each contribute certain inherent errors to the results, and failure to recognize and evaluate the variables has lead to considerable discrepancies regarding the usefulness and even the existence of the linear relationship with some viruses. Factors that have been studied, and which
FACTORS THAT INFLUENCE THE NEUTRALIZATION TEST

Influence the neutralization slope most markedly, are the type and age of the animal used for assay (relative susceptibility), route of animal or egg inoculation, serum treatment (duration of storage, length of heat-inactivation), and particular virus type examined. As illustrated in Figure 4, subtype variations seem to occur with ICH virus, for one strain (FV7233) showed a slope of 0.7, which is clearly different from the slope 0.4 obtained with the Cornell-1 strain. With the exception of virus strain characteristics that reflect inherent differences in the virus-antibody interaction, and the control of serum treatments, tissue culture methods have greatly reduced the errors attributable to variations in animal susceptibility. If relationships between various virus strains and their antisera are known, and a standard host type can be selected for each test, then discrepancies that arise might be more easily resolved.

Technical variations. When conditions for virus assay, incubation of serum-virus mixtures, and suitable virus dosages have been determined, the neutralization test should theoretically perform within a certain range of sensitivity, exclusive of technical errors. The inherent sensitivity of the test can be estimated by measuring the range of serum dilutions over which the response varies from zero to 100 percent infection. This
should be done using small dilution decrements (i.e., two-fold) and a suitable dose that is sufficiently large to give rise regularly to CPC in tissue culture, or uniform infection in laboratory animals. Inherent sensitivities of the neutralization test have been determined for ICH, distemper, hog cholera, and bovine virus diarrhea neutralization systems. Reports of such data with other tests have not been noted. Figure 5 shows a typical series of results for ICH in DKC tubes where the proportion of tubes showing CPC at each 0.3 log_{10} serum dilution is recorded. Here, 50 to 100 TCID_{50} 0.1 ml ICH virus was mixed in equal volumes with the serum dilutions and inoculated into 15 tubes per dilution. As indicated by the graph, the range of serum dilutions over which infection changed from essentially zero to 100 percent was slightly less than five-fold. The implication of these results is that not more than five-fold serum dilutions should be used in ICH neutralization tests in order to eliminate bias in the calculated 50 percent endpoints. Using the formula noted previously, the use of five-fold serum dilutions with three tubes per dilution should result in no more than a three-fold error. This procedure has been successful experimentally for ICH and also for distemper, bovine virus diarrhea, and hog cholera. The principal source of technical error in neutralization tests appears to be in the dilution schemes employed. This has also been noted for leptospirosis agglutination-lysis tests. Table I shows endpoint variations in the ICH test introduced by the use of different dilution schemes (five- versus ten-fold and single versus multiple pipets for dilution. As
**FACTORS THAT INFLUENCE THE NEUTRALIZATION TEST**

**TABLE I**
Comparison of ICH Virus Neutralization Tests on A Single Serum
By Varying Dilution Rate and Number of Pipets Used

<table>
<thead>
<tr>
<th>Dilution Rate</th>
<th>5-Fold</th>
<th>10-Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple pipets</td>
<td>Single pipets</td>
<td>Multiple pipets</td>
</tr>
<tr>
<td>4.2*</td>
<td>6.1</td>
<td>4.5</td>
</tr>
<tr>
<td>4.5</td>
<td>5.3</td>
<td>4.7</td>
</tr>
<tr>
<td>4.2</td>
<td>6.5</td>
<td>4.5</td>
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<tr>
<td>4.2</td>
<td>6.3</td>
<td>4.5</td>
</tr>
<tr>
<td>4.3</td>
<td>4.9</td>
<td>4.0</td>
</tr>
</tbody>
</table>

*-log_{10} serum dilution that neutralized 50 to 100 TCID_{50} ICH virus.

seen, the variance among tests was more than 10 times greater when a single pipet was used than when multiple pipets were used, both for the five- and the ten-fold dilution series. Titer variations were within the desired tolerance when five-fold dilutions were employed with a separate pipet used for each dilution transfer. However, when only one pipet was used for each entire dilution series, variation was great within sets and the observed titers were increased 10- to 100-fold over those occurring when a separate pipet was used for each dilution transfer. Obviously, titer variation due to residual carry-over of antibody is enormously large when a single pipet is employed. This was especially evident in the 10-fold dilution series. Since similar results have been obtained with other systems studies in our laboratory, it seems imperative that fresh pipets should be used between each serum dilution, as with the virus dilution series. Apparently this practice is not universally adhered to, since rather astronomical titers have been occasionally reported, especially as regards distemper. Other sources of variation that have been noted are inadequate mixing of the serum or serum virus mixtures, using various volumes of virus and serum in the neutralization system, varying the amount of virus-serum mixtures in the inocula, and varying the number of animals or culture tubes used for each test dilution.

**Interpretation of neutralization tests.** Where CPC in tissue culture or infection in a laboratory host is rapid, and there is evidence that "virus breakthrough" does not occur during the observation period, the "all or none" endpoint should be used, i.e., any evidence of CPC or infection should be scored as "not protected." With some viruses this is not possible, and it has been necessary to employ more subjective scoring criteria. For example, with some human adenovirus neutralization tests a final reading of two-plus, three-plus, or four-plus degeneration indicates lack of neutralizing capacity, while a final reading of one-plus or less indicates neutralizing antibody. Final endpoints are then computed by standard methods. We prefer the Spearman-Karber method because of its operational simplicity and certain mathematical characteristics.
Comment. While current attitudes appear to support the general association of serologic phenomena with immunity, doubt is still frequently raised when specific value judgements are attached to titer estimates. Such caution is probably warranted at the present time and skepticism will likely continue to shroud efforts to correlate quantitative serologic estimates with active resistance to virus diseases until test procedures are standardized to a common level of accuracy and serological values become meaningful in a comparative way. Standardization can be accomplished only after studies are made on the biologic conditions attending the various neutralization reactions. Table II shows a partial list of factors that are felt essential to know before establishing standard protocols for various virus neutralization tests, especially as regards the quantitative evaluation of the immune response. Until most of these basic criteria are known, serologic procedures will probably continue to be somewhat unique, inconsistent between laboratories, and therefore disputable. Most of the factors listed in Table II have been satisfied for canine distemper, canine hepatitis, foot-and-mouth disease, bovine virus diarrhea, and hog cholera neutralization test systems. On the other hand, little has been reported on the characteristics of the infectious bovine rhinotracheitis neutralization reaction, and current tests available leave much to be desired in terms of sensitivity and reliability. Naturally, certain viruses

<table>
<thead>
<tr>
<th>Factors Considered</th>
<th>CD**</th>
<th>ICH</th>
<th>FMD</th>
<th>BVD</th>
<th>HC</th>
<th>IBR</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Virus Growth Conditions in Assay Host</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>2. Virus Growth Curve</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>3. Sensitivity of Virus Assay System</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4. Kinetics of Neutralization</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>5. Virus Stability</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6. Neutralization Slope</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7. Sensitivity of Neutralization System</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8. Strain Differences Reflected in Neutralization Test Characteristics</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*+ = information found in search of literature
- = information not found

** CD = canine distemper
ICH = infectious canine hepatitis
FMD = foot-and-mouth disease
BVD = bovine virus diarrhea
HC = hog cholera
IBR = infectious bovine rhinopneumonitis
ER = equine rhinopneumonitis
possess characteristics that lend to more sensitive tests than others, as with bacterial systems, and those that are inherently variable should be critically evaluated as to their potential usefulness as regards diagnosis, vaccine evaluation, or estimation of immunity.

As the simpler criteria are studied (items one through seven in Table 2), the next logical step would be to evaluate strain characteristics in order to select reference test strains that show least variation under established test conditions. As an illustration of pitfalls that could occur with inadequate control of virus passage, distemper neutralization tests in embryonating eggs could be severely biased by employing a "standard" strain if it had been indiscriminately transferred for an unlimited number of passages or in an unusual host cell system. It has been shown, for example, that an egg-adapted strain (Onderstepoort) serially transferred in chick embryo cell cultures lost infectivity for the chorioallantoic membrane (CAM), for by the 40th passage in tissue culture the plaque-forming unit—CAM ratio was 72,000 to 1. Control of reference strains and passage histories, then, is an essential part of any program for test standards.

REFERENCES

This year your committee was setup to deal with biological products only, in line with the recommendation of our combined committee last year to separate the committee activity for biological products from the activity for pharmaceutical products.

Real progress has been made this past year in evaluation of licensed biological products at the National Animal Disease Laboratory at Ames. You will remember that the Committee for Biologicals and Pharmaceuticals included in their report in 1955 a strong recommendation be made by the United States Livestock Sanitary Association to the Secretary of Agriculture that laboratory facilities be made available to the Animal Inspection and Quarantine Division of the Agricultural Research Service for the purpose of check testing samples of licensed products produced by industry and for the standardization of testing methods to properly evaluate such products. In 1956 the Committee for Biologicals and Pharmaceuticals again emphasized the need for these facilities.

Last year you heard Dr. Van Houweling describe the facilities, the organization and planned operation of the laboratory, which included facilities and an organized group for the control testing of licensed biological products. During the past year this group at the National Animal Disease Laboratory has been very active and the following summary of activities pertaining to biological product testing was provided by Dr. John Hejl.

### SUMMARY OF ASSAYS CONDUCTED

<table>
<thead>
<tr>
<th>Type of Product</th>
<th>Number of Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large Animal</td>
<td>2,068</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>435</td>
</tr>
<tr>
<td>Small Animal</td>
<td>522</td>
</tr>
<tr>
<td>Poultry</td>
<td>842</td>
</tr>
</tbody>
</table>

This reports a considerable number of assays, quite varied in nature, which were conducted by the biologics group at the National Animal Disease Laboratory.

As a part of the activities of this group, a considerable number of assays were conducted in the examination of anaerobic cultures for purity, contamination and viability. These cultures were obtained from licensed manufacturers who used them for the production of anaerobic bacterins. Assays for anaerobic product potency were also conducted.
You can see from this activity that the quality of licensed biological products is now and will continue to be under better surveillance than was ever possible before laboratory facilities were available to the Animal Inspection and Quarantine Division to obtain objective data on quality of licensed veterinary biologicals.

This committee would like to recognize and commend the efforts of the A.I.Q.D. laboratory facilities and personnel as they thus far have functioned. Certainly the results of this effort will build greater confidence in all licensed veterinary biologicals. In fact, we think this A.I.Q.D. laboratory evaluation of veterinary biologicals is one of the greatest advances in preventive animal disease control in recent years. This committee pledges its support. We are also glad that the voice of this association and our past committees may have played some small part in this program for positive upgrading of veterinary biologicals.

We would also like to briefly report on research which may lead to new licensed veterinary Products.

RAM EPIDIDYMITIS IMMUNIZATION

The ram epididymitis problem has been under study intensively at the University of California by Drs. Biberstein, McGowan, et al. They have experimentally applied an immunization procedure for its prevention which was originally performed in New Zealand by Buddle. The immunization procedure consists of the simultaneous injection of an adjuvant bacterin prepared from the ram epididymitis agent and simultaneous injection of Brucella Abortus Strain 19 Vaccine. The organism isolated from ram epididymitis in California has not yet been named. The agent considered the cause in New Zealand was called Brucella ovis, however, this classification is in doubt. Field trials in California in the immunization of rams have been conducted using this adjuvant bacterin simultaneously injected with Brucella Vaccine with very encouraging results. Dr. McGowan, of the University of California, tells me that vaccinated rams when checked by palpation of the epididymis indicates the vaccine was highly effective in preventing the pathology characteristic of this disease and which is readily discernable by palpation. It would appear that this serious economic problem to the sheep grower may soon be under control.

HOG CHOLERA IMMUNIZATION

This past year a great deal of work has been done by Dr. Baker and his group at Cornell attempting to prove conclusively that protection against hog cholera can be induced by injection of a heterotypic virus. Field trials have been conducted in Florida and New York. The virus used in these trials was the Bovine Virus Diarrhea agent and this immunizing method has been reported on in the past and again on this program. Your committee and this association is interested in this phenomenon and the potential use that may be made of this type of inducing resistance to disease. It appears from the trials conducted that more work is needed to
apply this principle of immunization with heterotypic virus in a practical manner. Additional trials are planned for Hawaii. In the meantime, the hog cholera modified live virus vaccines available for the prevention of hog cholera have been doing an excellent job of preventing the disease where properly used. It is believed by your committee that these modified vaccines applied in the program developed for hog cholera eradication will permit great strides to be made if the vaccines are extensively used.

The following biological products were licensed this past year.

SPECIAL LICENSES

1. Avian Encephalomyelitis Vaccine
   This product was made available under a special license for one year for the further evaluation of its safety and efficacy. The special license terminates April 1, 1963.

2. Equine Pneumonitis Virus Vaccine
   This vaccine was also licensed under a special license for further evaluation of the product. The license terminates July 1, 1963.

REGULAR LICENSES

1. Lactobacillus Acidophilus
2. Flea Antigen
3. Rabies Vaccine, Killed Virus, Tissue Culture Origin
4. Staphylococcus Equine Bacterin

REFERENCES

ANAPLASMOSIS PROGRAM IN HAWAII

E. H. Willers
Honolulu, Hawaii

Many years ago a wise man said—"horse sense is just stable thinking." However we define it, good old horse sense is still the most important ingredient in any livestock disease control program. Our anaplasmosis program in Hawaii was no exception.

To get first things into the record first, I want to acknowledge that there would have been no anaplasmosis eradication program in Hawaii were it not for the work of Schoening, Moehler, Gates, Mott, Roby, and the many others in the Pathology Division of the old Bureau, later the Animal Diseases and Parasite Research Division (ADPRD) who after great effort developed the antigen and modified the techniques of the complement-fixation test to make it a reliable tool for the detection of anaplasmosis infected carrier animals.

It was Hawaii's good fortune that coincident with the diagnosis of our first clinical case of anaplasmosis in May 1954 that Dr. H. W. Schoening and the others in his group were preparing to announce that the test had finally been perfected to a point where it could be taken into the field.

After confirming our diagnosis the ADPRD offered to test and retest the infected herd if we would agree to dispose of the reactors by slaughter. This project became the forerunner of the first state-federal cooperative program for the control and eradication of anaplasmosis.

In the beginning, serum samples were sent to the Beltsville laboratory for testing while we in Hawaii performed transmission trials with splenectomized calves. The cattle in the infected herd and in two small adjacent herds of unknown status were divided into groups of about 20 head each. Blood samples were pooled from each group of cows and injected into a splenectomized calf. When the results of these procedures were examined and compared with the results of the CF test, it was found that the pools of blood that produced anaplasmosis in the inoculated calves had been drawn from groups of cattle, one or more of which were positive to the CF test. Conversely, the pools of blood that failed to infect calves had been drawn from cattle that were negative to the test.

The two adjacent herds of unknown status failed to reveal evidence of infection by either test method. The infected herd was retested at 60 day intervals and reactors removed. On the third retest no reactors were found. On the fourth retest, which would have been the second negative test, blood samples were again pooled for calf trial. Both the CF test, and calf trials were negative.

Concurrently with the testing program, vector control was maintained by fogging the area with insecticides for mosquito control and spraying the cattle individually to eliminate lice. Although we were not sure of the value of this procedure at the time later work definitely proved that control of the louse was essential to the program.
With this evidence that anaplasmosis could be eradicated from a single herd, it was not difficult to envision an eradication program for all cattle in the State. A survey was undertaken to determine the extent of infection in our dairy and beef herds. Herds that contained imported cattle, particularly from California, usually contained test reactors. No herds were found with reactors that did not contain imported cattle. Very few native cattle reacted positively. History of the reactors taken from the first herd tested showed that the majority had originated in a single shipment from California.

Insofar as it was possible for us to do so, at least one calf trial was conducted on each herd found to contain test reactors. In nearly all cases the calf trials confirmed the test results. Most of the failures involved young native animals. I will refer to these trials again later on.

With this information, it was clear that any control program would have to include the testing of all cattle intended for importation into Hawaii at the point of origin on the mainland. An agreement was soon worked out between ADPRD and the several western states whereby ADPRD would test serum samples at the Beltsville laboratory that would be collected, processed, and forwarded by the states on all cattle destined for Hawaii. Cattle negative to this mainland test would be eligible for shipment to Hawaii with the expressed condition that they would be put in quarantine on arrival and retested. Cattle negative to the quarantine test would be released to provisional quarantine on the premises of destination for a final test 60-90 days later. These requirements were later included in our import regulations.

At the suggestion of ADPRD the head of our veterinary laboratory was sent to Beltsville to study the CF test technique. Commencing in March 1955 all tests were run in the Hawaiian laboratory using antigen furnished by ADPRD. Duplicate serum samples were submitted to Beltsville for comparative testing. This comparative testing was continued on all serum samples collected until it was decided that test results in the Hawaiian laboratory were in sufficient agreement with the results in the Beltsville laboratory to permit the acceptance of the negative results obtained in Hawaii. After that all sera showing reactions in any degree or that were anticomplementary were submitted to Beltsville together with an equal number of negative sera from the same herds for comparative testing. This procedure is still being followed although the Laboratory Services Branch of Animal Disease Eradication Division (ADED) took over the routine comparative testing from ADPRD several years ago.

As soon as the State laboratory personnel became proficient in the test procedures we began collecting blood samples from each head of cattle slaughtered in the state. The testing of these samples constituted a continuing survey of all herds in relation to anaplasmosis much like the back tag testing procedure that is now being used in the brucellosis program.

A national meeting on anaplasmosis was called by the Agricultural Research Service in February 1955 in Chicago to develop field experiments with the states in the control of anaplasmosis by use of the CF test. Hawaii was invited to give a report on the results of our work up to that time. We
concluded our report by proposing that our field trial be enlarged to a full scale cooperative Federal-State eradication program. We based our proposition on the following:

1. Experience: We had proven that anaplasmosis could be eradicated from an infected herd.

2. Insularity: Because of its geographic position, Hawaii was able to thoroughly and carefully screen all animal imports.

3. Vectors: The absence of important vectors of anaplasmosis in Hawaii was a favorable factor.

4. Auction Sales: There were no auction sales organizations. Normal movement of cattle was direct from farm to market.

5. Separation of Counties: Each county in Hawaii was separated from the others by a large expanse of ocean which constituted a natural barrier to the uncontrolled movement of livestock between counties.

6. Rate of Spread: Test information indicated that the rate of spread of anaplasmosis from imported carriers to susceptible animals was very low.

On balance, these conditions seemed to favor the success of an eradication effort. There was also cogent reason for starting the program as soon as possible. Entomological records showed that 15 to 20 emigrant insects were found in Hawaii annually making it highly probable that a known or unknown vector of anaplasmosis could gain entrance at any time. (Twenty-two months later, a known vector Dermacentor albipictus was found in Hawaii. It was eradicated at considerable effort.)

Our proposal at the Chicago meeting was favorably received by the State and Federal officials in attendance. Details of a cooperative eradication program were agreed upon and I returned to Honolulu to present the States portion of the program to our legislature.

State authorization and appropriations followed; state and federal regulations were promulgated and a memorandum of agreement outlining the responsibilities of the two agencies was executed. The cooperative program became effective on November 16, 1955.

Our State regulation required that all cattle imported prior to January 1, 1955, the date when we began testing imports, should forthwith be tested for anaplasmosis. We particularly wanted to get all imported cattle tested as soon as possible since our experience showed that those cattle were most apt to be infected. Next in priority were those herds found to contain reactors as a result of the testing of blood samples collected at slaughter. We also drew blood samples from all cattle that were tested in the tuberculosis program. By following these procedures for several years, we believed that we could uncover all herds containing carrier animals. This proved, in retrospect, to have been a satisfactory approach. The number of reactors found has steadily declined year by year. During the past fiscal year, we tested 16,312 head of cattle, including 2,048 imports and found only five reactors. Three of these were detected on entry quarantine test,
and two showed up on the 60-90 day post-entry retest and were proven by calf trial to be carriers. We believe that anaplasmosis has been eradicated from Hawaii and that from now on our task will be to prevent reintroduction of the disease.

This task may become more complicated as time goes on. Some peculiar test reactions and calf trial results recently encountered on cattle in quarantine lead us to believe that some interference in the CF test is occurring, possibly due to prior use of drugs on these cattle. Experiments have been designed to shed some light on this possibility. We are concerned that unscrupulous efforts might be made to ship us infected carrier animals that have been drugged into a temporarily negative CF test status. We will also try to determine if this could happen accidentally in the course of treating the cattle for other disease.

This interference with the test is different from that observed by us in two herds in the islands. In the latter instance we were dealing with test negative cattle which when vaccinated for leptospirosis developed test reactions ranging from 1+ to 4+ in up to 20 percent of the cattle with as many as 50 percent of the remaining samples going anticomplementary. These reactions gradually subside over a period of months but can be elicited again by revaccination. We have been unable to develop evidence as to why this occurs or what causes it.

<table>
<thead>
<tr>
<th>Chart No. 1</th>
<th>SUBINOCULATION Calf Trial Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of Animals Tested</strong></td>
<td><strong>Origin</strong></td>
</tr>
<tr>
<td>14</td>
<td>Native</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
</tr>
<tr>
<td>19</td>
<td>Ret. of Imp.</td>
</tr>
<tr>
<td>13</td>
<td>&quot;</td>
</tr>
<tr>
<td>16</td>
<td>Imp.</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

*Both reactors were progeny of infected herds.*

Chart number 1 shows the results of calf transmission trials during the last five years. You will note that 28 head of 4+ reactors failed to infect calves. The majority of these reactors were under two years of age. It has been our experience that stained blood films made at the time that these young animals are showing the reactions to the CF test will exhibit eperythrozoa or hemobartonella or a specie of Theileria on the red blood cells. We also find that the test reactions tend to disappear in these young animals over a period of two to six months. We are therefore now of the opinion that, if we had our work to do over again, we would not bother to test young animals in a herd in which the adults are all negative to the test.

During the seven years that we have been engaged in the anaplasmosis eradication campaign we have conducted 185,543 tests on live animals
and 181,403 on blood samples collected at slaughter. 6,995 samples out of the total of 366,946 tested were forwarded to ADPRD or ADED for comparative testing. 5,415 or 77.4 percent agreed completely; 713 or 10.2 percent agreed within one degree of hemolysis; and 867 or 12.4 percent disagreed by more than one degree. Widest disagreement occurred at the time early in the program when the Hawaiian laboratory was not using fresh guinea pig complement as recommended and when several other elements of the test technique were out of harmony with the procedure at Beltsville. When corrections were made, comparisons improved. Even so, we have not been able to improve on the overall average during any annual or fiscal period due to the fact that we are forced by circumstance to test the sera shortly after phenolization. When the samples in disagreement are retested in the Hawaiian laboratory after the reports have returned from Beltsville, a much better comparison is made as illustrated in Chart No. 2.

Chart No. 2

FEDERAL-STATE COMPARATIVE TESTING COMPLEMENT-FIXATION TEST FOR ANAPLASMOSIS

<table>
<thead>
<tr>
<th>Period</th>
<th>No. Tested</th>
<th>Agree</th>
<th>Percent</th>
<th>1+ Difference</th>
<th>Percent</th>
<th>More than 1+ Difference</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 Years rerun</td>
<td>6,995</td>
<td>5,415</td>
<td>77.4</td>
<td>713</td>
<td>10.2</td>
<td>867</td>
<td>12.4</td>
</tr>
<tr>
<td>55-56</td>
<td>4,256</td>
<td>3,593</td>
<td>84.4</td>
<td>179</td>
<td>4.2</td>
<td>484</td>
<td>11.4</td>
</tr>
<tr>
<td>57-58</td>
<td>366</td>
<td>244</td>
<td>66.7</td>
<td>54</td>
<td>14.7</td>
<td>68</td>
<td>18.6</td>
</tr>
<tr>
<td>59-60</td>
<td>586</td>
<td>389</td>
<td>66.4</td>
<td>112</td>
<td>19.1</td>
<td>85</td>
<td>14.5</td>
</tr>
<tr>
<td>60-61</td>
<td>701</td>
<td>473</td>
<td>67.5</td>
<td>132</td>
<td>18.8</td>
<td>96</td>
<td>13.7</td>
</tr>
<tr>
<td>61-62</td>
<td>295</td>
<td>201</td>
<td>68.1</td>
<td>59</td>
<td>20.0</td>
<td>35</td>
<td>11.9</td>
</tr>
</tbody>
</table>

Result When Samples in Disagreement Retested:

<table>
<thead>
<tr>
<th>7 Years</th>
<th>6,995</th>
<th>5,573</th>
<th>79.7</th>
<th>775</th>
<th>11.1</th>
<th>647</th>
<th>9.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>55-56</td>
<td>4,256</td>
<td>3,643</td>
<td>85.6</td>
<td>215</td>
<td>5.0</td>
<td>398</td>
<td>9.4</td>
</tr>
<tr>
<td>57-78</td>
<td>366</td>
<td>268</td>
<td>73.2</td>
<td>50</td>
<td>13.7</td>
<td>48</td>
<td>13.1</td>
</tr>
<tr>
<td>59-60</td>
<td>586</td>
<td>412</td>
<td>70.3</td>
<td>106</td>
<td>18.1</td>
<td>68</td>
<td>11.6</td>
</tr>
<tr>
<td>59-60</td>
<td>791</td>
<td>547</td>
<td>69.1</td>
<td>195</td>
<td>24.7</td>
<td>49</td>
<td>6.2</td>
</tr>
<tr>
<td>60-61</td>
<td>701</td>
<td>499</td>
<td>71.2</td>
<td>143</td>
<td>20.4</td>
<td>59</td>
<td>8.4</td>
</tr>
<tr>
<td>61-62</td>
<td>295</td>
<td>204</td>
<td>69.1</td>
<td>66</td>
<td>22.4</td>
<td>25</td>
<td>8.5</td>
</tr>
</tbody>
</table>

There is one other set of statistics that I think might interest you. In our laboratory we estimate that it takes the full time of one technician to turn out 360 CF tests per day. In the field, if the herds are of sufficient size and facilities are properly set up, one veterinarian can collect more blood samples per day than the technician can test. Early in the program we encouraged our field staff to greater effort with free lunches or dinners for the one who set a new record. More recently we resorted to a perpetual trophy (slide). The present record holder tested 597 head in a single day. Hawaii will be very happy to make this trophy available to the United States Livestock Sanitary Association for presentation at the annual meetings to any veterinarian who sets a new record.
REPORT OF THE COMMITTEE ON ANAPLASMOSIS

M. N. Riemenschneider, Oklahoma City, Oklahoma, Chairman; W. E. Brock, Stillwater, Oklahoma; T. E. Franklin, College Station, Texas; R. G. Garrett, Austin, Texas; K. L. Kuttler, Reno, Nevada; O. H. Muth, Corvallis, Oregon; W. T. Oglesby, Baton Rouge, Louisiana; T. O. Roby, Clarksville, Maryland; W. L. Sippel, Kissimmee, Florida; F. B. Wheeler, Baton Rouge, Louisiana; E. H. Willers, Honolulu, Hawaii

Anaplasmosis continues to be a major problem to the Livestock Industry even though the disease does affect only the bovine animal. The Committee would like to call attention to the results of the survey that was conducted this past year by one of its members, Dr. W. T. Oglesby. Dr. Oglesby presented this report to the Fourth National Conference on Anaplasmosis at the University of Nevada, Reno. Quote, "Eighteen (18) states indicate that anaplasmosis is of major consequences to them. States where the disease was not reported to be a major problem, but where breaks occur now and then, almost without exception stated that the disease had been imported to them, usually with feeder animals, resulting in cases breaking in the feed lots.

"From the standpoint of cost, what did I learn?

1) Little has been done the country over in an effort to get a true picture of the incidence, though most states having the problem know it exists and they know much about its behavior.

2) Some 15 or 16 states gave enough information, in addition to indicating the disease of importance, to suggest that they are watching it closely.

3) Nine states carry the brunt of the cost of this disease. The attack rate in these states (measured by clinical disease) varies from state to state and year to year.

4) Losses in 1958 were most extensive—in area, volume, and cost—of any year reported. This is likely the year of heaviest losses ever recorded. Death losses that year were on the order of 50,000 animals and the monetary value of these animals something over $12,000,000. Taking the morbidity change at three times death loss cost (rather than two or four as referred to previously we have a figure of $36,000,000. Thus, in 1958 anaplasmosis cost our livestock producers $48 to 50,000,000.

5) Information is not nearly so complete for years before or after 1958, but estimates presented for 1959, '60 and '61 suggest losses were one-half to two-thirds of what they were in 1958.

"It would appear that we are all well in the range of reality in stating that anaplasmosis, in all its complexity, is costing the cattle owners of our country at least $34 to 35,000,000 every year."

Policies developed by the Agriculture Research Service outlining the requirements and guidelines for the use of standardized complement-fixation
antigen continues to be followed. The antigen is available without cost from the Animal Disease and Parasite Research Division of the Agriculture Research Service, United States Department of Agriculture, for research purposes. It is also available for diagnosis, control, screening procedures, and field trials under a Memorandum of Understanding between the cooperating laboratory or state and the respective division of the United States Department of Agriculture.

According to the report of the Animal Disease Eradication Division, there are presently nineteen states which have trained serologist to do Anaplasmosis serological testing. Of these, about fifteen are currently operating an active serological laboratory for Anaplasmosis diagnosis, others are getting organized to do so. Serological surveys of some degree are under way in eight states and a number of other states have indicated their interest in incidence surveys. Cooperative field trials are in progress in Texas, Mississippi, Wyoming and Louisiana, for a total of five herds under study in these states. This field trial work can be summarized as follows:

(1) A herd at Kerrville, Texas, is being studied to evaluate methods of Anaplasmosis control by management procedures and complement-fixation testing. It has been in progress for four years and being continued. The basic herd consists of infected cows (38); their calves are being tested when weaned. Negative heifer calves are kept and as of this year, they have replaced the original carrier cows. The purpose now is to see if the herd can be kept clean under the local vector conditions of that area.

(2) The Mississippi herds are privately owned and the work there is done jointly by the Entomology Research Division and the Agriculture Research Service and the Delta Branch Experimental Station and the ADP. The herd owners have provided without charge their animals for the experimental studies. For two years the study was done in each herd to evaluate the effect of daily spraying of Pyrethrins for vector control and to relate vector control to the extent of Anaplasmosis transmission. This procedure was found to be effective in reducing the transmission of Anaplasmosis in the infected herds. Both herds are now being studied for the ability of tetracycline antibiotics to eliminate the infected carrier cases of Anaplasmosis within each herd by daily feeding of this drug. These studies are still in progress and have not been completed at this time.

(3) The studies in Louisiana are in a dairy herd at Jeanerette. This work is cooperative with the Louisiana University and the dairy breeding research people of the Agriculture Research Service. No control efforts are being made and the work directed toward determining the economic effect of Anaplasmosis in a dairy herd.

(4) In Southwestern Wyoming, a part of a privately owned herd is being studied in an attempt to evaluate the capabilities of the Rocky Mountain wood tick as a vector of Anaplasmosis. The work has been in progress for four years. The results to date indicate a very low rate of transmission even though the Rocky Mountain tick occurs on the cattle each year. Deer from the ranch have been tested for the disease by calf inoculation, and thus far no infected deer have been found. The work has been done cooperatively by the Wyoming Veterinary Laboratory, the Entomology
Research Division of the Agriculture Research Service, ADP and ADED, the owner and the Wyoming Game Commission. This project looks like it may be terminated after this year because of a number of complicating circumstances.

Texas A & M College reports that they have tested only one herd this year of the seven segregated herds reported in 1961. They have received no adverse reports from any of the herd owners, apparently testing and segregating is continuing to work satisfactorily in their herds. Although the effect of dry weather this past summer has no doubt helped the program.

Dr. E. H. Willers, the State Veterinarian of Hawaii has submitted a report of that Program as follows:

"During fiscal year 1960-61, 8,801 complement-fixation tests for Anaplasmosis were conducted on dairy cattle in Hawaii. Only one animal, recently imported, was classed as a reactor. This animal had been negative on the import quarantine test but gave a 4+ reaction on the 60-day retest. The serological diagnosis was confirmed by calf trial. Out of the 5,463 tests that were conducted on beef cattle, none were classed as reactors. Four of the beef animals gave positive reactions on initial test but were subsequently proved by retesting or calf trial to be negative. Three cattle out of 2,057 imported during the year were classed as reactors on entry test and three others showing suspicious reactions were being held in quarantine as the year ended. Eight calf trials were completed during the year. Four of these resulted in positive diagnoses. These four reactors were the imports referred to above and were detected either on the entry quarantine test or on the 60-day retest."

The Committee circularized the fifty states and Puerto Rico in regard to the amount of money that is being spent in the various phases of Anaplasmosis testing, and research. Certainly this should be of interest in view of the financial losses as reported by Dr. W. T. Oglesby. The questionnaires were sent only to the Livestock Sanitary Official of the states. Twenty-five states reported. The Committee feels that this is not an exact dollar and cents figure, but it is an indication as to the amount of effort that is being put forth on the disease.

1. State money spent by the, or under the, direction of the Livestock Sanitary Official. This includes Laboratory for testing and field work .................. $ 39,573.00
2. State money spent thru the office of Livestock Sanitary Official for experimental work .................. 52,000.00
3. State money other than the two above—such as field investigations, drawing blood samples, drugs and travel.................................. 9,700.00
4. State funds utilized by Colleges and Universities for Anaplasmosis Laboratories .................. 3,500.00
5. State funds utilized by Colleges and Universities in experimental work on Anaplasmosis .................. 81,181.00
6. Federal funds being spent in cooperation with Universities or Colleges .................. 37,000.00
7. Other Federal money spent in cooperation with  
   Chief Livestock Sanitary Official Office ............ 20,181.00
8. Amount of money utilized from grants by Univer-
   sities ........................................ 40,500.00

GRAND TOTAL .................................. $283,635.00

Control procedures, including testing ... $124,954.00
Research ................................. $158,681.00

The Committee wishes to cite that there is continued interest by re-
search workers to further elucidate the nature of the Anaplasmosis agent. A
number of studies are being made as to causative agent. These were
brought out in the conference held at the University of Nevada, as well as
many other scientific and practical points pertaining to the disease
indicating that there is a steady interest and progress toward more
knowledge of Anaplasmosis. This work has brought us closer to our goal
of complete knowledge concerning the disease, the causative agent and the
control by testing, management and immunization. Progress has been
made in our knowledge of the vectors and their control and as to treatment
of the disease. Progress is very evident in the interest exhibited by cat-
tle owners in the disease and they are becoming acutely aware of the in-
roads on the economy of the industry. It is becoming more and more evi-
dent that we must assume leadership to prepare ourselves to assist the
cattle industry and in so doing the Committee wishes to submit the follow-
ing suggested "Uniform Procedures for the Control of Anaplasmosis."

STANDARD PROCEDURES FOR ANAPLASMOSIS CONTROL

Preface: The Anaplasmosis Committee of the United States Live-
stock Sanitary Association presents these recommendations, knowing that
control procedures in some parts of the United States are not feasible with
our present knowledge of the disease. The Committee does recognize that
there are areas in the United States where practical control measures
can be applied. Further, the Committee recognized that some standards
as to testing procedures, methods of identifying reactors, controlling the
movement of reactors and others should be established. With this knowl-
edge the Committee does make these recommendations.

1. The official test is the Complement-fixation test as recommended
   and approved by the United States Department of Agriculture.

2. The test shall be interpreted and reported (In 1 to 5 serum dilution)

   4+ No homolysis to 25 percent hemolysis = P
   3+ 26 percent hemolysis to 50 percent hemolysis
   2+ 51 percent hemolysis to 75 percent hemolysis
   1+ 76 percent hemolysis to trace hemolysis
   Trace to complete hemolysis = N
   Anti-complementary = AC
3. Age of animal to be tested—animals over three (3) months of age to be tested.

4. All animals tested shall be identified by an official ear tag or a registry tattoo for purebreds.

5. REACTIONS
   a. Reactors to be identified by a green tag using the uniform system of numbers with words Anaplasmosis Reactor on reverse side and an ear punch hole not less than one-half (1/2) inch in size to be placed in center of left ear, or if desired a hot iron brand "A", not less than three (3) inches in height placed on the left jaw.
   b. In case of treatment, the ear punch or brand to be omitted until termination.

6. TREATMENT—For the purpose of destroying the carrier state of Anaplasmosis the treatment shall consist of:
   a. The administration of five (5) milligrams per pound of body weight of one of the tetracycline antibiotics parenterally each day for ten (10) consecutive days.
   b. The administration of three (3) to five (5) milligrams per pound of body weight of one of the tetracycline antibiotics orally each day for sixty (60) days.

    Animals so treated to be tested not less than 120 days after the last treatment. Animals negative to the official test to be considered negative animals, animals so classified to have reactor tags removed.

7. Reactor animals quarantined and to be moved only on permit from the Chief Livestock Sanitary Official, as follows:
   a. Retained on premises where found
   b. Shipped direct to slaughter
   c. Moved to a known infected herd and retained under quarantine.

8. That all test material be under the control of the United States Department of Agriculture and the Chief Livestock Sanitary Official and only official testing to be done.

9. All biological material capable of increasing an animal's immunity to the disease be under the direct control of the United States Department of Agriculture and the Chief Livestock Sanitary Official.

10. The personnel collecting blood samples for Anaplasmosis testing receive the following instructions:

DIRECTIONS FOR COLLECTING AND SUBMITTING BOVINE SERUM SAMPLES FOR COMPLEMENT-FIXATION TESTING

PRECAUTIONS

Before attempting to collect blood serum samples proper provision should be made to safeguard against carrying any possible infection from one animal to another. Clean and dry instruments should be provided for each
animal. Bleeding needles must be clean and dry, nose leads, and other instruments used in obtaining blood from one animal must be cleaned and disinfected before being used on another animal. The hands of the operator must be kept clean and free of blood or other material at all times.

COLLECTING SERUM

As the serum is the constituent of the blood which is utilized in performing the complement-fixation test, it is extremely important that good specimens be forwarded to the laboratory for diagnosis. A good specimen of serum is essential to a conclusive and reliable test. To obtain a good clean specimen of serum the following procedure is recommended:

Draw a (10 mls.) full vial of blood (using a clean dry needle) from the jugular vein into a dry, clean Brucellosis test vial. Carefully set to one side and allow it to stand for at least thirty (30) minutes, or until complete coagulation has taken place. It is important to see that the blood is not disturbed until complete coagulation has occurred as the serum will not separate as readily if agitated before coagulation takes place.

Allow the clotted blood to stand until the clear yellow serum separates from the clot. Blood samples should not be allowed to stand in direct sunlight, especially in hot weather, as this results in hemolysis. Samples should not be allowed to chill after first drawn, as a lessened yield of serum will result. It is suggested that they be placed in a warm location after being drawn when the weather is cold.

When sufficient serum has separated from the clot, it should be poured off into a clean, dry vial, being very careful not to allow any red cells to mix with the serum. If after two (2) hours the clot has failed to contract sufficiently to express the necessary amount of serum, carefully loosen the clotted blood from the sides of the bottle or tube by means of a long wire or swab stick. The sample is then permitted to stand until the clot has completely contracted, leaving the clear yellow serum above. This is then poured off.

It is absolutely essential that good clear serum samples, that are as free of bacteria contamination as possible, be submitted for testing. Hemolysis and bacteria contaminations are among the more common things that occur in the serum sample to render it impossible to conduct the complement-fixation test. The veterinarian should take every precaution to obtain a sample in good condition, and free of any anti-complementary factor. The laboratory will endeavor to test all acceptable samples but cannot be expected to test samples that are hemolyzed or be responsible for samples containing anti-complementary factors. Please save yourself embarrassment and your client trouble by submitting good serum samples. The testing for Anaplasmosis to be most satisfactory should be done during the nonvector season. (It is thought that the horsefly is probably the most common vector for the condition in this state.) This doesn't preclude other
Committee on Anaplasmosis

Biting insects, ticks and accidental mechanical transmission. We realize that the test fails to react on serum from animals in the incubative stage of the disease, therefore, testing conducted during the vector season or during an acute break of Anaplasmosis certainly will not be of the same value as one conducted during the nonvector season.

General

A minimum of five mls. of clear serum should be collected for testing. All necessary forms to be filled out in their entirety and submitted to the official Laboratory with serum samples.

All animals to be identified by official ear tag or registry tattoo.

Reactors to be identified as directed by Uniform Procedures.

Reactor animals to be quarantined and moved as directed by Uniform Procedures.

11. In order to avoid confusion as to what is required in a control procedure, an agreement with the owner participating and veterinarian doing the bleeding be provided. These procedures for control are feasible in some geographical areas of the United States and not in others, but are the only known methods of control today.

Agreement for the Control of Bovine Anaplasmosis

This Agreement is between ___________________________ of ___________________________ County and the Chief Livestock Sanitary Official of ___________________________. The herd owner is interested in controlling Anaplasmosis in his herd and agrees that his cattle will be tested and managed under a system designed to isolate or eliminate infected cattle with the view of eventually eradicating the disease from the herd. It is recognized that insects (particularly, the horse fly) and the tick play a major role in the natural spread of this disease. The control of insects and ticks is recommended.

The following points are mutually agreed upon:

1. The owner agrees to provide for the collection of the blood serum samples from all the cattle in his herd over three (3) months of age, unless otherwise mutually agreed and the frequency of retests of the herd may be determined by the problems encountered. A second test to be conducted, approximately one (1) year after the first test, unless otherwise mutually agreed.

The testing should be done during the nonvector season. Testing done during the vector season is of limited value because the test fails to identify cattle in the incubation period.
2. An official Laboratory will test, or have tested, all testable blood samples for Anaplasmosis.

s. The owner agrees to have all reactor animals to the complement-fixation test for Anaplasmosis identified by an ear punch hole not less than one-half (1/2) inch in size placed in the center of the left ear and an Anaplasmosis reactor tag placed in the left ear. Such tagging and punching to be done within fifteen (15) days of receipt of blood test results. (Note exemption to ear punch if option to treat animals is employed).

4. The owner agrees to furnish the services necessary for restraining cattle for the purpose of collecting blood samples or other examinations.

5. Reactor cattle are quarantined to owners premises. This is necessary under the laws and policies.

Reactor cattle shall be moved from premises on a permit from the Chief Livestock Sanitary Official and then only for immediate slaughter, or to another known infected herd then on permit and quarantined at destination.

6. The owner agrees that none of the cattle covered by this agreement will be vaccinated with any type of live Anaplasmosis organism or vaccine for the duration of this agreement, unless mutually agreed.

It is mutually agreed that good management practices as outlined will minimize the accidental transmission of Anaplasmosis within the herd and shall be practiced.

The Veterinarian collecting blood samples for this program and or personnel performing other services in the herd shall follow practices necessary to prevent the accidental transmission of Anaplasmosis within the herd and from this herd to other cattle as follows:

1. Blood samples from each animal will be collected with a separate (clean, dry) needle.
2. Nose leads shall be disinfected after use on each animal.
3. Animal vaccinations and parenteral injections made in animals in the herd for any and all purposes shall be carried out by using separate clean, dry needles on each animal.
4. Tattooing, ear notching and any similar practice, shall be done with instruments which have been thoroughly rinsed, cleaned, and disinfected after use on each animal.
5. Castrating, spaying, and other surgical procedures shall be done with instruments which have been rinsed clean and disinfected with antiseptic solution, or sterilized by other means, after use on each animal.
6. Dehorning techniques shall be followed which prevent transfer of blood from one animal to another.
7. The operator's hands shall be washed after handling each animal where possibilities exist for contamination with blood from infected animals.
The owner has selected to follow the plan indicated below:

1. THE OWNER AGREES that all cattle demonstrated to be reactors to the complement-fixation test for Anaplasmosis shall be maintained in a separate group from animals which are found to be negative to the test. The segregated infected herd shall be kept on the owner’s premises in a location removed from the portions of the herd which are negative to the test.

2. Combining the negative herd and the infected herd during the nonvector season. The mixing or running together of the infected herd and the negative herd during the nonvector season. During the balance of the year the animals to be separated as a negative herd and a reactor herd. This separation should be a sufficient distance apart or the circumstances such as to minimize and possibility of transferring the disease, particularly, by the horsefly and other vectors from the infected herd to the negative herd.

Treatment of infected (reactor) animals. The treatment to be used shall consist of one of the following as they are the only treatments known at this time that offers promise of clearing infected animals.

1. The administration of five (5) milligrams (mgs) per pound of body weight of one of the group of tetracycline antibiotics parenterally each day for ten (10) consecutive days.

2. The administration of three (3) to five (5) milligrams (mgs) per pound of body weight of one of the group of tetracycline antibiotics orally each day for sixty (60) days.

The animal to be tested not less than 120 days after the last treatment. If the animal is negative, it may be added to the negative herd as a negative animal. Under this agreement animals to be treated would not be ear punched. After passing the negative test as above the reactor tag to be removed.

Identifying reactors to the Anaplasmosis test. Reactors to the test may be identified by a hot iron brand "A", not less than three (3) inches in height on the left jaw and an Anaplasmosis reactor tag placed in the left ear.

THE FOLLOWING IS PART OF THE ANY PLAN SELECTED

Purchased animals or additions to the negative herd shall be made only after such animals have passed two (2) consecutive negative tests at not less than sixty (60) days apart. This procedure shall also apply to the introduction of offsprings from the infected herd. During the period between the required negative tests these animals shall be maintained in a separate location from either the reactor or negative herds.

Negative animals which might leave the owners premises for show, breeding, or any other purpose, shall be treated as purchased animals on return to the herd.
In the event of any animal having a suspicious reaction to the complement-fixation test for Anaplasmosis, such animal must be maintained in a separate location from the negative herd until additional testing clarifies their status. If, on retests, the suspicious animal is found to be a reactor, it shall be placed permanently in the reactor herd. If, however, the suspicious animal has two consecutive negative retests at a period of not less than sixty (60) days between each test, it may be returned to the negative herd.

The blood samples will be collected under the supervision of a veterinarian and the methods used are known to be safe, and the animals will be under the care and maintenance of the owner. The owner agrees to abide by the terms herein set forth at his own expense and without recourse to the Official Agencies.

THIS AGREEMENT shall become effective on ____________, 19____, but may be modified within limitations by mutual agreement, by the parties hereto and shall apply for a period of one (1) year or until the second test of herd is completed. Modification proposals

________________________________________

________________________________________

________________________________________

________________________________________

Modifications must be accepted in writing by the Chief Livestock Sanitary Official.

OWNER OR AGENT

BY

ADDRESS

TITLE

As attending Veterinarian, I agree:

(1) That the owner is responsible for fees in connection with the bleeding of the herd and for the tagging, ear punching and quarantining of reactors. Further, that quarantine releases to move reactor animals will be issued by the Chief Livestock Sanitary
Official. Any variation of fees in Area Brucellosis Counties will be noted in writing on this agreement and any and all modifications must be accepted in writing by the official agencies.

(2) That all animals over three (3) months of age will be bled.

(3) That the bleeding and other procedures will be performed with clean instruments in such a manner as to prevent the spread of the disease from one animal to another.

(4) To identify all reactors to the complement-fixation test with an Anaplasmosis reactor ear tag in the LEFT ear, and to ear punch a hole not less than one-half (1/2) inch in size in the middle of the LEFT ear unless treatment option is selected. These identification procedures to be done within fifteen (15) days on receipt of test results.

Chief Livestock Sanitary Official

Signature of Veterinarian

Address

Date

12. In order to avoid confusion as to the requirements for a diagnostic test only, it is suggested that the owner sign the following agreement. This is to answer the problem of diagnostic verification and for possible interstate movement requirements.

AGREEMENT FOR DIAGNOSTIC TESTING FOR BOVINE ANAPLASMOSIS

I, ____________________________________________, of ______________________________ County request to have a complement-fixation test for Anaplasmosis conducted for diagnostic purposes on one or more cattle from my herd.

I AGREE to have all reactor animals to the test identified by an ear punch hole not less than one-half (1/2) inch in size placed in the center of the LEFT ear and an Anaplasmosis reactor tag placed in the LEFT ear. Such ear punch not required if official treatment is done. Such tagging and punching to be done within fifteen (15) days of receipt of blood test results.
I UNDERSTAND that reactors to the Anaplasmosis test are quarantined to my premises and I AGREE that they will be moved only on permit from the Chief Livestock Sanitary Official and ONLY for immediate slaughter or under quarantine to known infected herd.

Owners Signature


Address


Date


Chief Livestock Sanitary Official

The Committee in no way wishes to suggest any particular control program; they are only desirous of suggesting what can be done with the present knowledge. They are interested in this Association assuming leadership in establishing these pertinent procedures that there may be a continuity of procedure as we progress with this disease and its control.

With these facts in mind the Committee wishes to make the following recommendations:

1. That adequate funds be provided to carry out the following research:
   (A) The study of known vectors and possible unknown vectors as to their life cycles and the use of insecticides. That this work be expanded by close cooperation between the various Animal Disease Workers and Entomologists.
   (B) Intensified research be continued and expanded to determine the exact nature of the causative agent. It is obvious that without this information production of any immunizing agent, the understanding of the pathology and the successful treatment and the control of the disease are difficult, if not impossible.
   (C) The Anaplasmosis problem varies in terms of incidence of transmission and reservoir in the different geographical areas of the country. Therefore, the Committee believes there is an urgent need for continued and broadened experimental studies in the various geographical areas, and recommends that such steps be taken that by expanding the efforts we may extend our knowledge of the vectors and reservoirs of infection. This information would provide for the development of feasible control and eradication procedures.
(D) That research be continued to improve the complement-fixation test and broaden its use and availability to the various states.

(E) That further investigation of a simplified test for Anaplasmosis be done.

(F) That continued and expanded research be conducted to develop an immunizing agent.

(G) That efforts be made to develop an effective inexpensive treatment.

2. That the present procedures for licensing anaplasmosis immunizing and diagnostic products be reviewed by the appropriate committees of the United States Livestock Sanitary Association with the view toward strengthening the regulations to reduce the possibility of such products being released prematurely.

3. That all testing for anaplasmosis be done officially by restricting all diagnostic products and testing to official laboratories.

4. That all immunizing agents against anaplasmosis be used officially under the authority of the Chief Livestock Sanitary Official.

5. That the Agriculture Research Service, United States Department of Agriculture continue to make available sufficient Anaplasmosis antigen to supply the cooperating laboratories, research and other experimental work.

6. That the Agriculture Research Service, United States Department of Agriculture continue and expand their training program for serologists in order that adequate trained personnel be available to all possible cooperating laboratories.

7. The Committee urges the adoption of the uniform procedures as recommended and that the states adopt these procedures where applicable to their area and their activities relating to Anaplasmosis control.

The Anaplasmosis Committee urges that every possible effort be made to implement the recommendations in order that we may reduce the economic loss to the cattle industry brought about by this very devastating and complex disease.
In our report to this Association last year, a realistic timetable was presented for completion of the cooperative State-Federal brucellosis eradication program. This called for the entire Nation to be Modified Certified by 1965, and Brucellosis-Free by 1975. Unfortunately, the establishment of these goals did not have the stimulating effect anticipated in some of the States where the certification effort was lagging. It seems advisable, therefore, to take a good hard look at the direction in which the program is going.

There is no question but that nationwide Modified Certification can be achieved by the end of fiscal year 1965 if the current level of financial support is maintained, and available tools and procedures are energetically applied in all parts of the country. Figure 1 projects the progress made during the past year and provides a reasonably clear picture of what the

*Chief Staff Officer, Brucellosis Eradication, Agricultural Research Service, United States Department of Agriculture.
national status will be in 1965. This indicates that 352 counties will fail to attain certification by June 30, 1965, unless the present trend is altered. The downward curve reflected in the graph can be reversed only through renewed efforts in those areas still to be certified. This would be possible by intensifying the certification program in 11 States. Figure 2 identifies these areas in which progress has been delayed.

While it is recognized that some of these states have special problems, they are no greater than those faced in the past by many other states which were among the leaders in achieving Modified Certified status. Moreover, certification procedures have been improved and broadened over the years, until there is a practical method available to deal with brucellosis under a wide range of conditions. For example, a careful study of the remaining problem areas indicates that early adoption of the market cattle testing program on a broad scale would insure attainment of the 1965 certification goal.

The brucellosis eradication program in the United States is based primarily upon the American tradition of "freedom of choice." Under this system it is expected that education and enlightenment, rather than force and coercion, will pave the way to success. The fact that all areas have not requested a complete eradication program is evidence of a deficiency in educational efforts—failure to reach the cattle owners with the facts; and failure to act affirmatively at every opportunity. The Scandinavian countries already have eradicated brucellosis, and now have turned their attention to other pressing problems of animal disease research and eradication.
TABLE I

Cooperative Brucellosis Data

<table>
<thead>
<tr>
<th>Activities</th>
<th>Fiscal Year</th>
<th>Percent Change for 1962</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1961</td>
<td>1962</td>
</tr>
<tr>
<td>Blood Tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herds-Lots</td>
<td>1,332,651</td>
<td>1,551,680</td>
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<tr>
<td>Reactor Herds-Lots</td>
<td>58,068</td>
<td>59,571</td>
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<tr>
<td>Percent</td>
<td>4.4</td>
<td>3.8</td>
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<tr>
<td>Cattle Tested</td>
<td>13,418,657</td>
<td>11,966,324</td>
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<tr>
<td>Reactor Cattle</td>
<td>139,894</td>
<td>126,839</td>
</tr>
<tr>
<td>Percent</td>
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<td>1.06</td>
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<tr>
<td>Ring Tests</td>
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<tr>
<td>Herd Tests</td>
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<tr>
<td>Suspicious Herd Tests</td>
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<tr>
<td>Percent</td>
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<td>1.6</td>
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<tr>
<td>Vaccinations (Calfhood)</td>
<td>6,721,296</td>
<td>6,740,344</td>
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<tr>
<td>Certification of Counties</td>
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</tr>
<tr>
<td>Modified Certified</td>
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<tr>
<td>New and Reinstated</td>
<td>216</td>
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<tr>
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<td>56</td>
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<tr>
<td>Total</td>
<td>62</td>
<td>113</td>
</tr>
<tr>
<td>Total Certified Counties</td>
<td>2,216</td>
<td>2,406</td>
</tr>
</tbody>
</table>

( )% difference

PROGRAM ACTIVITIES

A comparison of program activities in fiscal years 1961 and 1962 is given in Table I.

Blood Testing: The most significant change occurring in fiscal year 1962 was the 16.4 percent increase in the number of lots blood tested. This increase is mainly the result of expanded market cattle testing. The decrease in the number of cattle blood tested reflects a change in program procedures from "down the road" area testing, to greater dependence on screening devices, such as brucellosis ring testing and market cattle testing.

Ring Testing: The total number of ring tests decreased in 1962, in spite of the fact that many states had increased the frequency of such tests. This reflects the continuing decrease in the number of commercial dairy herds in the United States. The percent suspicious ring tests decreased at a gratifying rate, as did the total suspicious herd tests. This trend underlines the effectiveness of ring testing in identifying herds most likely to contain Brucella infected animals.
**Calf Vaccination:** The 6,740,344 official calf vaccinations reported in 1962 establish a new record for this phase of the brucellosis program. However, this figure represents only about a 55 percent coverage of the eligible calves, and should be increased still further. High level vaccination will continue to play an important role in the brucellosis program until eradication is assured.

**BRUCELLOSIS ERADICATION PROGRAM**

**Figure 3**

**AREA CERTIFICATIONS**

The present status of the Modified Certified Area program is shown in Figure 3 and Figure 4. As the number of certified counties increased over the years, the problem of recertification has become progressively difficult. For example, in 1962 it was necessary to recertify 642 counties. This aspect of the program is requiring more and more attention in most States. In this regard, it is of particular concern that 48 counties lost their certified status in 1962. This is 20 percent of the total counties newly established and reinstated during the same period. There are no provisions within the Uniform Methods and Rules to continue the certified status of delinquent counties beyond the expiration date. It is essential therefore that all States keep abreast of their recertification schedules in order that required work will be completed in all counties prior to the date certifications expire. It should be pointed out that cattle moving interstate
Figure 4

from delinquent areas no longer enjoy the privileges associated with currently qualified certified areas.

There was a net gain during 1962 of only 190 Modified Certified counties. If this trend toward fewer counties being certified each year continues, program goals will be delayed. For this trend to be altered, it will be necessary for all interested groups to support fully an expanded effort to bring all remaining counties into the area certification program.

CERTIFIED-FREE AREAS

The Certified Brucellosis-Free Area program advanced still further during 1962. Fifty-six new counties were qualified during the year, making a total of 118 counties in 14 States that have achieved this goal. There is every indication that favorable progress along these lines will continue and accelerate in the future. The results already attained in establishing and maintaining Certified-Free Areas demonstrates conclusively the feasibility of eventually freeing all areas of brucellosis, provided adequate screening procedures are applied on a continuing basis. In this regard, the need for market cattle testing as well as ring testing in all areas is becoming increasingly evident.
Following its incorporation into the cooperative brucellosis eradication program in February 1959, the market cattle testing program has advanced more slowly than anticipated. Almost three and one-half years later, as of June 30, 1962, only 17 States were employing this procedure for certification purposes to any appreciable extent. These States are shown in Figure 5. If this trend continues, it will be at least 10 years before market cattle testing will be utilized fully by all States. A delay of this magnitude should be avoided at all costs. Unless this program is universally adopted on or before 1965, it is doubtful that the 1975 goal for final eradication can be realized. It is strongly urged, therefore, that all States consider the advantages of market cattle testing and support its expanded use to the fullest extent possible.

During 1962 there were 1.8 million tests conducted under the market cattle testing program. This is an increase of about 800,000 over the number reported for fiscal year 1961. In general, there has been a significant improvement in tracing market cattle reactors to herds of origin. During the past year about 94 percent of all reactors disclosed under the program were successfully traced. This compares with approximately 87 percent successful tracebacks of the same class of animals in 1961.

As a result of market cattle testing in 1962, 17,647 infected animals were disclosed at markets and in herds of origin. This represents 13.9 percent of all reactors reported in the United States during the same year.
The market cattle testing program also accounted for 16.2 percent of all blood tests conducted in 1962. Thus, the testing of market cattle compares favorably with other procedures utilized in the eradication program and when fully adopted will prove to be one of the most effective tools available for disclosing the last infected herd.

A new motion picture entitled "Market Cattle Testing for Brucellosis" has just been completed and will have its initial showing at this meeting. As the title indicates, the theme of this picture is the utilization of market cattle testing for initial certification and recertification of areas. I should like to take this opportunity to express my sincere appreciation to three prominent cattlemen, namely, Bob Laramore of Wyoming, Bob Johnston of Colorado, and Oda Mason of Wyoming, for their fine cooperation in making this movie possible.

BRUCELLOSIS SURVEY IN RANGE AND SEMI-RANGE COUNTIES

For the past 18 months a new survey has been conducted to assess again the brucellosis situation in each infected herd disclosed in range and semi-range areas. Data for this study were submitted from 89 counties in 14 States at the time recertifications were requested. The States involved are:

- Arizona
- Arkansas
- Colorado
- Florida
- Idaho
- Montana
- Nevada
- New Mexico
- North Dakota
- Oregon
- South Dakota
- Utah
- Washington
- Wyoming

The purpose of the survey is to determine the efficiency of Strain 19 vaccine in limiting the spread of brucellosis within infected herds and throughout certified range and semi-range counties.

The animal population of the 89 counties is 2,463,024 cattle in 60,924 herds. During the three-year certification period, 25,843 blood and ring herd tests, representing 607,683 cattle, were conducted. This testing involved 42.4 percent of the herds and 24.7 percent of the cattle. Brucellosis was identified in 2.6 percent of the herds, and 0.45 percent of the cattle tested were reactors.

Of the 55,200 exposed cattle in the infected herds, 16,406—or 29.7 percent, had been vaccinated. Of these, 242—or 1.48 percent, were infected. Of the 38,794 nonvaccinated animals among the known exposed population, 2,521—or 6.5 percent were infected. On this basis, the apparent protection afforded by vaccination in the infected herds was 77.2 percent. When broken down into beef and dairy breeds, the protection from vaccination in known infected herds is 84.6 percent and 70.7 percent respectively. This significant difference can be explained, in part at least, by variations in the concentration of populations in these herds and differences in herd management practices.

In the case of 275 infected beef herds which contained both vaccinated and nonvaccinated cattle, only 0.9 percent of the 12,449 vaccinates were infected, while 5.3 percent of the 26,663 nonvaccinated animals in these
same herds were reactors. Brucellosis was found also in seven beef herds in which all animals had been vaccinated. Nine infected animals were disclosed out of the 800 cattle contained in these herds.

These limited data reflect the serviceable protection afforded by Strain 19 vaccination in both beef and dairy herds. They also indicate the relationship between vaccinal protection and the degree of exposure to which vaccinates are subjected. The concentration of animals in most infected dairy herds provides an environment conducive to severe exposure and consequent breaks in vaccinal resistance.

THE PROBLEM HERD PROGRAM

Brucellosis epidemiologists are being trained and assigned to new areas as the Problem Herd Program expands to additional Certified States. More than one-half of the Certified States now have a comprehensive Problem Herd Program in operation, and others are planning early participation.

Twelve Certified States participated recently in a survey of activities carried out under the brucellosis problem herd program. These states reported that 81 percent of the brucellosis outbreaks respond to usual program activities. Half of the remainder are readily freed of the disease by correcting obvious failures to observe sound principles of disease control and eradication. Only 10 percent of the infected herds are considered true problem herds.
Although the number of problem herds is relatively small in relation to the total herds in the Certified States, they become increasingly important as eradication is approached. This is especially true in the Certified Brucellosis-Free Areas where prompt action is essential to eliminate quickly any outbreak of infection that occurs. It is for this reason that brucellosis epidemiologists are being trained and assigned to new areas as the problem herd program expands into additional Certified States. The battery of supplemental tests being used in the States conducting active problem herd programs has proven highly effective in freeing these herds of infection. On the basis of information developed in these states, procedures are available to eradicate brucellosis even under the most difficult situations. However, none of the supplemental tests can be accepted alone as the basis for final judgment in problem herd work. Moreover, it is essential that these tests be applied and interpreted by trained epidemiologists in order to insure accurate decisions. In this connection it is interesting to note that 86.6 percent of all condemnations in problem herds are being accounted for by blood serum tube and plate agglutination tests. The simultaneous application of both tests has proven extremely useful in the Problem Herd Program.

The primary purpose of supplemental testing in problem herds is to identify obscure reservoirs of infection that have perpetuated the disease. It is to be expected, therefore, that in the initial stages of problem herd investigations there may be a significant increase in the number of animals found to be infected. When these hidden sources of infection are identified and removed, the problem quickly ceases to exist.

Within the past few months, a new color film entitled, "Exposed! Brucellosis in Problem Herds," has been completed and is available for distribution. It depicts in some detail the epidemiological procedures employed in brucellosis problem herd investigations. We believe such a movie is timely and should be useful for encouraging early adoption of a Problem Herd Program in all Certified States.

SWINE BRUCELLOSIS ERADICATION

The swine brucellosis eradication program is off to a fine beginning. Dooly County, Georgia, has been established as the first Validated Brucellosis-Free Area in the country. Of the 376 breeding herds tested in the county, only five were found to be affected with brucellosis.

California has embarked upon a swine brucellosis eradication program designed to cover the entire State by 1965. Two counties, Del Norte and Mono, have completed areawide testing of all breeding swine and have qualified as Validated Brucellosis-Free Areas.

It is evident from the experience gained to date that swine brucellosis will, for the most part, be dealt with as a herd problem rather than a problem of the individual reactor animal. In most heavily infected herds it has been necessary to eliminate all exposed animals before the disease was eliminated. It appears that in this type of herd, the disposal of individual blood-test reactors will have limited application. Repopulation of premises can be accomplished at a minimum of cost, except where
purebred swine are involved, and entire herd replacement should be the method of choice in dealing with brucellosis in all commercial breeding herds.

It is important that the swine brucellosis eradication program receive a great deal of attention in all areas at this time. Every effort should be made to encourage swine producers to establish Validated Brucellosis-Free Herds. Plans also should be made for participation on an area basis as soon as practicable.

Certificates and metal signs for display on the premises of Validated Brucellosis-Free Herds will be available soon for use in all States.

It has been conservatively estimated that the economic losses caused by swine brucellosis each year are at least $10 million. Moreover, the eradication of this disease will contribute materially toward the final elimination of human brucellosis. Public Health Reports indicate that approximately 60 percent of the reported cases of brucellosis in humans are now traceable to swine exposures.

ANTICIPATED PROGRESS DURING FISCAL YEAR 1963

It is difficult to realistically estimate future accomplishments in the brucellosis eradication program. Unfortunately, the optimistic goals reported one year ago were not met during fiscal year 1962. Recent estimates of achievement for fiscal year 1963 indicate that there will be approximately 194 new Modified Certified Brucellosis Counties by the end of the year. This compares with a net increase of 190 in 1962. It is expected that approximately 57 counties will attain Certified Brucellosis-Free Area status during 1963, for a total of 175. If these goals are reached, there will be a combined total of 2,600 certified counties by June 30, 1963.

With a net increase of only 194 certified counties predicted for 1963, and with 747 yet to be certified as of July 1, 1962, it can be seen that the bovine brucellosis eradication program must be accelerated considerably if the goal of nationwide certification is to be attained in 1965. There is no question but that this goal can be attained if all areas are brought into the program in the near future.

COMMENTS

Excellent progress has been made over the past six (6) years in the cooperative State-Federal brucellosis eradication program. However, it is disturbing to note the current decline in certain aspects of field operations. This is reflected in the unexpectedly low number of area certifications recorded during fiscal year 1962. Should this trend continue it will be difficult, if not impossible, to attain the 1965 goal for nationwide certification.

It has been demonstrated repeatedly that the incidence of brucellosis either advances or declines—it never remains static. In other words, a so-called "holding operation" will never be practical with this disease. The obvious solution, therefore, is to completely eliminate all reservoirs
of infection as quickly as possible. Eradication in the case of bovine brucellosis can be achieved with available knowledge and tools. This is being shown clearly in the 139 Certified Brucellosis-Free counties that have been established in 13 States, Puerto Rico and the Virgin Islands.

Final eradication of brucellosis from the United States will require extensive use of existing screening devices. Between the brucellosis ring testing and the market cattle testing programs we have efficient and practical procedures for the early detection of new brucellosis outbreaks. By employing them intelligently and at a satisfactory level, the problem of brucellosis eradication can be simplified greatly. Without them, the prospects for final eradication are very dim, indeed.

Procedures for utilizing market cattle testing in the initial certification of areas were adopted by this Association at last year's meeting in Minneapolis. These were promptly approved by the Agricultural Research Service and are now a part of the official recommendations outlined in the Uniform Methods and Rules for brucellosis eradication. It is urgent that these procedures be employed as soon as possible, especially in those range and semi-range States where area certification work has been delayed. This method provides an efficient and practical means of accomplishing certification. Its value in brucellosis eradication has been fully confirmed through extensive use in some of the States for area recertification.

Strange as it may seem, the attainment of modified certification is fraught with certain hazards. This relates to the natural tendency to relax and become complacent after this stage has been reached. Modified certification actually means that bovine brucellosis has been brought under control and nothing more. With only a little more effort, properly applied, the eradication program can be advanced at this point. Here is a disease we can live without, if we so desire.

The "problem herd" program continues to provide valuable information for use in the 10 percent or less Brucella infected herds that do not respond readily to routine field operations. In fact, it now appears that we have a battery of supplemental tests that will disclose the most obscure types of infection. All Modified Certified States are urged to develop a competently supervised brucellosis "problem herd" program as soon as possible. The Animal Disease Eradication Division stands ready to cooperate fully with all states along these lines.

The States of Georgia and California are to be complimented on their interest in eradicating swine brucellosis. It is anticipated that Dooly County, Georgia, will be only the first of many other areas throughout the country that will achieve Validated Brucellosis-Free status. This is assured in the case of California where a proposed Statewide program is already operating in 17 counties. With eradication being the final goal of the brucellosis eradication program, it is essential that consideration be given to all livestock species. Outside of cattle, the other important reservoir of Brucella infection in the United States is swine.

It is believed that guidelines developed in the Dooly County project can be applied to advantage in other areas seeking validation. This study indicated that existing procedures recommended in the Uniform Methods
and Rules for swine brucellosis eradication are effective when properly applied.

With the accomplishments already made in the State-Federal brucellosis eradication program, we can do nothing less than strive toward the elimination of the last infected herd in the United States. The opinion of some skeptics that subacute diseases, such as brucellosis, cannot be eradicated has been refuted by field experience. Each year, experience confirms further past indications that brucellosis is an eradicable disease. This means that we can achieve one of the greatest accomplishments ever made in the field of livestock diseases by completing the brucellosis eradication program.
STUDIES ON BRUCELLA AGGLUTININS IN BOVINE SERUM


Ames, Iowa

The heat inactivation test (HIT) has been proposed as a supplement to the standard seroagglutination tube test (STT) for bovine brucellosis eradication\(^1\,^5\). Experiments utilizing the HIT have shown two types of Brucella seroagglutinins that can be differentiated on the basis of their heat stability at 65°C for 15 minutes\(^2\,^4\). Ultracentrifugation studies employing a sucrose density gradient technique have shown a high correlation between the heat labile seroagglutinin and the high molecular weight seroagglutinin; similarly the heat stable seroagglutinin has been shown to have a low molecular weight\(^6\). The purpose of the present paper is to summarize recent research at the National Animal Disease Laboratory on these two types of seroagglutinins and to evaluate their immunologic, epidemiologic and diagnostic significance.

The first experiments involving the HIT were conducted on serum samples from 143 artificially exposed cattle and 410 naturally exposed cattle with the following results\(^1\).

1) Positive HIT serologic reactions (50 percent or more agglutination in the 1:25 dilution) were obtained from all STT-suspect and reactor cattle from which *Brucella abortus* was isolated.

2) Negative HIT serologic reactions were obtained from 74 percent of STT-suspect cattle from which no *B. abortus* was isolated.

3) Negative HIT serologic reactions were obtained from 97 percent of the STT negative cattle from which no *B. abortus* was isolated.

Additional studies over a period of several months showed consistently negative HIT results on sera from 11 cattle with low level STT titers in known brucellosis-free herds\(^1\).

Later postvaccinal serologic studies were conducted on 32 calves\(^2\). These calves were vaccinated subcutaneously, when four to eight months old, with 50 billion organisms of *B. abortus* Strain 19. Blood samples were collected twice weekly after vaccination. The HIT and the STT were performed on the fresh sera. Endpoint titers were determined by both tests. The STT titer of each serum was compared to its HIT titer and thus the relative amounts of heat labile and heat stable seroagglutinins were determined. Figure 1 shows the mean seroagglutinin postvaccinal response of the 32 calves and the proportions of both types of seroagglutinins produced. The amount of heat labile agglutinins present in a sample were determined by subtracting its HIT titer from its total or STT titer. There was considerable variation in the relative amounts and the persistence of

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both the heat stable and heat labile seroagglutinins. Most of the early postvaccinal agglutinins were heat labile. The maximum levels of heat labile seroagglutinins were detected between 21 and 28 days postvaccination. The heat labile seroagglutinins frequently persisted at low levels for several months. Previously the heat labile seroagglutinins had been termed "nonspecific"\(^1\), since they agglutinated some antigenically related organisms as well as Brucella and were found in serums of cattle known to be brucellosis free. Our studies have shown that heat labile seroagglutinins may also be produced by cattle as a direct result of specific stimulation with *Br. abortus*\(^2\).

The next experiments involved postexposure serologic studies on 79 pregnant heifers\(^4\). Midway through their first gestation 57 vaccinated and 22 nonvaccinated heifers were exposed conjunctivally to approximately \(7 \times 10^5\) cells of virulent *Br. abortus* Strain 2308. Blood samples were collected routinely before exposure and twice weekly during the post-exposure period. Proof of infection was based upon the isolation of *Br. abortus* from one or more of the following sources at parturition: dam's blood, milk, colostrum, uterine contents, fetus or tissues obtained at necropsy\(^3\). Figure 2 shows the results of the studies on the serums from 20 nonvaccinated heifers that became infected. Most of the seroagglutinins produced during the first 30 days postexposure were heat labile. After 30 days postexposure the amount of heat stable seroagglutinins increased rapidly until 60 to 120 days when approximately 100 percent of the seroagglutinins were heat stable.
MEAN SEROAGGLUTININ RESPONSE OF
20 INFECTED NONVACCINATED CATTLE

Figure 2
MEAN SEROAGGLUTININ RESPONSE OF
19 INFECTED VACCINATED CATTLE

Figure 3
The mean seroagglutinin response of 19 vaccinated cattle that became infected is shown in Figure 3. Here the response occurred sooner than in the nonvaccinated infected cattle, but in other respects the patterns were very similar. This was interpreted as an anamnestic response.

The mean seroagglutinin response of 38 vaccinated heifers that resisted the exposure is shown in Figure 4. Heat stable seroagglutinins were produced for a short time, reached a peak at about 60 days and receded below detectable levels by 120 days postexposure. Heat labile seroagglutinins were produced at detectable levels for a much longer postexposure period than were the heat stable seroagglutinins.

![Graph showing mean seroagglutinin response of 38 noninfected vaccinated cattle](image)

**DISCUSSION**

A question arises as to the significance of the presence of heat stable or heat labile seroagglutinins. Calves that produced relatively high amounts of heat stable seroagglutinins after vaccination with *Br. abortus* Strain 19 failed to show any significant difference in their immune response to exposure with virulent *Br. abortus* than calves that produced lower levels of heat stable seroagglutinins. Similarly the production rate and persistence of postvaccinal heat stable or heat labile seroagglutinins failed to give an indication of the degree of immunity established with *Br. abortus* Strain 19. Furthermore, we have observed a limited number of calves which produced neither heat stable nor heat labile postvaccinal seroagglutinins in detectable amounts, yet they later were resistant to virulent *Br. abortus* Strain 2308.
On the basis of these studies the presence of heat stable seroagglutinins is an indication that cattle have been exposed to *Br. abortus*. The presence of heat labile seroagglutinins may or may not be the result of stimulation by exposure to *Br. abortus*, since such agglutinins have been found repeatedly in the serums of cattle in brucellosis-free herds as well as in brucellosis-infected cattle. The immunologic significance of the heat labile seroagglutinin is still unknown and should be investigated further. Where doubt exists as to the brucellosis status of an animal producing only heat labile seroagglutinins a HIT retest in 15 to 30 days is strongly indicated and may be of considerable diagnostic value in determining the animal's brucellosis status.

**SUMMARY**

Recent research at the National Animal Disease Laboratory has been directed toward differentiation of *Brucella* agglutinins in bovine serums on the basis of their heat stability at 65°C for 15 minutes. By this criterion a heat labile and a heat stable seroagglutinin have been demonstrated. Ultracentrifugation revealed that the heat labile seroagglutinin had a higher molecular weight than the heat stable seroagglutinin.

The heat labile agglutinin was found in the serums of cattle known to be brucellosis-free; in the serums of calves after vaccination with *Brucella abortus* Strain 19; and in serums of heifers with persistent post-vaccinal seroagglutinin titers. It was also found in the serums of cattle recently exposed to virulent *Brucella abortus* and in serums of cattle that became infected. The heat stable agglutinin was found only in the serums of cattle that were infected or exposed to *Brucella abortus*. The heat labile seroagglutinins were predominant at the onset of the disease, whereas the heat stable seroagglutinins became predominant as infection progressed. Heat stable *Brucella* seroagglutinins were an indication of exposure to *Brucella abortus*, whereas the significance of the heat labile seroagglutinins was not as readily apparent.

**REFERENCES**

EPIDEMIOLOGIC STUDIES OF BOVINE BRUCELLOSIS IN PROBLEM HERDS IN MINNESOTA


St. Paul, Minnesota

The prevalence of brucellosis in cattle in the United States has been dramatically reduced in the past twenty years through the cooperative efforts of the cattle industry, government agencies, and interested organizations and professions. Eradication program procedures—Uniform Methods and Rules—based on the best available scientific knowledge and good animal disease eradication practices, have been modified as new information and methods offered demonstrable advantages for the program.

In progressing to the present stage of eradication the standard plate serum agglutination test (SPT) has been utilized as the primary laboratory diagnostic aid for detecting and classifying individual animals as reactors, suspects, or negative. This laboratory aid to diagnosis has been quite accurate, rapid, and economical in the mass testing programs in the general cattle population as evidenced by the great reduction in prevalence of reactor animals. However, occasional discrepancies have been observed since it is unusual for any single serologic test to detect with complete accuracy the presence of specific agglutinins for Brucella in all animals under all conditions that may exist at a given point in time.

Some aspects of this situation were noted as early as 1948 in areas of very low prevalence in northern Minnesota on the basis of epidemiologic observations followed later by the demonstration of nonspecific agglutinins for Brucella in a few reactor animals. Other investigators have reported Brucella infection in some animals which were not disclosed as reactors by standard serologic procedures. The problem of detecting carriers—infected animals which do not respond as reactors to one or the other of the standard serologic tests—which may serve as the reservoir and continuing source of infection in herds becomes increasingly important as prevalence declines to very low levels and attention is devoted to finding and removing every last vestige of infection in the population.

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The authors gratefully acknowledge the assistance of Dr. Frank Stiles of the National Animal Disease Laboratory in confirming Brucella isolates, the technical assistance of Ione White and Pat Scanlon, and the cooperation of the area and district veterinarians in Minnesota.
should be emphasized that this type of carrier animal appears to comprise only a small proportion of the cattle population.

PROBLEM HERDS

In general, the term "problem herd" is used when standard brucellosis eradication procedures are conscientiously carried out and yet suspicious or positive reactions to milk and/or blood serum tests continue to occur repeatedly or periodically or when suspicious and positive reactions are observed in herds with no history of infection for several years. The term is not static and the type of problem may vary with (a) the type and frequency of blood serum and milk testing programs in the area (b) the type of infection and prevalence in an area (c) the type of cattle, environmental conditions, management and movement of animals.

Problem herds being considered in this report are essentially those where the reservoir and continuing source of infection appeared to be carrier animals which were not disclosed as reactors by the standard serologic test routinely used for detection of infection. These herds have been placed in two broad groups based on the reactions to the Brucella ring test (BRT) of milk and the SPT of serum which were the standard tests when the studies of BRT problem herds with so-called "false" suspicious BRT reactions were started in Minnesota in 1959.

Group I - Milk BRT Problem Herds

This group corresponds closely to the problem herd categories four and five as defined by Mingle in 1960 as: (a) herds which are persistently suspicious to the milk ring test, but in which only suspects to the blood serum agglutination test are revealed or (b) herds which are repeatedly suspicious to the milk ring test, but in which no blood agglutination test reactors or suspects are found or (c) herds which are persistently suspicious to the milk ring test, but blood agglutination test reactors have been found previously at periodic intervals with suspicious ring tests during the intervening periods.

Group II - Seroagglutination Test Problem Herds

This group corresponds closely to categories one, two, and three as defined by Mingle in 1960. Herds in this group have the following history: (a) infection has persisted and reactors were disclosed on the last four or more consecutive herd blood tests (b) reactors have been observed sporadically for over a year and several suspects were disclosed on each herd blood test with or without reactors (c) currently infected herds which have had periodic outbreaks of brucellosis with few if any suspects found on intervening herd blood tests.

Since herds in group I and group II did not seem to respond to standard procedures and recommendations, investigation appeared necessary to elucidate the cause of the problem and speed eradication efforts.
METHODS AND PROCEDURES

Herds for these studies were selected by reviewing Minnesota records to determine the extent and type of problem and classification. Those meeting the criteria for classification in group I and group II were selected for intensive study using all available procedures as outlined below.

A standard form and protocol were developed for collecting data to evaluate a given herd situation and to accumulate information for later analysis by type of problem herd and area. This included herd history, history of individual animals, results of blood serum and milk tests, and other factors which appeared pertinent to aid in detecting the reservoir, source of infection and mode of transmission. Information was evaluated by a veterinary epidemiologist who made the recommendations for the herd. These were implemented in cooperation with the area veterinarians.

Milk Test Procedures

1. The standard test was the Brucella ring test (BRT)\textsuperscript{17}. The standard BRT was applied to pooled milk to detect suspicious herds and then to the milk from individual cows to detect the suspicious cow or cows in the herd.

2. The supplemental milk test used in this study was the serial dilution method of conducting the BRT\textsuperscript{17}. Quarter milk samples were collected aseptically from the cow or cows with Brucella agglutinins in the milk and shipped in refrigerated containers to the laboratory. A portion of the milk was removed and the BRT serial dilution test was conducted on each quarter milk sample and on a simulated composite milk of each BRT suspicious cow to establish an end titer for the Brucella agglutinins in the milk. These results—ranging from 1:1 to 1:3200—provided information as to relative quantity of agglutinins in the milk from each quarter and were used for evaluation as part of the overall pattern of serologic results.

Blood Serum Test Procedures

All of the seroagglutination procedures were conducted on every serum collected from cows in the study herds as well as on the serum collected whenever tissues were obtained for bacteriologic examination from study animals sent to slaughter at South St. Paul.

1. Standard tests were the standard plate seroagglutination test\textsuperscript{16} (SPT) and the standard tube seroagglutination test\textsuperscript{16} (STT).

2. Supplemental tests were the acid plate antigen test\textsuperscript{14} (APA) at pH 3.75, 3.5, 3.25, 3.0, the heat inactivation test\textsuperscript{2} (HIT) and the rivanol precipitation test\textsuperscript{1} (RIV). These tests were developed to detect in bovine serum the presence of high molecular weight agglutinins (16-19S macroglobulins)—often termed nonspecific agglutinins for Brucella\textsuperscript{6}.

The results of both the supplemental and standard tests were carefully interpreted on the basis of the overall pattern of test results and the epidemiologic investigation.
3. Animals referred to in the results presented in this report were infected with *Brucella* spp. as demonstrated by isolation of the agent from milk or tissues or both. These animals were selected for this report on the basis of serologic and cultural results and herd history from among the animals and herds studied. These isolations were confirmed through the cooperation and courtesy of the National Animal Disease Laboratory.

RESULTS

1. Group I Problem Herds

Methods and procedures outlined for this study were applied to Minnesota herds classified as group I. This report concerns 42 animals in 36 group I herds selected on the basis of isolation of *Brucella abortus* where the SPT did not indicate any reactors although the herds had a history of repeated milk BRT suspicious reactions. As determined by the serial dilution method the milk BRT titers of these animals are summarized in Figure 1.

![Figure 1. Distribution of Brucella ring test titers among 42 selected animals* from group I problem herds and 67 animals** from group II problem herds in Minnesota July 1, 1960 to August 31, 1962.](image)

*Animals from which Brucella organisms were isolated with nonreactor serum plate titers.

**Animals from which Brucella organisms were isolated.
The BRT titer of 90 percent of the milks (38 of 42) was 1:25 or greater while two had a titer of 1:6 and two milks were 1:3 and 1:1 respectively. In the case of milk titers below 1:6 these animals were detected on individual BRT and another animal or animals were also contributing to the consistent herd BRT titer as well. These results indicate that in this type of herd the majority (90 percent) of milk titers from infected animals were greater than 1:25 but even those with milk titers of 1:6 or lower may be infected animals.

Results of blood serum agglutination procedures for group I animals are presented in the form of reaction patterns to the SPT, STT, and supplemental tests in Table I. The reactions of pattern number one were applicable to 27 serums (64 percent) and the reactions in pattern number two were applicable to 15 serums (36 percent).

All of the 42 animals were detected as positive by one or more of the supplemental tests (APA, HIT, RIV) yet none were classed as *reactors* by the SPT.

**TABLE I**

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Standard Plate Test</th>
<th>Standard Tube Test</th>
<th>Supplemental Tests**</th>
<th>Number of Animals</th>
<th>Percent of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Pattern II</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>27</td>
<td>64%</td>
</tr>
<tr>
<td>Pattern III</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>15</td>
<td>36%</td>
</tr>
<tr>
<td>Pattern IV</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>42</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Blood serum agglutination titer classified animal as "reactor" when + appears. **One or more of the three supplemental tests were positive when + appears.

From these patterns it is apparent that the STT of blood serum of these forty-two animals did not classify 36 percent (15 of 42) as *reactors*. Thus only 64 percent of the infected animals would have been condemned by the STT alone. If one were willing to rely on a positive result of any one of the supplemental tests, all of the 42 animals (100 percent) would have been detected as having specific agglutinins for Brucella in the serum and 95 percent of the serums (40 of 42) were positive for two or more of the supplemental tests. As indicated in Table III, 62 percent (26 of 42) of the animals had been vaccinated with strain 19 *Brucella abortus* vaccine—24 at four to eight months of age and two beyond eight months of age.

It was also found that only eight of the 42 isolates were Type II *Brucella abortus*.

By definition the herds in group I were chronic BRT suspicious herds and the results of the field investigation revealed that a suspicious BRT reaction in the milk from these herds had been observed for an average of 5.4 years (range of two to nine). Although many of these herds had severe outbreaks at one or more periods in their history, an average of 2.3 years
(range of 1:6) had elapsed without an SPT reactor prior to selection as study herds. The extent of previous infection is indicated by the fact that the reactors removed in previous outbreaks constituted 48 percent of the number of animals (484 of 990) presently in the herds.

In these herds reactors were disclosed continuously or periodically over an average of 5.6 years (range of two to nine) but on the average three, one year, intervals were observed when no reactors were disclosed. This periodicity of outbreaks suggests the continuing presence of one or more carrier animals in these herds. The average age of the 42 carrier animals in group I was 6.4 years (range of four to 13) and in every case, one or more carrier animals had been in the herd when previous infection was observed.

2. Group II Problem Herds

The procedures outlined previously were applied to the Minnesota herds classified as group II and 67 animals from 37 herds were selected on the basis of isolation of *Brucella abortus*.

Results of the milk BRT by the serial dilution method are presented in Figure 1 and depict the distribution of milk titers from a dilution of 1:1 to 1:800 or greater for the 62 animals from which milk was obtained. The BRT titers of 78 percent of the milk samples were 1:25 or greater while 11 percent were 1:12, five percent were 1:6, and six percent were less than 1:6. In only one case did the milk from one of the 62 animals fail to demonstrate a BRT reaction of 1:1 or greater.

These BRT results for group II herds indicate that a BRT reaction may not be observed with 22 percent of the milk samples at a dilution of 1:25 or greater.

Blood serum agglutination test results are presented, Table II, in the form of reaction patterns to the SPT, STT, and supplemental tests (APA, HIT, RIV). The reactions shown in pattern number one were applicable to 37 (55 percent) of the serums in group II animals. Pattern number two reactions were applicable to 18 (27 percent) of the serums and the reactions in pattern number three applied to 10 (15 percent) of the animals. Pattern number four shows that two animals (three percent) were detected as reactors by the SPT and the supplemental test but not by the STT.

<table>
<thead>
<tr>
<th>Pattern of Blood Serum Agglutination Test Reactions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Plate Test</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Pattern I</td>
</tr>
<tr>
<td>Pattern II</td>
</tr>
<tr>
<td>Pattern III</td>
</tr>
<tr>
<td>Pattern IV</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

*Blood serum agglutination titer classified animal as "reactor" when + appears.
**One or more of the three supplemental tests were positive when + appears.
In the serums from group II herds, it is observed that a total of 58 percent of the animals were SPT reactors, a total of 82 percent of the animals were STT reactors, and that 100 percent were classed as supplemental positive by a combination of the APA, HIT, and RIV.

Table III indicates that 42 (62 percent) of these group II animals were vaccinated with strain 19*Brucella abortus* vaccine.

<p>| TABLE III |
| Vaccination Status of 109 Animals in Group I &amp; II From Which <em>Brucella Spp.</em> Were Isolated |</p>
<table>
<thead>
<tr>
<th>Number of Animals</th>
<th>Number Vaccinated</th>
<th>Percent Vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>42</td>
<td>26*</td>
</tr>
<tr>
<td>Group II</td>
<td>67</td>
<td>42</td>
</tr>
<tr>
<td>Total</td>
<td>109</td>
<td>68</td>
</tr>
</tbody>
</table>

*Two of these animals were vaccinated when more than eight months of age.

In this series of animals from group II herds, nine of the 67 isolates were Type II* Brucella abortus.*

Results of field investigations and histories of these herds indicated that infection had been present continuously or periodically on the average of 6.5 years (range 1:12). The number of animals in any one herd from which isolations were made ranged from one to six animals. Isolations were made from two to four animals in a number of the herds. The average age of the infected animals was 3.3 years with a range of 1.5 to 12 years. The periodicity of reactors at varying intervals and the history of suspects indicated that carrier animals—as judged by the 42 percent of animals not disclosed as reactors by the SPT—were a major source of infection for the new reactors. Approximately the same percent of carriers were disclosed, regardless of whether reactors were disclosed on four or more consecutive tests or periodically with no reactors on intervening tests.

**DISCUSSION**

In evaluating the significance of milk BRT reactions observed in this study, particularly those concerned with the group I chronic BRT suspicious herds, it appears that the milk BRT may be far more efficacious in detecting infections than previously reported4,12. Previous reports by one group of investigators7 indicated that in two counties in Wisconsin, between 81 percent and 90 percent of the herds with suspicious BRT reactions did not contain SPT seroagglutination reactors, however the criteria was the SPT which may have a low efficacy in chronic BRT herds as shown by the results presented here. The milk BRT appears to have a high degree of efficiency as judged by isolation of *Brucella spp.* when applied to certain problem herds in the population as a standard and a
supplemental test and supports strongly the premise that any herd of more than six animals with recurring or chronic herd milk BRT suspicious reactions should be investigated more thoroughly with the supplemental BRT on individual animals, and animals with titers of 1:6 or greater should be investigated further with multiple serologic procedures, culture methods, etc.

Other investigations with the whey plate test support this premise, however the whey plate test without dilutions below 1:25 may fail to detect BRT titers of 1:12 or less which may provide important criteria for evaluation of an animal in conjunction with other serologic findings and history. It should be emphasized however that the whey plate test or individual animal BRT are not consistent indicators of infection in all types of herds but are useful as supplemental tests when applied to individual animals and evaluated in conjunction with all other information. The data suggest that any BRT suspicious reaction in a herd of six or more animals should be considered indicative of infection until investigated and shown otherwise.

Epidemiologic findings in chronic BRT suspicious herds indicate that animals shedding Brucella in the milk may well be the source and reservoir of continuing infection in the herd as evidenced by the fact that single or multiple reactors were found periodically in most of the group I herds and generally a carrier animal of four to 13 years of age was found to have been present throughout a period of two to nine years. The possibility of transmission from udder to udder and the hazard posed by these chronic BRT suspicious cows has been supported by other investigators.

It is of interest to note that vaccination with strain 19 Brucella abortus vaccine appeared to increase the difficulty of detecting infected animals with the SPT and the STT in a definitive manner since 62 percent of these animals from problem herd groups I and II were vaccinated. Interpretation of seroagglutination results for these vaccinated animals was not possible without the aid of supplemental tests and epidemiologic evaluation. From one viewpoint, detection of exposure experience with Brucella would be simplified if vaccination were not practiced in areas of very low prevalence where the risk of exposure is negligible.

These data showing that the SPT and the STT failed to disclose as reactors 64 percent (70) and 25 percent (27) respectively of the 109 infected animals selected from these chronic problem herds should not be interpreted as meaning that these tests are not useful in the general population but rather that neither of these tests alone is sufficient to cope with selected chronic problem herds in areas of low prevalence.

As approached in these studies the problem was alleviated by (1) using the standard BRT on milk and the SPT and STT on serum to indicate the level of agglutinins prior to applying the supplemental tests (2) using the supplemental BRT on milk from individual animals in the herd to obtain a relative titer from 1:1 to 1:3200 to aid in making a diagnosis (3) using three supplemental seroagglutination tests (APA, HIT and RIV) which indicated the presence of 7 S agglutinins for Brucella at a 1:25 dilution of serum without undue interference from the so-called nonspecific reactions which may be due to higher molecular weight agglutinins (macroglobulins) for
Brucella (4) using the herd history as an integral part of the findings (5) using bacteriologic culture procedures to provide guidelines for serologic evaluation and (6) using all the information obtained in the first five steps to develop an epidemiologic pattern for evaluation by the veterinary epidemiologist followed by a diagnosis and recommendations for handling the problem herd. This sixth step is important since the supplemental tests have a fairly definite pattern of sensitivity for macroglobulins which are present in early infection and this may confuse the interpretation of the supplemental tests if epidemiologic evaluation does not precede diagnosis and recommendations. In the authors' experience, the SPT appears to have more sensitivity in detecting early agglutinins resulting from infection than the STT. This is another reason why one should have the complete pattern of reaction plus the history to make an effective epidemiologic evaluation.

By using all these procedures it appears possible to detect otherwise undisclosed infections in areas of low prevalence and yet distinguish and differentiate significant agglutination reactions to the SPT and STT which are not indicative of present infection with Brucella. These procedures require more time and competence than the standard ones, however, in areas of low prevalence the number of problem herds and animals is quite small and more complex and definitive procedures can be applied by specially trained personnel without a significant increase in costs. As the number of infected animals is reduced in the population more discriminatory procedures are mandatory if eradication of brucellosis is to be achieved.

SUMMARY

These epidemiologic studies of selected "problem herds" in Minnesota indicate that methods and procedures are available that will aid in eliminating recognized problems and implement the brucellosis eradication program.

Data presented from two groups of 73 problem herds and 109 animals imply that when one is concerned with detecting all Brucella infected animals in areas of low prevalence to eradicate the disease, special attention and procedures must be applied to detect carrier animals—infected animals which are not classified as reactors by one of the standard (SPT, STT) serologic tests—that may serve as a continuing reservoir and source of brucellosis infection. In areas of very low prevalence of brucellosis infection it is also important to recognize and distinguish so-called "non-specific seroagglutination reactions" which are not indicative of present infection as judged by attempts at isolation of the organism, supplemental tests and herd history.

The data demonstrate that, in handling problem herds in areas of low prevalence, the supplemental tests (APA, HIT, RIV) combined with standard tests and herd investigations are valuable and essential adjuncts in making an epidemiologic evaluation to detect infected animals with a high degree of accuracy.
REFERENCES

CALIFORNIA'S SWINE BRUCELLOSIS AREA VALIDATION PROGRAM PROGRESS REPORT


Sacramento, California

Effective July 1, 1962, a statewide swine brucellosis eradication program was launched in California. The program began in 15 northern counties of the State. The counties were declared by regulation of the California Department of Agriculture to be swine brucellosis control areas. In such areas, the testing of breeding swine in every herd is carried out to determine the brucellosis status of the herd. Prior to this control area program California, like other states, had relied on a voluntary participation of swine owners.

The United States Department of Agriculture is cooperating with the State of California in this effort and we expect the program to move southward through the State embracing all counties within the next two years, involving a swine population of more than 320,000. The swine brucellosis eradication program is but another phase in the complete eradication of brucellosis from California.

The bovine eradication program was officially launched September 26, 1957, but was preceded by 10 years of compulsory vaccination of dairy as well as vaccination of a high percentage of beef calves. Like the present swine brucellosis eradication program, control areas were established in the northern counties and the bovine brucellosis program moved in an orderly fashion southward including the entire state by July of 1960. At that time it became apparent that several California counties were without any infected cattle and that certified brucellosis-free status was in sight. The question arose whether or not brucellosis existed in any other animals in those counties. Fortunately, brucellosis of goats has not been known to exist in California.

A limited survey of market swine in the early summer months of 1961 was conducted. By the sixth of June, 544 breeding sows from 23 ranches in 10 counties were tested at slaughter. These animals were primarily from large garbage feeding establishments. Results of this survey revealed 53 percent of the slaughtered animals carried a titer.

The results of this survey, along with proposed swine brucellosis control regulations, were immediately presented to the statewide swine disease committee. This committee, composed of 18 members representing

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various aspects of the swine industry, voted favorably toward adoption of these regulations. Following statewide hearings conducted in February of 1962, the proposed regulations were adopted and became law.

The California swine brucellosis eradication program makes full use of the validated herd plan recommended by the United States Livestock Sanitary Association and approved by the United States Department of Agriculture. A voluntary herd plan has been in effect in California for many years and is encouraged in both control as well as non-control areas. This plan is becoming quite popular and validated herds have increased five-fold in number since the adoption of this swine brucellosis control regulation on July 1 of this year. Those producers who are exhibiting or selling replacement or purebred animals found this validated plan a desirable aspect of the over-all herd program.

In addition to the validated herd plan, the program makes full use of a procedure for the testing of breeding swine at slaughter. Herds not included in either the validation plan or the market swine testing plan are individually tested. Herds found to be infected are placed under State hold order and State or Federal veterinarians work out a plan for the owner to follow in order to establish a brucellosis free herd. The plans are Plans 1, 2 or 3 of the validated herd plan recommended by the United States Department of Agriculture.

The disposal of reactors or the infected herd to slaughter is at the convenience of the owner and in accordance with one of the approved plans. Premises are required to be cleaned and disinfected following removal of reactors. No indemnity is provided. When all of the herds in a county have been tested and the infection is found to be in less than 3 percent of the herds, the area is declared to be a validated brucellosis free area. This validation is recognized for a period of three years.

In control areas the swine are bled by practicing veterinarians under contract with the State of California. The fee is $1.25 per head. Those not bled by practicing veterinarians are bled by state or federal personnel. The majority of bleeding is done by the vena cava method.

Supporting the California area eradication program are three other regulations affecting the movement of swine throughout the State.

1. (795.3) Controls the interstate movement of swine. All breeding swine over four months of age moved within the state must be either from a validated free herd or negative to a brucellosis test.

2. (795.4) Controls the movement of breeding swine into California. The requirements are similar to those of the intrastate regulation.

3. (795.5) Controls the breeding swine at fairs and purebred sales. Here the restrictions are more severe. The swine must be from a validated free herd or from a herd in which all of the breeding swine over four months of age have been tested within 30 days and have found to be negative. This regulation was one demanded by our purebred swine producers and has been a great stimulus to encourage the statewide eradication effort.

Our efforts thus far have been very encouraging. Already two counties, Del Norte and Mono, have been declared validated brucellosis free areas.
Preliminary results of testing to date show 290 herds representing 13,082 swine have been tested, three herds have been found to be infected and 61 reactors were found in these herds. We anticipate that six to eight counties will reach validated free area status soon. There are now 87 validated herds.

Approximately one-third of the testing thus far has been by contract practicing veterinarians and the balance by State or Federal regulatory personnel.

We organized our regulatory personnel into two teams of two men each; one to bleed and one to restrain animals and keep records.

A special hog-restraining chute was developed. It has proven extremely useful in testing herds of 20 or more eligible sows and boars. A snare was used on smaller herds.

One important observation was that herd owners seemed extremely interested in disinfection procedures used by blood-testing personnel between herds. They appeared fearful that disease might be carried from herd to herd. In view of this, all items of equipment used, including boots, etc., were scrubbed with an antiseptic solution before moving off the premises.

We realize that we are in a pilot program and there will be changes that will need to be made. Our goal is the eradication of brucellosis from California. We have already achieved a modified certified brucellosis status from the entire State, and we do not believe that any reservoir of swine brucellosis should be allowed to exist. Therefore, we have launched our brucellosis eradication program and we encourage other states to join us.
REPORT OF THE COMMITTEE ON BRUCELLOSIS


Charles F. Kettering, of General Motors, when illustrating the value of having the facts and knowing what to do with them, said, "The French Government spent 500 million dollars in the Panama Zone, and did not get a canal; we spent 400 million dollars, and did get a canal. Why did our engineers succeed while the French engineers failed? Because of just one fact which our engineers knew, but which the French did not know. Our engineers knew that mosquitoes carry malaria and yellow fever. These two diseases were what made the Panama Zone uninhabitable, so our engineers cleaned out the mosquitoes, and then proceeded to dig the canal."

In dealing with any problem, the first step is to get the facts, the second is to analyze them from the standpoint of what you wish to accomplish, and the third is to act on them.

It has always been the policy of the United States Livestock Sanitary Association, as they have met in annual convention down through the years, to gather all the facts available pertaining to a particular disease that might be under discussion for eradication or control. However, no program of disease eradication can be carried on successfully year after year unless every fact known to science and research has been considered and incorporated into the program. Each year as we meet in convention suggested new plans, rules and regulations as a result of research and controlled experiments are presented to your committee for discussion. This year has been no exception to the rule. Resolutions and suggestions for the improvement of the program have been received during the past twelve months from those vitally interested located in different sections of our country. All of these suggestions and resolutions have been considered in public hearings which started Monday of this week at 9:15 a.m.

We believe that everyone has had an opportunity to present his case and every proposal for a change in our uniform rules and regulations has been carefully considered, and we have endeavored to include in this report any and all amendments and recommendations that we as a committee are convinced will be of material benefit and help in carrying out the program of brucellosis eradication during the years ahead.
Your Committee realizes that those working in the field on this program must be guided to a great extent by the information furnished them through this report. If we are going to continue to fight this battle of disease eradication we also must accept the fact that we must have the knowledge and experience of those working in the field, whose job it is to put into effect the knowledge that we have gleaned and analyzed down through the years relative to the methods that we employ in eradicating disease from our livestock population. It is further the belief of your Committee that if the program of brucellosis eradication is to be brought to a successful conclusion, the accelerated program which is now in effect must continue without interruption. To accomplish this it is evident that sufficient Federal and State funds must be provided yearly.

You will note that Doctor Mingle’s report on the State-Federal Brucellosis eradication program states that we now have 30 States and two territories on the certified list. Four States have been added to this list since we met in convention a year ago.

The coveted goal of attaining a Modified Certified Brucellosis Area status is the desire of every State. Upon achieving it, however, a State or County may be in its most dangerous period. There is a real prospect of a let-down in the vigorous effort to attain final and complete eradication. While State and Federal expenditures can be reduced considerably following attainment of a modified certified status, industry and professional pressure must be continued not only to attain final eradication in all of our States, but to prevent losing hard fought ground.

Therefore, with these thoughts in mind, your Committee makes the following recommendations, additions and amendments to our present uniform rules and regulations. We submit these to you believing if they are adopted they will assist materially in completing the job that has been so successfully carried on since its inauguration.

**BOVINE BRUCELLOSIS ERADICATION**

Proposed Changes in the Uniform Methods and Rules, 1962

**PART I: DEFINITIONS**

"Positive" or "Reactor"

(1) Official vaccinates, thirty (30) months of age and over, or official vaccinates under thirty (30) months of age that are parturient (springers) or post-parturient that disclose complete agglutination reactions in the blood titer dilution of 1/200 or higher.

(2) All other cattle more than six (6) months of age that disclose a complete agglutination in the blood titer dilution of 1/100 and higher.

"Suspect"

(1) Official vaccinates thirty (30) months of age and over or official vaccinates under thirty (30) months of age that are parturient (springers) or post-parturient that disclose complete agglutination in the 1/100 dilution and less than complete in the 1/200 dilution.
(2) All other cattle more than six (6) months of age that disclose agglutination in the 1/50 and less than complete agglutination in the 1/100 dilution.

"Negative"

(1) Official vaccinates thirty (30) months of age and over or official vaccinates under thirty (30) months of age that are parturient (springers) or post-parturient that disclose reactions of not more than complete agglutination in the 1/50 dilution.

(2) All other cattle more than six (6) months of age that disclose a reaction of less than incomplete agglutination in the 1/50 dilution.

The "Herd" Test

The herd test shall include all cattle over eight (8) months of age except steers, spayed heifers, and official vaccinates under thirty (30) months of age which are not parturient (springers) or post-parturient.

"Official Vaccinate"

A female bovine animal vaccinated against brucellosis with an approved Brucella vaccine while from four (4) through eight (8) months of age, or a female bovine animal of a beef breed in a range of semi-range area vaccinated against brucellosis with an approved Brucella vaccine while from four (4) to twelve (12) months of age, under the supervision of a Federal or State veterinary official, permanently identified as such a vaccinate, and reported at the time of vaccination to the appropriate State or Federal agency cooperating in the eradication of brucellosis.

Identification of Vaccinated Animals

Official vaccinates shall be tattooed or branded as follows:

(1) The tattoo shall be applied in the right ear and shall be the U. S. Registered "shield and V." The "shield and V" shall be preceded by a numeral indicating the quarter of the year followed by the last digit of the year in which the vaccination was done, for example, "1 0" indicates the first quarter of calendar year 1960.

(2) If the brand is used, then the "V" shall be applied in four different positions—one each year over a four-year period to indicate in which year the vaccination was done. The fifth year will repeat the first year, and so on indefinitely. In 1962 the "V" shall be placed with the open end facing upward and so on clockwise indefinitely.

PART II. RECOMMENDED PROCEDURES

Section I. Individual Herd Plans

Plan A. (1) Blood testing of herds, with permanent identification and prompt disposal of positives for immediate slaughter and optional vaccination of calves, and/or

(2) Milk ring testing of dairy herds at intervals of three (3) to six (6) months with all suspicious herds handled according to paragraph (1).
Note: Herds that have passed three (3) successive satisfactory milk ring tests at intervals of not less than four (4) nor more than six (6) months may be considered as having met the brucellosis requirements of Plan A for Grade A milk production.

Plan B. Testing of cattle, permanent identification, and temporary retention of positives pending their disposal for slaughter, with vaccination of calves. Positives may be retained in a quarantined herd for a period not to exceed three (3) years from the date retention of positives was started. All Plan B herds should be retested at least every six months. Plan B shall be discontinued effective July 1, 1964.

Plan C. Calf vaccination without test of any part of the herd. This plan is to be confined to those herds in which the movement of animals is restricted by special permits issued by the State Livestock Sanitary Authority. Plan C shall be discontinued effective July 1, 1964.

Section II. Participation on Area Basis

A. When 75 percent or more of the cattle owners representing at least 51 percent of the cattle in an area have placed their cattle under any one or a combination of the three plans, then the remaining owners shall select a herd plan. The period under which individual herd plans are in effect on an area basis should not exceed three (3) years, at which time the area is obligated to adopt the Modified Certified Brucellosis Area plan. Effective July 1, 1964, only Plan A of the individual herd plans will be recognized under this paragraph.

PART III. INDIVIDUAL CERTIFIED HERD PLAN

Section III. General Provisions

A. Official vaccinates under thirty (30) months of age other than those which are parturient (springers) or post-parturient, are not required to be tested, or if tested are not required to be negative. All other official vaccinates classed suspect may be retained in isolation for retesting until their final determination is made.

B. Additions to certified herds or herds with negative tests shall be limited to the following:

1. c. (1) Official vaccinates under thirty (30) months of age other than those which are parturient (springers) or post-parturient, on certificate of vaccination; all other official vaccinates if negative within thirty (30) days prior to addition.

1. c. (2) All other cattle on evidence of negative retest not less than sixty (60) days from date of negative herd test.

2. c. (1) Official vaccinates under thirty (30) months of age other than those which are parturient (springers) or post-parturient, on
certificate of vaccination; all other official vaccinates if negative within thirty (30) days prior to addition.

F. Cleaning and disinfection

Premises shall be cleaned and disinfected under regulatory supervision within fifteen (15) days following removal of reactors. An extension of time may be considered under extenuating circumstances.

PART IV: MODIFIED CERTIFIED AREA PLAN

The provisions of the individual certified herd plan that relate to quarantining, cleaning, and disinfecting shall apply to the Modified Certified Brucellosis Area plan. The extent of the area shall be determined by the cooperating State and Federal agencies. All tests for area certification shall be performed within an eighteen (18) month period. When an area has been legally designated as working toward Modified Certified Brucellosis Area status, the following rules shall apply:

PART IV, Section I, Paragraph B

B. An area may be declared a Modified Certified Brucellosis Area by the application of two (2) milk ring tests (BRT) not less than six (6) months apart, together with a blood test of herds suspicious to the BRT, such other herds as are not included in the milk test, and herds in which the BRT does not represent a majority of the cattle in the herd. The number of positives must not exceed one percent of the cattle and the herd infection rate must not exceed five percent. Infected herds shall be quarantined until they have passed one negative blood test at least thirty (30) days following removal of the cattle classed positive, except cattle consigned for immediate slaughter under permit.

PART IV, Section I, Paragraph C (1)

C. (1) Range and semi-range areas may qualify as Modified Certified Brucellosis Areas for a period of three (3) years if as the result of a blood test of all dairy cattle, all purebred cattle, and not less than 20 percent of the range and semi-range cows over three (3) years of age in each herd, the number of positive does not exceed one (1) percent of the area cattle population (excluding steers and spayed heifers) and five (5) percent of the herds. Two or more semi-annual milk ring tests with blood tests of suspicious herds may be substituted for blood tests of individual dairy herds. The 20 percent test will be discontinued June 30, 1964. After that date the number of animals to be tested in range and semi-range herds shall be based upon Graph CA 4-4, page 24.

PART IV, Section II, Paragraph C (2)

C. (2) Areas may be maintained in a certified status for additional periods of three (3) years provided: subparagraphs (a), (b), (c), (d), (e), (f), and (g), are unchanged.
PART IV, Section III, Paragraph A

A. Cattle from officially Certified Brucellosis-Free Herds or Areas and cattle from negative herds in Modified Certified Brucellosis Areas may enter Modified Certified Brucellosis Areas without being retested for brucellosis. All such cattle shall be individually identified.

PART IV, Section III, Paragraph B

B. Cattle from noncertified areas may enter a Modified Certified Brucellosis Area or an area in the process of such certification when all animals in the herd of origin were negative to the official blood agglutination test for brucellosis within ninety (90) days of the date of entry. Individual animals to be moved must be negative to a final retest at least thirty (30) days from the date of the previous herd test and within thirty (30) days of entry. Official vaccinates under thirty (30) months of age which originate in herds not known to be affected with brucellosis other than those animals which are parturient (springers) or post-parturient need not meet the test requirements of this paragraph.

PART IV, Section III, Paragraphs C and D

Note: Paragraphs C and D are deleted as they are now included in paragraphs A and B.

PART IV, Section III, Paragraph E

Note: This paragraph is now re-numbered paragraph "C."

C. All other cattle over eight (8) months of age, including official vaccinates over thirty (30) months of age and those under thirty (30) months of age which are parturient (springers) or post-parturient, except steers, spayed heifers, and cattle intended for immediate slaughter, shall be required to pass a negative officially recognized blood agglutination test for brucellosis within thirty (30) days prior to the date of entry. They shall be maintained in quarantine separate and apart from all other cattle and be retested in not less than thirty (30) nor more than ninety (90) days after date of entry. Should reactors be found in cattle held in isolation for retest, they shall be consigned for immediate slaughter and all exposed cattle shall be continued in isolation until they have passed a negative rest not less than thirty (30) days following removal of reactors.

PART V, Section II, Paragraph B. 4.

B. 4. The number of herds found infected during the entire certification period does not exceed one percent of the area herd population, or one herd, whichever is greater. If the area is making a concerted effort through effective screening programs and extensive epidemiology to locate infected herds and eradicate the disease, only the infection disclosed during the last eighteen (18) months of the certification period will be counted.
PART V, Section II, Paragraph C

C. If the percentage of infected herds exceeds the limit set forth in paragraph B. 4., the area shall revert to Modified Certified Brucellosis Area status and must re-qualify in accordance with Section I of PART V.

CHAPTER II
PORCINE BRUCELLOSIS ERADICATION—UNIFORM METHODS AND RULES

PART I. Section I.

Validation is made on the basis of two (2) consecutive negative tests on the entire breeding herd 30-90 days apart. This includes all breeding animals six (6) months of age and over. This validation holds for twelve (12) months and applies to Specific Pathogen-Free pigs from such herds.

ESTABLISHING AND MAINTAINING VALIDATED BRUCELLOSIS-FREE AREAS

Section I

A. Definitions

(1) Reactor or Positive Swine
Any swine disclosing a complete agglutination reaction in the blood titer dilution of 1/100 or higher; and any swine in an infected herd or herd of unknown status having a complete reaction in the 1/25 dilution or higher.

(2) Infected herds
Any herd that discloses one or more swine showing a complete agglutination reaction in the blood titer dilution of 1/100 or higher.

(3) Negative swine
a. Any swine from an infected herd or herd of unknown status that discloses no agglutination in blood titer dilution of 1/25 or higher.
b. Any swine from a validated or negative herd that discloses agglutination no higher than incomplete at 1/100 dilution.

(4) Negative herds
a. Any herd that discloses no swine having agglutination reactions higher than incomplete at the 1/100 dilution.
b. Any herd in which at least 10 percent of the breeding swine have been tested annually in the Market Swine Testing Program for three consecutive years and no reactions disclosed.

(5) Herd test
a. Shall include all breeding swine six months of age and older.
b. All swine tested shall be identified with an approved ear tag, tattoo, or other approved procedures.
B. General
(1) Blood samples are to be tested only by cooperating State-Federal laboratories.
(2) All activities conducted privately or as part of the official program such as results of agglutination tests must be reported promptly to State and Federal cooperating agencies.
(3) Infected herds are to be held in quarantine until freed of brucellosis.
(4) Reactor swine are to be permanently identified by ear tag and brand.
(5) Reactor swine must be sold directly to slaughter establishments for immediate slaughter only within 15 days of date of identification. An extension of time may be granted under extenuating circumstances.
(6) Buildings, farrowing pens, equipment, etc., are to be cleaned and disinfected following the removal of reactor swine or the entire herd.
(7) Replacement swine may be added without test if procured directly from Validated Brucellosis-Free herds or negative herds in Validated Brucellosis-Free Areas.
(8) All other replacement swine shall have passed a negative test and be held in isolation until passing a second negative test at least 30 days after the first in cases of boars and open gilts or after farrowing for bred sows and gilts.
(9) All swine kept for feeding purposes shall be kept separate and apart from all breeding swine.
(10) The official brucellosis eradication program shall be supervised by full-time employed State and/or Federal veterinarians.

Section II. Area Validation

A. All Breeding herds in the eradication area must qualify under one of the following within an 18-month period.

(1) Negative herd test, including all breeding swine six months of age and over.
Note: Owners desiring a Validated Brucellosis-Free Herd will be required to have a second negative herd test 30-90 days after the first.

(2) Establish negative herd status in the Market Swine Testing Program.
Each year for no less than three years 10 percent of breeding swine in the herd or a minimum of one animal, whichever is greater, shall be blood tested.

(3) Infected herds are to be held in quarantine until freed of brucellosis.
Owners of infected herds have the option of selecting one of the three recommended plans described under Chapter II, Porcine Brucellosis Eradication Uniform Methods and Rules, paragraph "B."
The eradication area will qualify as a Validated Brucellosis-Free Area provided the herd infection rate has not exceeded three percent during the 18-month period and provided all herds in the area have attained a negative status prior to the date of declaration as a Validated Brucellosis-Free Area.

Section III. Area Revalidation

A. The designation, "Validated Brucellosis-Free Area" will be granted for a 3-year period subject to removal if the requirements for maintaining this designation are not carried out.

B. Requirements for maintaining a Validated Brucellosis-Free Area:
(1) Test of all breeding swine 6 months of age and over during last 18 months of validation period, or
(2) During each year of the 3-year validation period, each herd within the designated area must blood test at least 10 percent of the breeding swine over six months of age or a minimum of one animal, whichever is greater. If one-half of the required tests are not reported by the end of the first 18 months of the 3-year validation period, it will be necessary to blood test all breeding swine in the herd before the expiration of the 3-year period.

C. Herds found to be infected during the period an area is designated a Validated Brucellosis-Free Area:
(1) Infected herds are to be placed under quarantine until freed of brucellosis.
(2) Owners of infected herds have the option of selecting one of the three recommended plans for eradication of brucellosis described in Chapter II, Porcine Brucellosis Eradication-Uniform Methods and Rules, paragraph "B."

D. General
(1) Reactor animals are to be permanently identified and moved under permit for immediate slaughter only at approved slaughtering establishments.
(2) Buildings, farrowing pens, and equipment, etc., to be cleaned and disinfected following removal of reactors or the entire herd.
(3) The accumulated level of infection for a Validated Brucellosis-Free Area may not exceed five percent of the herds or one herd, whichever is greater, in the area over the 3-year validation period.

E. Movement of swine into Validated Brucellosis-Free Areas
(1) Swine originating in Validated Brucellosis-Free Herds or negative, nonvalidated herds in Validated Brucellosis-Free Counties may enter a nonvalidated herd without test provided such swine are moved directly and have not been in contact with swine of lesser status.
(2) Swine originating in herds not described in paragraph (1) must have passed a negative test within 30 days prior to date of entry,
be held in isolation, and pass one negative test within 30-60 days before entering the herd.

(3) Swine for feeding purposes may be held on negative, nonvalidated and validated, herd premises without test provided such swine are held in complete isolation, separate and apart from all breeding stock.

(4) Swine consigned to slaughtering establishments in a Validated Brucellosis-Free Area are to be transported directly to the holding pens at the slaughter establishments.

(5) All swine entering a Validated Brucellosis-Free Area shall be transported in vehicles properly cleaned and disinfected prior to loading unless consigned directly to a slaughter establishment.
TRANSMISSION OF RINDERPEST VIRUS FROM EXPERIMENTALLY-INFECTED CATTLE TO PIGS

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Little is known concerning the susceptibility of pigs of European breeds to rinderpest virus as a result of contact with infected cattle. According to Scott et al.,\(^1\) natural infection of European pigs with rinderpest virus has not been reported. Nicholas and Rinjard,\(^2\) Robertson,\(^3\) Curasson,\(^4\) and Carmichael\(^5\) have reported success in experimental transmission of rinderpest to pigs of European origin. Scott et al.\(^6\) have reported experimental transmission of rinderpest virus to pigs of European origin by parenteral inoculation, feeding, and infection of pigs and cattle by contact with infected pigs. DeLay, et al.\(^7\) have shown that crossbred Yorkshire-Tamworth pigs develop viremia and a mild transitory febrile response after inoculation with rinderpest virus. It was concluded, however, that because of the mild clinical response, infection in pigs could easily escape detection.

This report describes contact transmission of rinderpest virus from experimentally-infected cattle to pigs and observations on the survival of infective virus in tissues of convalescent pigs.

MATERIALS AND METHODS

Viruses

Rinderpest virus, Pendik, received from Kenya, East Africa, as accession 214 and passaged twice in cattle at Plum Island Animal Disease Laboratory (PIADL), was used to infect cattle and pigs. This virus has been recognized as one which is readily transmitted among cattle. The inoculum was a suspension of mesenteric lymph nodes (MLN) from infected cattle diluted 1:1 with buffer.

Rinderpest virus, Kabete 0, PIADL passage two was used as challenge virus for cattle. Both the Kabete and Pendik viruses were lethal for cattle in one-ml. amounts at \(10^{-5}\) dilution.

The Nakamura III strain of rinderpest virus was used for virus neutralization (VN) tests in rabbits.

Animals

The animals used were as follows: pigs, Tamworth, four to seven months old; cattle, grade Herefords, 18 months old; rabbits, adult New Zealand white.

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TRANSMISSION OF RINDERPEST VIRUS

**Materials**

Liquid thioglycollate and National Institutes of Health (NIH) agar were used to test for bacteriological contamination in pig tissues. The buffer used was 5.52 Gm. of NaH$_2$PO$_4$·H$_2$O and 9.56 Gm. of Na$_2$HPO$_4$ per liter which had a pH of 6.8. Sodium citrate 3.8 percent, one ml. per ten ml. of blood was used as an anticoagulant.

**Serology**

The VN tests in rabbits were done according to the method of Scott and Brown, 1958.$^3$ The agar gel precipitin test was conducted in accordance with the method described by White, 1958,$^9$ and modified by Stone and DeLay.$^{10}$ The serum was from rabbits hyperimmunized with Nakamura III rinderpest virus.

**EXPERIMENTAL PROCEDURE**

Each of two steers, housed with 15 pigs, was inoculated intramuscularly (IM) with at least 1,000 bovine lethal doses (BLDs) of rinderpest virus. The inoculation site was shaved, and washed before and after inoculation with four percent acetic acid which, in preliminary trials, appeared to be virucidal.

Four pigs in another isolation room were inoculated IM with 10,000 BLDs of rinderpest virus, Pendik, to test the reaction of pigs to the virus. Viremia was accepted as evidence of infection in pigs.

To preclude infection other than by natural contact, temperatures of pigs in contact with rinderpest-infected cattle were not taken. All 15 pigs were tested for viremia and rinderpest antibodies on the tenth day after the donor steers were inoculated with rinderpest virus. Blood was collected from the vena cava after shaving and disinfecting the skin. A pool of citrated whole blood from the 15 pigs was injected, in 120-ml. amounts, into each of two steers.

Virus detection tests were conducted with tissues from pigs at 10 and 38 days after inoculation of steers. Each of two normal steers received 40 ml. (IM) of a 30 percent suspension of spleen and MLN from seven pigs killed at 10 days. Forty ml. of a 50 percent suspension of spleen, MLN and bone marrow (femurs) from four pigs killed at 38 days were injected IM into each of two normal steers. The tissues were taken from the pigs and suspensions prepared in accordance with the procedure described by DeLay et al.$^7$

To evaluate further the carrier status of pigs infected by contact, two susceptible steers were placed in the contact exposure room with four convalescent pigs six days after the infected donor steers had been removed. This room had been cleaned but not disinfected in the interim.

**RESULTS**

Two donor steers in direct contact with 15 pigs developed signs of illness within four days after inoculation with rinderpest virus. One steer
was found dead six days postinoculation (DPI) and the other animal was killed *in extremis* seven DPI.

Two steers, inoculated with a pool of blood from the 15 contact pigs 10 days after exposure to rinderpest infected cattle, developed a febrile reaction and typical rinderpest lesions. Two steers injected with tissue suspensions taken from pigs 10 days after exposure to the two donor steers developed signs and typical lesions of rinderpest. Agar gel diffusion tests with rinderpest hyperimmune serum and MLN suspensions from the steers were positive.

Tissues taken from four pigs at 38 days after exposure to donor steers were not infective for cattle.

Bacteria were not detected in suspensions of pig tissues tested in NIH agar and thioglycollate.

Two susceptible steers failed to develop neutralizing antibodies or signs of rinderpest during 18 days confinement with four rinderpest-convalescent pigs in the room initially used to house infected cattle.

Blood taken six DPI from four virus control pigs was infective for cattle.

Neutralizing antibodies were found in sera of convalescent pigs at 31 and 38, but not at 10 days after exposure (Table I).

**DISCUSSION**

The presence of virus in tissues of pigs 10 days after exposure to rinderpest-infected cattle suggests that pigs could, during early convalescence, contribute to the spread of the disease. Since pigs do not develop marked signs of rinderpest, control of the disease would be further complicated particularly in livestock production areas heavily populated with cattle and pigs.

The failure of cattle to become infected after 18 days exposure to four rinderpest convalescent pigs in a room which six days previously contained infected cattle, was unexpected. Failure to demonstrate infective virus in the room may have been due to a 13-day interval between exposure of pigs and introduction of infected cattle, fragility of virus (Scott1), and/or daily washing of the room.

**SUMMARY**

Pigs described in this report became infected with rinderpest virus after exposure to experimentally-infected cattle. Virus was found in tissues of pigs at 10, but not 38 days following exposure to cattle injected with the virus.

Rinderpest virus neutralizing antibodies were found in sera of pigs at 31 and 38, but not at 10 days after contact exposure to inoculated cattle.
TABLE I

Results of Virus Neutralization Tests in Rabbits with Sera from Animals after Exposure to Rinderpest

<table>
<thead>
<tr>
<th>Virus Dilutions</th>
<th>10^-2</th>
<th>10^-3</th>
<th>10^-4</th>
<th>10^-5</th>
<th>10^-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sera</td>
<td>Titration*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit hyperimmune rinderpest antiserum</td>
<td>2/2**</td>
<td>2/2</td>
<td>2/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Serum pool - 3 pigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-exposure to infected cattle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>killed 10 days post-exposure</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Serum pool - 4 pigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-exposure to infected cattle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>killed 10 days post-exposure</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>Serum pool - 4 pigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-exposure to infected cattle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>killed 31 days post-exposure</td>
<td>2/2</td>
<td>1/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Serum pool - 4 pigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-exposure to infected cattle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>killed 38 days post-exposure</td>
<td>2/2</td>
<td>1/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Serum pool - 2 steers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-exposure to convalescent pigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>killed 34 days post-exposure</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
</tbody>
</table>

*Nakamura III lapinized virus.
**Rabbits infected/rabbits tested.

REFERENCES

REPORT OF THE COMMITTEE ON FOREIGN ANIMAL DISEASES


Serious and devastating exotic-type diseases of animals are now assuming a greater relative importance than ever before. These diseases at times are described as "emerging" diseases, a new term which seems to indicate new disease situations.

In January 1962, an African type of foot-and-mouth disease virus (SAT-1-FMD) was reported in the livestock located on a group of islands in the Persian Gulf off the east coast of Arabia. The disease was later reported in Iraq, Israel, Lebanon, Jordan, and Syria. It continued to spread until in June it was diagnosed in Turkey and Iran. This is the first time that SAT-1-FMD virus has become established outside Africa.

In 1959, African horse sickness appeared in the Middle East and in 1960 spread throughout the near and Middle East with devastating effects on the equine population and becoming established for the first time outside Africa.

In 1957, the deadly African swine fever was reported in the Iberian Peninsula, appearing first in Portugal and subsequently spreading into Spain. Again, an African disease being reported outside that continent.

During the last year equine piroplasmosis was diagnosed for the first time in the United States. To date there have been nearly 100 confirmed cases, all confined to southern Florida. The Florida cases have been caused by Babesia caballi which is transmitted by ticks. Efforts at controlling the disease are underway through control of the vectors, and through research aimed at improving methods for diagnosis and control.

The presence of any one of these diseases has serious effects on the areas or country in which it appears. They are of international economic importance because of the loss of food resources, loss of markets by quarantines and embargoes essential for control. These diseases can cause major disturbances of normal trade in livestock and products. Their presence influences the economic development of the more primitive countries where the farmer is dependent on a lone horse, oxen, or water buffalo for his livelihood.

These disease outbreaks have added new complexities into the world health situation and they point out the danger of sudden invasion by epizootic diseases into countries where the diseases have never before appeared. International trade and commerce are continually expanding and the opportunity for the movement of insect vectors and contaminated product that carry disease to distant places increases. These emerging diseases can enter the United States from any area of the world. For example,
as the African diseases escape from that continent and gain entrance into the world trade channels, they become a constant and very serious threat to the livestock and economy of this country, as well as other countries of the world.

Professionally, all veterinarians have a responsibility to continually keep this threat in mind. These diseases can no longer be regarded as exotic African curiosities of purely academic interest. We must realize their significance. We must be able to recognize them quickly in order to prevent or control their spread. We must, in effect, understand and know them thoroughly.

The United States Livestock Sanitary Association President, Dr. A. P. Schneider, in his 1961 address at Minneapolis, suggested that the Association's 1954 publication, Foreign Animal Diseases, be revised to bring it up-to-date on those diseases for which new information is now available and to eliminate those diseases no longer considered foreign to this country. He pointed out the unique character of the publication and mentioned its wide use. As a result of his suggestion, nine members were designated as a Committee on Foreign Animal Diseases, reactivating the former Committee on Exotic Diseases.

The value of a handbook on foreign animal diseases to regulatory personnel, practitioners, and educators here in the United States as well as in foreign countries has been repeatedly cited by a number of authorities. The wide distribution of the first edition is indicative of the use made and interest shown in this publication. The impact of extensive economic losses has caused grave concern in many areas and a new look at disease preventive measures long considered by many authorities as adequate to protect a country or region. Consequently, regulations as well as inspection and quarantine procedures once relatively fixed are now in a fluid state and should be reviewed in a document such as this.

International participation in control programs for various diseases which may involve many veterinarians never before participating in situations of more than provincial interest is expanding. It is essential that the scope and effect of such programs be brought to the attention of veterinarians in order that the profession may draw on informed people, particularly in emergency situations. Therefore, the Committee plans to include a substantial section on international aspects of diseases, including the technical activities currently underway in disease control programs.

Members of the Committee on Foreign Animal Diseases have discussed the design of the revision to permit more effective coverage of material, deletion of sections covering diseases no longer considered foreign to this country, inclusion of sections on exotic diseases not appearing in the first edition, and remodeling of charts and appendices to reflect current situations. In addition to such proposed changes, it has been suggested that material will be presented in the form suggested in the American Institute of Biological Sciences' Style Manual for Biological Journals, and that each section of the book will include a guide to the literature that will provide the interested scientists with a ready and current reference to more sophisticated literature.
Individuals with knowledge and experience in foreign animal diseases were contacted and requested to participate in the work of the revision. Several draft manuscripts have been prepared and are now being reviewed by members of the Committee or experts whose assistance was solicited by the Chairman. Judging from the response from members of the Committee and other participating scientists, most of the draft material to be included in the revision should be received by January 1, 1963. It is the objective of the Committee that all material for inclusion shall be received and put in proper form for publication by mid-1963, and that a target date coincident with the next United States Livestock Sanitary Association meeting shall be set for publication.

The Committee wishes to draw attention to the financial requirements for publishing and printing. It suggests that the Executive Committee and the Committee on Programs and Policy study and recommend methods of meeting this expense. It should be noted that a part of the cost of publication will be recovered through sales of the book. The work involved in preparation of material will be through voluntary contribution by a number of experts.

It is understood that a number of copies of the 1954 edition are still available. It is suggested that since the current revision will outmode the original report the current supply, if adequate, be given to the veterinary college libraries.

Finally, the Committee is of the opinion that a part of the value in a publication of this nature depends on how current its contents may be. This implies that periodic revision is essential. Since this Association's accomplishments in keeping abreast of current animal disease situations has resulted in considerable prestige, the Committee recommends that revision be scheduled at regular intervals in the future. Such an effort would not entail nearly as much work and time as this first revision which comes after a period of 9 years during which time significant changes and findings in the epizootiological aspects of a number of diseases have occurred.
ANAPHYLAXIS IN CATTLE RECEIVING
SERUM-FREE LEPTOSPIRAE

R. L. Morter, D.V.M., Ph.D., B. L. Valentine, M.S., and T. Tapacio,
D.V.M., Ph.D.*

Leptospirosis has been recognized as an important disease of cattle
with economic losses resulting from abortions, decreased lactation, re-
duced gains, and deaths. Deaths most frequently occur in young animals
of all breeds or feed lot cattle.

Commercial bacterins have been effective in protecting cattle of all
ages against subsequent exposure to *Leptospira pomona*1,2,3. The bacterins
do not alter the course of the disease in infected animals. The spread of
leptospirosis in infected herds can be controlled by prophylactic immuni-
ization. Temporary immunity induced by the bacterins necessitates re-
peated immunization4,5. In geographic areas where the disease is endemic
or in problem herds, cattle are frequently reimmunized at six to nine
month intervals.

Most commercial bacterins are prepared from cultures grown in fluid
media containing 10 percent rabbit serum. The final concentration of rab-
bit serum in the bacterin is dependent upon the processing methods and
varies from traces to the 10 percent in the original medium.

The use of *L. pomona* bacterins has been attended by signs suggestive
of anaphylaxis. Revaccination of cattle 10 days after initial injection with
*L. pomona* bacterin was accompanied by signs of hypersensitivity.6 Bink-
ley7 indicated rabbit serum to be the cause of anaphylactic reactions as-
sociated with leptospiral bacterins.

Anaphylaxis is a manifestation of an antigen–antibody reaction. The
small quantity of antibody detected in cattle by agglutination-lysis tests
following vaccination does not indicate the degree of sensitization which
may occur following use of the bacterins.

Animals can be sensitized with many antigens, including those of bac-
teria, although serum or serum protein fractions are most commonly em-
ployed to induce systemic anaphylactic reactions experimentally.

To ascertain if *L. pomona* antigens are capable of causing systemic
anaphylactic reaction, calves were experimentally exposed to *L. pomona*
and subsequently exposed to serum free preparations of disrupted lepto-
spirae. One of two experimental groups of calves received antihistamines
to further clarify the nature of the reaction.

Submitted as Journal Paper No. 2004, Purdue University Agricultural Experi-
ment Station, Lafayette, Indiana.

*Present address: College of Veterinary Medicine, University of the Philip-
pines, Quezon City, Philippine Islands.
Preparation of leptospirae

Leptospirae were grown in modified Chang's medium prepared from tryptose (Difco) 1.0 gm., liver extract concentrate (1:20) 0.7 gm., disodium hydrogen phosphate (Na$_2$HPO$_4$·7H$_2$O) 2.0 gm., potassium dihydrogen phosphate (KH$_2$PO$_4$) 0.4 gm., NaCl 4.0 gm., distilled H$_2$O 1,000 ml. The pH was adjusted to 7.2 - 7.4. Four hundred ml. of the medium was dispensed in 1,000 ml. prescription flasks and autoclaved for 20 minutes at 121 C. Following the addition of 10 percent sterile rabbit serum the flasks were held at 56 C. for 30 minutes in a water bath and incubated for 24 hours at 37 C.

The strains of L. pomona used were: Johnson, L. W. and 3050. Each flask was inoculated with 10 ml. of a seven day culture of L. pomona incubated for 10 days at 28 C. and examined for bacterial contamination. Fluid thioglycollate media (Difco B256) and blood agar plates served as contamination control media. The leptospirae were harvested at 0 C. in a continuous flow centrifuge at RCF=34,800 g. with a flow rate of 40 ml./minute. The sedimented cells were washed three times with approximately 40 ml. of isotonic phosphate buffer and resuspended in the phosphate buffer to one percent of the original culture volume.

The cells were disrupted by sonication at 10 kc./sec. for five minutes and stored at -20 C. Nitrogen was determined by micro-Kjeldahl technique.

Assay for rabbit serum

The cell preparations were assayed for residual rabbit serum by a double diffusion agar gel precipitation test. The agar gel was prepared by dissolving Noble's Agar (Difco) in a phosphate buffer saline solution and dispensed in 20 ml. amounts in 100 mm. petri dishes. Commercial sheep anti-rabbit globulin serum (Nutritional Biochemical Corporation, Cleveland, Ohio) was used in a 1:10 dilution. Antiserum was placed in a center well and the antigens (rabbit serum or leptospirae cell preparation) in six peripheral wells. Precipitin bands were observed unstained or following staining with 0.5 percent nigrosin.

Experimental Animals

Eleven dairy and beef calves, 10 to 12 months of age, served as experimental animals. Three immunized calves and two nonexposed animals received an I.V. injection of disrupted cells of two strains of L. pomona (Table I). The immunized calves had received L. pomona living or dead preparations four to five and one half months previously.

To determine the effectiveness of antihistamines in alleviating the anaphylactic reactions six immunized calves were treated approximately 20 minutes prior to I.V. or S.C. injection with L. pomona cell preparations (Table II).
TABLE I

<table>
<thead>
<tr>
<th>Calf Number</th>
<th>Months Post Infection</th>
<th>Titer</th>
<th>Strain L. Pomona</th>
<th>Inoculum mg. N</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$4^1_2$</td>
<td>$10^3$</td>
<td>3050</td>
<td>27.08</td>
<td>Severe</td>
</tr>
<tr>
<td>2</td>
<td>N.I.*</td>
<td>$10^0$</td>
<td></td>
<td>6.00</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>$5^1_2$</td>
<td>$10^3$</td>
<td></td>
<td>14.33</td>
<td>Severe</td>
</tr>
<tr>
<td>4</td>
<td>N.I.</td>
<td>$10^0$</td>
<td>Johnson</td>
<td>14.33</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>$10^1$</td>
<td></td>
<td>14.33</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

*N.I. = non-infected controls

TABLE II

<table>
<thead>
<tr>
<th>Strain</th>
<th>Route</th>
<th>Calf</th>
<th>Treatment</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.W.</td>
<td>S.C.</td>
<td>A</td>
<td>*Chlorprophenpyrimadine</td>
<td>Mild</td>
</tr>
<tr>
<td></td>
<td>S.C.</td>
<td>B</td>
<td>Maleate 20 mgs.</td>
<td>Moderate</td>
</tr>
<tr>
<td>3050</td>
<td>S.C.</td>
<td>C</td>
<td>**Thenylpyramine</td>
<td>Mild</td>
</tr>
<tr>
<td></td>
<td>I.V.</td>
<td>D</td>
<td>Hydrochloride.</td>
<td>Moderate</td>
</tr>
<tr>
<td>Johnson</td>
<td>I.V.</td>
<td>E</td>
<td>0.5 ml./100 lbs.</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>I.V.</td>
<td>F</td>
<td></td>
<td>Moderate</td>
</tr>
</tbody>
</table>

*Schering, Chlor-Trimeton
**Pitman-Moore, Pyrahistine

RESULTS

Rabbit serum was not present in detectable quantities in the cell preparations. Zones of precipitation formed between the dilutions of rabbit serum and the anti-rabbit globulin serum. Bands of precipitate were not detectable with any of the cell preparations (Figures 1 and 2).

During the intravenous injections of serum free leptospires signs of acute anaphylactic shock occurred in all three immunized calves. Both strains of leptospires produced similar results. Muscular tremors were followed by dyspnea and acute ataxia. Two calves, numbers one and five, lost consciousness and respirations ceased for one to two minutes. Epsistaxis was observed in calves one and three (Table I). All three calves recovered. Dyspnea continued for one half to one hour. Eighteen hours post injection the respirations were normal and the calves were eating.

Antihistamines reduced the severity of anaphylaxis. All six calves had muscular tremors and dyspnea but none collapsed. The symptoms
were more pronounced in the calves that received the I.V. injection than they were in those inoculated S.C. The strain of *L. pomona* employed had no apparent effect on the severity of the reaction.

**DISCUSSION**

The agar gel precipitin test results indicate that the leptospirae cell preparations were relatively free of rabbit serum. A sensitivity of one to two ug/ml. for a rabbit globulin: antiglobulin system with methods similar to those employed has been reported. Had a minute amount of rabbit serum been present in the cell preparation, it probably would not have produced the anaphylaxis.

The acute respiratory embarrassment elicited in the immunized calves by S.C. and I.V. injections of the serum free leptospirae was typical of an anaphylactic reaction. The reaction was observed in animals that had had previous exposure to rabbit serum from injection of whole cultures and in those calves that had received injections of washed cells. The participation of a leptospiral cell component in an anaphylactic type antigen-antibody reaction was strongly suggested.
Individuals vary greatly in the amount of antigen required to cause anaphylaxis, viz. the 100 fold variation in amount of antigen that will induce the reaction in guinea pigs. Some individuals are susceptible to anaphylaxis when given less antigen than was administered to produce sensitizing antibodies. It is possible that in sensitized cattle the bacterin could contain an anaphylactic inducing dose of antigen.

CONCLUSION

Evidence suggests that cattle were subject to sensitization by *L. pomona* antigens since subsequent exposure to *L. pomona* cell preparations was followed by signs of acute anaphylaxis. The severity of the reactions were moderated by the administration of antihistamines. The rule of a cellular component of *L. pomona* in this type of reaction is strongly suggested.
REFERENCES


SOME ASPECTS OF BOVINE LEPTOSPIROSIS CONTROL*


Pullman, Washington

Bovine leptospirosis may exist as an unrecognized herd infection until blood tests or clinical signs suggest the presence of infection. In many beef herds, the first observed evidence of infection may be abortions, and since infection may not result in abortion, this criterion is not reliable. The use of Leptospira pomona bacterins appears to have reduced the occurrence of leptospirosis outbreaks in the Northwest. Recurrence of leptospirosis has been reported in herds two or three years after interruption of the leptospirosis vaccination program.1 The recognition of other bovine serotypes in the United States2,3 obviously raises the question of polyserotype bacterins. As further information becomes available concerning the role of these serotypes in the disease picture, a more complete answer should be available. This is particularly exemplified by a report that L. hardjo was still present in the urine of a calf 499 days after experimental inoculation.4

Concomitant infections and persistence of leptospirosis AL titers may often confuse the etiology of disease outbreaks. Extensive studies suggest that the hemolytic test5,6 may be a satisfactory serologic means for the recognition of cattle which harbor L. pomona. Since this involves a genus specific antigen, any of the serotype infections should be detected. Identification of the infecting serotype requires use of microscopic slide, plate or tube tests employing specific serotype antigen(s).

Vaccines

Bacterins have been evaluated in a number of investigations and found to stimulate an effective immunity in a herd for about 12 months7,8,9,10 Persistence of immunity for 20 months10 has been reported. Vaccination is recommended for cattle in areas where leptospirosis is diagnosed, in feedlots, or for animals consigned to sales or fairs. Vaccination should be carried out about two to three weeks preceding shipment. In the event of an outbreak, all clinically healthy animals should be vaccinated. Obviously further acute infections associated with hemoglobinuria, abortions, or death may occur, since the bacterins should not be expected to protect

*Since these comments are not intended to be complete review of the literature, only limited numbers of references have been cited.

**The authors are associated with the Washington Agricultural Experiment Stations, Department of Veterinary Science and Department of Veterinary Microbiology, College of Veterinary Medicine, Washington State University, Pullman. Work conducted under project 1176.

These investigations were supported in part by: Research grant E707-C4, from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, Public Health Service.

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animals already infected or those that become infected before the bacterin immunity developed.

Some practitioners \(^1\), \(^1\) report that abortions cease within a week after vaccination. Yet, other reports \(^2\), \(^3\) indicate that abortions may continue for three to five weeks. Extensive degeneration of some aborted fetuses indicate that fetal death would have occurred several weeks before the actual abortion. On the basis of experimental studies the continuation of abortions should be expected. \(^4\), \(^5\) If immunity is closely associated with demonstrable AL antibodies, experimental studies suggest that immunity may be delayed for 10-14 days or more after vaccination. \(^6\) Actual exposure trials suggest that practical immunity may sometimes be present by the seventh day following vaccination with a bacterin. \(^5\)

Calves nursing bacterin-vaccinated dams have been found immune to exposure. \(^6\) In experimental studies, AL antibodies have been demonstrated for more than six months in calves dropped by recovered and even actively infected dams. \(^6\) Often the titer of the colostrum and calf's blood will be three to ten times higher than the humoral antibody level of the dam. Calves born to cows, six* years after recovering from infection, may still demonstrate a high humoral antibody level shortly after birth. These titers sometimes persist at reduced levels up to 12 months. \(^6\)

On subsequent vaccinations, an allergic response, apparently associated with foreign serum proteins in the bacterin, has been described. Through removal of rabbit serum proteins from the bacterins, it is reported that danger from allergic reactions has been reduced. \(^7\)

Early work indicated that an attenuated live vaccine (ALV) may afford a satisfactory immunity for at least 17 months. More recent studies indicate that immunity to exposure was observed for at least 54 months. \(^8\) Since demonstrable AL antibodies are present by the fourth day, an earlier immunity is expected than when the bacterin is employed. \(^9\)

Experience from comparative bacterin and ALV studies indicates that the live culture was about three times more effective than the bacterin in reduction of abortions. \(^9\) No evidence of transmission has been observed under experimental conditions, although a mild nephritis has been observed in biopsied renal tissues of cattle vaccinated with ALV. Although AL antibodies are usually demonstrable in the urine of naturally infected animals, \(^9\) cattle given ALV have not excreted such agglutinins during our observations. \(^9\)

Control of Animal Traffic

Herd additions and replacements should always be quarantined for observation and blood testing to avoid herd exposure during early phases of infection. The use of bacterin on new herd additions has been suggested, but some experimental studies \(^9\) indicate that vaccination during the shedding state increases the level and duration of shedding. Intensive antibiotic therapy for valuable stock might be considered if economically feasible, since such procedures are known to eliminate leptospirosis. \(^2\)

If the quarantine of infected bovine herds is based on the hemolytic test, control should be quite effective since this test apparently recognizes the shedding animals. However, we should not overlook the possible

*Recent tests indicate at least eight years.
significance of wildlife as a source of infection, which might complicate quarantine procedures. Adequate control of wildlife populations should reduce dangers from disease transmission to domestic livestock. However, the role of rodents and wildlife has not been clarified.\textsuperscript{2,3,22}

\textit{Reduction of Stress}

We all recognize many factors that are considered important in producing stress. Cold, rainy weather, muddy barnyards and poor quality forage have been associated with some of the severest leptospirosis outbreaks we have observed.

The use of tranquilizers, electrolytes and/or antibiotics before shipping or hauling of animals has been suggested\textsuperscript{23,24} and under guidance of the clinician would serve a very useful function.

One cannot overlook the role of vitamin A in the maintenance of health and as an antistressor. Vitamin A deficiencies may exist in spite of a satisfactory NRC carotene level in the feed.\textsuperscript{25} Even the presence of low vitamin A levels in the feed is of questionable value to the animal with a low level storage, since carotene may not be effectively converted to vitamin A. Since vitamin A is such an important constituent of the feed ration, and extensive evidence indicates that conversion of carotene may not be satisfactory because of possible interfering materials or a lack of carotene, adequate preformed vitamin A should be added to the feed.\textsuperscript{26} Water soluble products which offer definite advantages under some conditions should be considered.

Stress as a triggering mechanism for leptospirosis in apparently recovered animals, should be considered.\textsuperscript{27} We have evidence that the renal tissues may harbor \textit{L. pomona} at least 126 days post inoculation.\textsuperscript{4} Other findings indicate that \textit{L. pomona} leptospirosis was demonstrated for 108 days in a cow infected in a field outbreak,\textsuperscript{19} and that biopsied kidney tissues from an inoculated cow were positive by culture on the 104th day after infection.\textsuperscript{27} Serological evidence from the serum hemolytic test suggests that latent infection may exist for over a year.\textsuperscript{19} Most animals cease demonstrable shedding of \textit{L. pomona} within three months (utilizing present laboratory methods) and become negative to the serum hemolytic test within five months.\textsuperscript{5}

\textit{Chemotherapy}

Broad spectrum antibiotics in the feed will prevent leptospirosis in the ox.\textsuperscript{21} Acute bovine leptospirosis is often treated with broad spectrum antibiotics, penicillin or dihydrostreptomycin. Leptospirosis appears to be most successfully treated with dihydrostreptomycin, although broad spectrum forms are also effective.\textsuperscripts{28} Toxemias have complicated the recovery of cattle experiencing abortions associated with acute leptospirosis and retained fetal membranes.\textsuperscripts{11,12,29} Practitioners have employed antibiotics and vitamin-mineral supplements as therapeutic measures for these cases.\textsuperscripts{11,12,29}
Control of Surface Waters

The significance of water-borne leptospiroses was emphasized by early workers.\(^1\) As anticipated \textit{L. pomona} has been demonstrated in surface drainage waters from ranges pastured by infected herds.\(^2,3\) We have observed severe outbreaks apparently associated with extensive contamination of a pond or reservoir during the late summer. Certainly the danger of infections can be reduced by correction of drainage around watertanks, in pastures and farmyards and by limiting access to ponds and flowing streams. Irrigated pastures should be rotated if possible to favor self-sterilization of waterpools.

Summary

A general review of certain factors affecting prevention and control of bovine leptospirosis is presented. Particular emphasis is given to the use of vaccines, control of animal traffic, reduction of stress, chemotherapy and control of surface waters.

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4. Roth, Earl: Louisiana State University, Baton Rouge, Louisiana: Personal communication. 1962.
REPORT OF THE COMMITTEE ON LEPTOSPIROSIS


Previous reports of this Committee were considered; however discussion was centered on the more important aspects of leptospirosis as related to diagnosis and control. On most points the collective opinion of this Committee remains the same. It appears in order to restate that the consensus of opinion is that leptospirosis is not amenable to eradication. We do, however, feel that the economic losses to the livestock industry can be reduced by an accurate, early diagnosis by proper management practices and by the intelligent prophylactic use of available bacterins. Consequently, the maximum improvement of diagnostic tests and prophylactic measures is of utmost importance.

Bacterins prepared from *L. pomona* have been widely used but few critical studies of its efficacy have been reported. Some observations on variability in the immune response of different vaccines has come to the attention of this Committee. We are following with interest the evaluation of bacterins at the National Animal Disease Laboratory. The anticipated introduction of more stringent testing procedures may tend to upgrade the immunogenic value of these bacterins. It would be highly desirable to have controlled efficacy trials conducted in cattle or swine. This may well be done as a cooperative effort between several agencies or on a regional research basis between several states.

Consideration should be given to the evaluation of macroscopic plate antigens presently available. They should be evaluated for uniformity, sensitivity, stability and specificity. These recommendations are prompted by reported discrepancies between the results obtained with the agglutination-lysis test and the plate tests. The plate tests appear to serve a useful purpose but should be used as a herd diagnostic tool rather than on an individual animal basis in the cases of cattle and swine.

We reviewed the knowledge concerning anaphylaxis in cattle associated with the use of leptospiral bacterins. Initially it was thought that the rabbit serum component of the bacterin was the sole cause of these reactions, but evidence obtained recently suggests that cellular components of the leptospiral organism may play a causative role in these reactions. These observations serve to emphasize the need to exercise caution in the use of these bacterins.

We still recognize the need of a simple test that will accurately detect a carrier animal and also recognize the need of an economically feasible and effective method for eliminating the carrier state.
Leptospira pomona remains the most important recognized cause of leptospirosis in cattle and swine. Leptospira canicola and L. icterohaemorrhagiae are recognized as causes of leptospirosis in dogs. In a few instances, L. canicola and L. hardjo have been found associated with signs of disease in cattle. Serological evidence of a high incidence of infection with the latter or a related serotype among cattle in some Southeastern states has been established. The significance of these findings should be investigated.

There are other leptospiral serotypes present among wildlife in this country that so far have not been found associated with signs of diseases in domestic animals. It is significant to note that some of these serotypes cause disease in cattle or swine in other parts of the world. Hyos serotypes present among striped skunks and opossums in this country have been identified with mild porcine leptospirosis in Australia, Hungary, Switzerland, Argentina and the Belgian Congo. Leptospira icterohaemorrhagiae, found principally in rats in this country, is associated with infection of swine in the Netherlands and England. Leptospira grippotyphosa, present among raccoons, opossums and striped skunks, is associated with bovine leptospirosis in Israel and Russia. Leptospira autumnalis, which causes bovine leptospirosis in Japan is found among opossums and raccoons in this country. All pathogenic leptospires apparently pose a public health hazard.

The above information is included in this report for the purpose of creating an awareness of these serotypes that may be potential infection hazards. The significance of this is more fully appreciated when one considers the dynamic aspect of the leptospiroses. Changes in virulence and host predilection of the organism may alter its spectrum of host-parasite relationships so that disease is produced in domestic animals. In addition, changes in ecological factors and livestock management practices may produce changes in host-parasite relationships.

We respectfully submit this report to the Executive Committee for approval and suggest that the work of this committee continue.
Bovine mastitis, while by no means a newly recognized disease, is surrounded by more confusion and greater lack of well organized and logical attempts toward control than possibly any other dairy cattle anomaly. It is also the most prevalent and costly disease of dairy cattle and of greater economic importance to the dairy industry than any other cattle disease with which we are confronted.

According to figures published in 1954 by the Agricultural Research Service in its report on "Losses in Agriculture," it was estimated that mastitis cost the dairy industry nearly a quarter of a billion dollars. Further analysis of mastitis loss estimates indicated animal loss as 12 percent of total cattle disease losses and milk loss accounting for 71 percent of milk losses caused by all cattle diseases.

Present day estimates are much greater. Based on figures obtained from the April, 1962, issue of Milk Production, Disposition and Income, 1960-61, USDA Statistical Reporting Service, Crop Reporting Board, covering milk cow population, milk production, and farm value of milk produced, a loss of over a half billion dollars due to mastitis is indicated. These figures represent only the loss of animals and milk and do not include the cost of treatment.

There is no question but that the economic welfare of the dairy cattle industry and the veterinary practitioner is closely associated with the control of bovine mastitis. It is important that the veterinary profession be actively and aggressively associated with mastitis control as with other disease control projects.

In addition to the economic importance which should stimulate a sense of urgency in controlling mastitis, a number of problems concerned with regulatory policy as well as the public health aspect of the disease exist. All regulatory agencies require that milk shall be procured from disease free animals. Mastitis, in its broad sense, is an inflammation of the udder and as such is a symptomatic condition resulting in the production of abnormal milk. As a physiological disorder of the mammary gland, associated with various infective organisms, it may be classified as a disease transmissible from animal to animal and potentially to man. Since its incidence is extremely high, strict enforcement of existing regulations could have a tremendous impact on the dairy industry in the loss of available milk supplies. From the public health view, human infection may result from the consumption of raw milk containing organisms which are often involved in bovine mastitis. Food poisoning may result from enterotoxin which may be present as a result of the growth of staphylococcus aureus.

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Such food poisoning outbreaks, though rare, have been reported abroad as well as in some of our mid-western states as a result of consumption of certain dairy products.

The presence of antibiotics in milk as a result of treatment has also been considered as a public health problem. It has been estimated that one out of every ten people in the United States are prone to antibiotic sensitivity. Persons who develop sensitivities to certain antibiotics may experience severe allergic reaction after consuming milk or milk products containing antibiotic residues.

Concern over both the economics and public health aspects of this disease prompted the Executive Board of IAMFS to sponsor a mastitis action conference in the Fall of 1960 for the purpose of presenting a review of the problem of bovine mastitis to interested leaders of the dairy industry, to health and agricultural agencies of federal, state and local government, and to certain professional groups in the hope of initiating a uniform national effort for the control of this disease. As a result of this action which considered the public health, economic, research, and regulatory aspects of this disease, the National Mastitis Council was formed and later incorporated as a non-profit organization to serve as a national force in furthering mastitis research and control.

The first activity of the newly formed council was to determine what is presently being done in the fields of research, education and control programs. Questionnaires were sent to several potential sources of information in each state. These sources included state health departments and Departments of Agriculture, colleges of agriculture and veterinary medicine, agricultural experimental stations and extension services. The return from these questionnaires was gratifying and at least showed an enthusiasm on the part of respondents in regard to the problem of bovine mastitis and that a significant effort is being made with widespread planning for further control activities.

Most states have some type of educational program capable of distributing literature on suggested mastitis control measures. Activities were shown to vary from well defined state-wide all-encompassing programs (which are quite rare) to those of a very localized, highly restricted activity such as quality checks on raw bulk milk with little or no follow-up activity. The lack of similarity in existing programs and varying degrees of effectiveness indicated a need for development of a uniform and practical approach to the problem.

Unlike tuberculosis and brucellosis which are caused by specific organisms and readily lend themselves to test and elimination procedures, mastitis is a disease which may be associated with a variety of organisms and aggravated by a multitude of stress factors. There are, therefore, certain points which are imperative when considering a well organized program to combat this disease. If we restrict a program to any one phase of the problem without giving attention to the over-all picture, definite limitations will occur.

A well organized program should be based on several factors or phases:
1. A well developed educational program.

2. Screening tests through examination of milk supplies as an indication of the existence of mastitis in individual herds.

3. Diagnosis of mastitis either physically and/or bacteriologically in the herds indicated to have problems.

4. Installation of an over-all good management program to include regular inspection of the milking equipment by qualified technicians.

An approach which ignores any one of these phases will have little success. If we should follow the diagnostic approach without any recognition given to management and prevention, our success would be nullified because it takes something more than diagnosis and treatment to control bovine mastitis. On the other hand, if we approach it from management alone, without recognizing the fact that it is a disease of the cow's udder associated with many types of bacteria, again our success will be limited. These organisms will continue to cause trouble unless they are recognized and dealt with accordingly in the manner indicated.

Furthermore, should we introduce an educational program without any attention given to recommendations for control programs, no great success can be expected.

A successful approach needs the participation of the veterinary profession. Too often our programs, while stimulating interest on the part of dairymen, have resulted in an increase of treatment without veterinary supervision. There has probably been no greater encroachment upon the practice of veterinary medicine than through empirical efforts by laymen abetted by commercial interests through the sale of treatment materials to dairymen and accompanied by slight, if any, alleviation of the disease.

Based on the experience of apparently successful programs, the council through the efforts of its Committee on Programs and Procedures has developed a proposed outline for a successful control program which is being recommended as a council activity for use by state and local groups. This program is governed by four cardinal points:

1. Committee formation  
2. Education  
3. Pilot studies  
4. Program planning

Local level participation is imperative to reach our ultimate goal. The council is attempting to promote and aid in the development of State Advisory Councils, comprised of all interested groups, to work with the national organization in applying the principles of good mastitis control on a local level. From such an advisory committee can be developed a governing body, assisted by sub-committees for specific activities such as program development and evaluation; education; farm management; milking equipment installation and operation and therapeutic and laboratory procedures. Sub-committees should be composed of members from vocational and professional groups related to the dairy industry. Provisions may be made to include members whose interests are vital to the welfare of the dairy industry and the health of the public.

Education is of prime importance, especially on initiation of the program. Those in the dairy industry must be alerted to the fact that a
program is to be established and what the scope of activities will be. Each segment must be informed as to the benefits which may be gained and the responsibilities each will assume to derive these benefits. It is also necessary to train each person to perform his task. This is particularly true of the dairyman. From an educational standpoint, the dairyman is the most important cog in the wheel. How is this program going to affect him? How can he use it? Programs must be concise, clear cut and practical. What the dairyman needs to know and be sold on is the necessity for control, what the disease costs him, what the service he will require costs and the expected results. The success or failure of a mastitis control program in the final analysis rests in his hands. You can do anything you will in setting up a mastitis control program . . . you can take it to the dairyman . . . but if he does not use it . . . if he does not cooperate . . . the results will not be worth the effort.

Education as we speak of it means more than the dissemination of informative material to the dairyman. It means orientation and instruction, as well as short courses for the dairy farm advisors, veterinarians, specialists and technicians. They must be able to organize, promote and provide training in the program; hence their training must provide for orientation as to the purpose, scope and objectives of the program as well as instruction in the use of milking machine analyzing equipment, correct milking procedure, management, farm surveys and screening tests.

Veterinarians may have a part in any phase of the program but may be more directly concerned with the diagnostic, therapeutic, and laboratory procedures. They should also be familiar with the program to avoid conflicting information being disseminated to the dairyman.

Sanitarians, laboratory technicians and other professional personnel should be trained in mastitis testing procedures and utilized according to their capabilities and the existing need for their services. The laboratory technician will necessarily be limited to familiarization with the accepted tests used in the program. The sanitarian, however, may be utilized in many areas of activity such as farm surveys, milking equipment inspections, milking procedures, sanitation, etc.

Pilot studies are a must to accomplish two basic functions in the programs: first, to train personnel in order to carry out their activities in the program and to train others; and, second, to evaluate the effectiveness of the program in a given area and allow for corrections in procedure before a general program is initiated. Demonstration herds should be set up at the beginning of the program, possibly one in a county, with a gradual increase until there are many.

A well balanced program will involve several distinct phases, each of which is equally important.

The farm survey must be done to correct any deficiencies in the environment which will cause trauma to the cow's udder, and other stress factors.

The milking machine is a critical item in mastitis control. It must be thoroughly checked, using the best available testing equipment. The dairy farm advisor or equipment dealer should be well trained and qualified to do this work. The dairy farm advisor, however, should limit his
activity to pointing out the deficiency and advise the dairyman to have his equipment dealer make necessary corrections.

*Milking procedures* are a vital factor. Equipment may be in excellent condition but careless or improper use may be a causative factor in mastitis. Milking time inspections are recommended to determine whether proper methods and procedures are followed.

*Screening tests* which indicate the leucocyte level in milk are a most valuable aid for the detection of mastitis and may even be termed the first requisite in the development of a good program. This procedure can be done by the direct microscopic count or by using one of the chemical methods such as CMT, Catalase, or Modified Whiteside test. The screening test may be used with bulk milk as an indication of mastitis in the herd, as well as on an individual cow basis to indicate the presence of inflammation in the udder.

*Bacteriological analysis* of aseptically drawn samples is also a necessity. Isolation and identification culturing should be done along with sensitivity tests and made available for the veterinarian to utilize in conjunction with treatment of the herd.

Cows should be treated by a veterinarian who is familiar with the objectives of the program. The choice of a veterinarian should be left to the discretion of the owner. Those practitioners, however, who will be called to care for mastitis cases should make a sincere effort to cooperate with the rules and procedures which are set up for the program, if it is to be successful. The veterinarian fits into the picture solidly. Without adequate veterinary service, any program will have distinct limitations. He is the one person qualified to carry out the diagnostic and treatment procedures and should be cognizant of and cooperate with the various approaches and activities of the project.

This program, which we have available for you today, is quite detailed and all-encompassing in its scope. We are presenting it as a guide to those states which have requested assistance. It may be altered to conform to the needs of a given area. If one part does not fit the situation in some state, perhaps the other sections will be valuable. The basic principles, however, must remain intact if success is to be expected. The personnel utilized will depend upon the availability of people interested in combating this disease. However, no program should be attempted without professional assistance and advice.
REPORT OF THE COMMITTEE ON MASTITIS

H. G. Geyer, Columbus, Ohio, Chairman; H. S. Bryan, Michigan; J. T. Drayer, Columbus, Ohio; R. W. Metzger, Syracuse, New York; R. J. Schroeder, Downey, California

For numerous years, the United States Livestock Sanitary Association has devoted a portion of its program to the problem of bovine mastitis. In all instances, it has been recognized that this disease of dairy cattle has had a direct effect on the economy of the industry, as well as public health implications.

With the advent of antibiotics, it was believed by some that the "miracle" drugs would eliminate the problem. Today, experience has demonstrated that this is not true; in fact the general and promiscuous use of these agents has added to the cost and complexity of the disease, and as a sequel, created additional public health hazards.

When one attempts to review the literature, one finds many varied and conflicting reports relative to apparently similar aspects of the disease. This has, undoubtedly, added to the over-all confusion, and in some instances, an actual deterrent to progress.

These factors were basically covered in the report "An Evaluation of Existing and Proposed Mastitis Control Programs and Proposals of the National Mastitis Council" by Dr. R. W. Metzger at the 65th Annual Meeting of the United States Livestock Sanitary Association, November 1961.

It is obvious that if real progress is to be made in this field, concentrated efforts must be expended through some co-ordinating medium. Since reasonable progress has been made in this direction by the National Mastitis Council, Inc., your committee having met at Miami, Florida, during the American Veterinary Medical Association Convention, through general correspondence and again at the 66th Meeting of the United States Livestock Sanitary Association, wishes to present its report in the form of the following resolution:

WHEREAS, bovine mastitis is accompanied by general confusion and lack of well organized and scientifically logical attempts toward control; possibly more than any other dairy cattle anomaly; and

WHEREAS, it is one of the most prevalent and costly diseases of dairy cattle, and in accordance with Agricultural Research Service statistics, represents an annual loss of more than one-half billion dollars in milk and animals alone, and which estimates do not encompass the additional cost of the several therapeutic agents, notwithstanding the problems created for the regulatory agencies, as well as the public health implications; and

WHEREAS, the presence of antibiotics in milk as a result of treatment is also considered a public health problem; and,

WHEREAS, the newly formed National Mastitis Council, Inc., has accepted the challenge to determine what is being done in the fields of
research, education and control programs, and has set forth principles upon which a well organized control program should be established; and

WHEREAS, the principles of this program may be adopted in whole, or in part, by the regulatory agencies and affiliated interests of the several states; and,

WHEREAS, the results of the application of these principles in the several states may be appropriately evaluated and the information relative thereto be disseminated to all interested groups and agencies;

NOW, THEREFORE, BE IT RESOLVED: That the Executive Committee of the United States Livestock Sanitary Association concur in directing that the Mastitis Committee of this Association expend its efforts in collaborating with the National Mastitis Council, Inc., and concur in having the Chairman of the Mastitis Committee represent the United States Livestock Sanitary Association, as a director on the National Mastitis Council, Inc. Board, and lend such other assistance as the Executive Committee of this Association deems appropriate for the expeditious resolution of the problem.
Considering the widespread and rapidly expanding use of artificial insemination in cattle, it is important that every possible effort be made to minimize or eliminate the possibility of transmitting pathogenic microorganisms through its use. The Code of Minimum Standards for Health of Bulls and Hygiene of Bull Studs Producing Semen for Artificial Insemination which was proposed by the American Veterinary Medical Association's Committee on Artificial Insemination and adopted by the National Association of Artificial Breeders provides recommendations for controlling tuberculosis, brucellosis, trichomoniasis and leptospirosis. The degree of compliance with these recommendations varies amongst the various artificial insemination (A.I.) studs in the United States. Specific recommendations are not proposed for controlling infectious diseases other than those just mentioned. These problems are handled by the following recommendation: "Problems of a special nature, not included in the specific recommendations hereinafter, should be handled in accordance with the best of modern scientific principles." Although this sentence covers a wide range of disease problems, it is not suggested that the health code be altered at this time to take care of other specific problems. Many of the reproductive diseases due to viral and bacterial agents are in need of further research before more specific recommendations can be proposed.

The purpose of this paper is to discuss seminal vesiculitis and its possible significance in artificial insemination. Seminal vesiculitis is one of the more commonly neglected reproductive diseases of cattle. This stems in part from the failure of many practitioners to perform routine rectal examinations on bulls. The usual excuse for this neglect is that the increased use of A.I. has greatly reduced the number of bulls on farms with subsequent limitation of examination opportunities. It is more likely that the failure of dairymen to provide adequate facilities for handling dangerous animals and a lack of training and experience in this area are the more important reasons for neglecting reproductive diseases of the bull. Furthermore, so few veterinarians are conducting research on reproductive problems in cattle that limited new information is being obtained and transmitted to the veterinary profession. Perhaps a six-month to one-year post-graduate course in reproductive physiology and pathology would be advisable for individuals who plan to specialize in sterility work in cattle. Such a course would be especially valuable for those veterinarians who plan to become associated with the A.I. industry.

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Seminal vesiculitis is not difficult to diagnose providing the diagnostician is thoroughly familiar with the internal reproductive organs of the bull. In a normal bull the seminal vesicles may be palpated rectally as two resilient, lobulated, elongated, structures which are located on the floor of the pelvis. In mature bulls they extend slightly beyond the rim of the pelvis. The glands are situated in the urogenital fold lateral to the ampullae. In sexually mature young bulls the seminal vesicles are approximately six to eight cm long and one to two cm in diameter. In mature bulls the glands may be 12-15 cm long and three to five cm in diameter. It is not unusual for the seminal vesicles to be uneven in size and shape in normal bulls. Thus one must be careful not to diagnose vesiculitis merely on the basis of a small to moderate variation in size of the vesicles.

REVIEW OF THE LITERATURE

W. L. Williams, W. W. Williams, and H. L. Gilman conducted some of the earliest research on seminal vesiculitis and stressed the significance of the condition in regard to its adverse effect on fertility. They suggested that the seminal vesicles are more frequently infected than other areas of the male genital tract with the exception of the penis and prepuce.

Buck, Creech and Ladson reported the isolation of Brucella abortus from the seminal vesicles of four bulls and concluded that the seminal vesicles were the organs most likely to be infected in the bull. Jepsen and Jorgensen examined 12 bulls with brucella infection of the reproductive organs and demonstrated that seven of these bulls discharged brucella organisms in the semen. Six of these bulls had brucella infection in the seminal vesicles. Lagerlof, Hedstrom and Hoflund described the clinical signs observed in bulls with seminal vesiculitis. They reported that there are no signs of the condition in the majority of chronic cases. In their experience, fever and peritonitis around the seminal vesicles were observed in some of the acute cases. They stated that bulls with acute seminal vesiculitis may have clinical signs similar to those observed in bulls with traumatic reticulitis. Consequently, they stressed that the seminal vesicles should always be examined in bulls with signs of peritonitis. According to their experience, painful processes in the seminal vesicles did not diminish the capacity for copulation but fertilizing capacity was almost always decreased. They described the clinical signs and pathologic changes in cases due to streptococci, Corynebacterium pyogenes, Br. abortus, and Mycobacterium tuberculosis. Blom and Christensen examined the reproductive tracts from 2,000 sexually mature slaughtered bulls. Twenty one (one percent) bulls had inflammatory lesions of the reproductive organs. Of these animals, 17 (0.9 percent) had seminal vesiculitis thus providing evidence that the vesicular gland is by far the most frequent site of inflammatory processes in the genitals of the bull. Br. abortus was recovered from 11 cases and C. pyogenes from two. The etiology was not established in four cases.

Van der Sluis found 11 cases of seminal vesiculitis and 24 cases of vesiculitis combined with orchitis in a series of 828 bulls examined between
1946 - 1956. He reported that he had not observed lowered fertility from using bulls with vesiculitis. Van der Kaay described one case of vesiculitis due to *C. pyogenes* and one due to *Pseudomonas aeruginosa*.

**MATERIALS AND METHODS**

This report concerns 16 (4.66 percent) cases of seminal vesiculitis found in a series of 343 bulls which were subjected to either surgery of the seminal vesicles or post mortem examinations including gross and microscopic examination of the vesicles. Ten of the bulls were used for natural service and six for artificial insemination. The bulls were classified into three groups according to their place of origin. Seventeen bulls had been used for artificial insemination in studs outside of New York State. Sixty-five came from New York State farms and had been used for natural service. The remaining 288 bulls in the series had been used in the local A.I. association. Four bulls were submitted to surgery for removal of infected seminal vesicles. The surgical technique and fertility of the bulls following surgery will be reported in another paper. The rest of the bulls were submitted to post mortem examination. Detailed fertility and health records were obtained for most of the A.I. bulls and some of the natural service sires. Bacteriological examinations were conducted on most of the grossly detectable cases of vesiculitis.

**RESULTS**

Seminal vesiculitis was diagnosed in 16 of a series of 343 bulls. Four of the bulls were submitted to surgery. One seminal vesicle was removed from each of two bulls and both seminal vesicles were removed from the other two animals. The surgery was successful in all cases as judged by the absence of leukocytes in the semen following surgery. Three of the bulls have been returned to service and the fourth is recovering from surgery. Sufficient data is not available at present to evaluate the success of the operation as it relates to fertility.

In the series of 288 bulls from the local stud, three cases (1.04 percent) of vesiculitis were diagnosed. One of these bulls was operated upon for bilateral seminal vesiculitis.

Three (17.65 percent) of the 17 bulls from A.I. studs outside of New York State had vesiculitis. Two of these were corrected by surgery.

Ten (15.38 percent) natural service sires in a series of 65 bulls examined were diagnosed as having vesiculitis. Unilateral seminal vesiculectomy was performed on one of these bulls.

Nine of the cases occurred in Holsteins which is the predominant breed in this section of the country. Two occurred in Guernseys and Ayrshires, and one each in the following breeds: Jersey, Charolais and Angus.

**Etiology**

The seminal vesicles from 11 bulls were cultured for bacteria. No bacterial growth was obtained in four cases. Two of the bulls had antibiotic
therapy prior to cultural examination. *Corynebacterium pyogenes* was isolated from the vesicles of five bulls. *Streptococcus bovis* was isolated from one case and *Proteus mirabilis* from another. The seminal vesicles were not cultured in five cases. Four of these were slight cases which were not recognized on gross examination. *Brucella abortus* was not isolated from any of the bulls and the blood serum and semen agglutination tests were negative for brucellosis on all bulls tested.

**Pathology**

Complete post mortem examinations were not conducted on all of the bulls. Therefore, some of the lesions which may have been associated with the vesiculitis could have been overlooked. However, in the group of 12 bulls which were necropsied, two had chronic traumatic gastritis and one of these also had vegetative endocarditis. One bull had chronic bronchopneumonia, one pyelonephritis, one urethral calculi and another had suppurrative polyarthritis with vegetative endocarditis. Thus, six of the bulls with seminal vesiculitis had concurrent lesions outside of the reproductive tract. Of the 12 bulls which were necropsied, four had bilateral and two had unilateral epididymitis. All of the epididymal lesions were primarily in the tail of the gland with partial involvement of the body of the epididymis in some of the animals. Two of the bulls which were submitted to surgery had bilateral vesiculitis and both vesicles were removed.

The degree of involvement of the seminal vesicles varied from mild inflammatory changes to the formation of large abscesses. In the four cases which were not diagnosed clinically there were small to medium sized lymphocytic foci and scattered macrophages and plasma cells in the interstitial tissue. A few neutrophils were present in the lumina of the glands. Two of these bulls had anomalies of the spermatozoa which accounted for their infertility. The third bull had chronic bronchopneumonia and the fourth had urethral calculi. Thus the failure to diagnose vesiculitis in these cases was not of serious concern. The rest of the bulls had vesiculitis which was diagnosed on clinical examination. The seminal vesicles in some of the animals were approximately of normal size, but in most of these cases they were more firm than normal due to fibrosis. The glands which were two to three or more times larger than normal usually contained abscesses. *Corynebacterium pyogenes* appeared to produce a more severe tissue reaction than *Str. bovis* or *Proteus mirabilis*. Periglandular inflammation and adhesions were more pronounced in the cases due to *C. pyogenes*. The histologic changes consisted of neutrophilic infiltration of the lumina of the glands with varying degrees of neutrophilic infiltration of the interstitial tissue. The secretion was frequently retained and inspissated. Macrophages laden with debris were also found in the lumina of the glands. Metaplasia of the glandular epithelium was common in the chronic cases. Lymphocytes, monocytes and plasma cells were numerous in the interstitial tissue. Large areas of necrosis and vascular lesions were observed in a few glands. Intimal proliferation and recanalization of arteries had occurred in these cases.
Symptoms

Detailed histories were not available for all cases. Some animals apparently developed the infection with very few or no manifestations of illness. In some of these abnormal semen containing flakes and flocculi of material was the first sign of the disease. Brief histories of a few cases will be presented to illustrate the range of manifestations observed in the cases under consideration.

On January 13, 1960, a six-year-old Angus bull was observed kicking his abdomen with his right rear leg. He showed a moderate amount of tenesmus and pain on defecation. There was partial anorexia. The penis was somewhat relaxed and accompanied by a penile drip. The owner claimed that the animal had not maintained weight and that his libido had been poor since coming off pasture in the fall. The bull was submitted to the Large Animal Clinic of the New York State Veterinary College a few days later. Upon rectal examination, the seminal vesicles were found to be enlarged, hard, and painful. The tail of the right epididymis was hard and about two times normal size. The bull was sent to slaughter a few days later.

An 11 1/2-year-old bull was presented to the Large Animal Clinic for examination. Bilateral seminal vesiculitis was diagnosed. This bull had had an operation for traumatic gastritis eight years previously and had flocculi in the semen for a period of several years. Although many ejaculates were discarded, this bull was frequently used for artificial insemination during the time when flocculi were present in the semen. The bull began to refuse to mount six years following the operation and was submitted to the Large Animal Clinic two years later. Since bilateral vesiculitis and possible chronic traumatic gastritis with extensive adhesions were diagnosed, this bull was sent to slaughter.

The third case involved a three-year-old Holstein bull. Of the first 38 cows bred by this bull, 36 conceived to one service and all the cows calved normally. Subsequently an infertile cow in a neighbor's herd was bred naturally to this bull. Two days later the bull became ill and remained so for three days. Three weeks later flocculi were observed in the semen when it was collected for freezing. Since that time 28 cows were bred naturally and two artificially to this bull. Seven (23 percent) of these cows aborted from three months to term. Unfortunately none of the fetuses were submitted for examination. One failed to conceive and two had retained placentas. The diagnosis of unilateral seminal vesiculitis was made by the local veterinarian and was confirmed in the Large Animal Clinic. The affected right seminal vesicle was removed surgically. The bull is now being used for natural service in the herd of origin.

Diagnosis

To be able to diagnose seminal vesiculitis it is necessary for the clinician to know how normal seminal vesicles feel on rectal palpation and how they vary in shape and size in animals of different ages. This information can be gained only by examining a large number of normal bulls.

In the acute case of vesiculitis there will be swelling of the affected vesicle and pain on palpation. We observed one case in which the pain was
so severe that the animal dropped to the floor during the examination. The semen in the acute cases will usually contain leukocytes either in clumps or scattered throughout the semen sample. Blood may be present.

In chronic cases, the lobulations will become less distinct and the affected vesicle will become hard. In some cases a large abscess will form which will fluctuate on palpation.

Occasionally the diagnosis may be difficult to establish by rectal palpation alone and there may be very few or no leukocytes in the ejaculate. The diagnosis can be established in these cases by massaging the vesicles and collecting the material as it drips from the penis. In most bulls with vesiculitis there will be a considerable increase in the leukocytes in an ejaculate following massage. The technique of massaging the vesicles is one of the most useful methods for verifying a doubtful case of vesiculitis. A rectal examination should be part of the routine examination of all bulls with signs of peritonitis.

The semen should be cultured in an attempt to establish the etiology. All bulls with vesiculitis should have their blood serum and semen tested for brucella antibodies and animals having a brucella titer should be slaughtered.

Seminal vesiculitis should not be diagnosed merely on the presence of leukocytes in the semen. These may originate in the urinary system or in the prepuce and penis. The virus of infectious pustular vulvovaginitis and various bacteria may cause acute balanoposthitis with the production of numerous neutrophils which become mixed with the semen during ejaculation. Thus a thorough examination of the penis should be included in the routine examination.

DISCUSSION

According to our observations and those of Blom and Christensen¹ the incidence of seminal vesiculitis in the general population of bulls is approximately one percent. Although this is a rather low incidence, the disease may be of serious concern when it occurs in a bull which is used for artificial insemination. In these cases, pathogenic organisms may be transmitted to a wide population of cattle. The damaging effects of such a dissemination of pathogens may not be detected by the 60-90 day non-return rate which is used as the criterion of fertility in A.I. The presence of trichomoniasis in A.I. bulls is a good example of how a venereal disease may be transmitted by artificial insemination and not be detected by the non-return rate. The dilution and antibiotic treatment of semen would tend to lessen the incidence of disease transmitted, but would not necessarily eliminate dissemination completely. Furthermore, it is possible that the cows might conceive and abort at some time beyond the 60-90 day period. The 23 percent abortion rate observed in one herd considered in this report is suggestive that this might occur.

The incidence of vesiculitis in the series of 17 bulls from A.I. studs outside of New York State was 17.7 percent, and 15.4 percent in 65 bulls which had been used on farms. Although the number of bulls in this series
is rather small, it suggests that the incidence of vesiculitis in bulls with fertility problems may be larger than is currently recognized.

Some of the bulls in this study were used for artificial insemination while they had vesiculitis. Although the fertility of some of these bulls appeared to be good, bulls with seminal vesiculitis should not be used for artificial insemination. Our preliminary studies indicate that surgery may offer a better opportunity for correcting the condition than other methods of therapy which have been tried.

The pathogenesis of the disease has not been established. Williams suggested that calfhood infections may persist in the reproductive organs in young bulls and serve as foci of infection in sexually mature bulls. He also proposed that the infection may gain entrance into the seminal vesicles via the urethra in mature bulls. In our series of 12 bulls which were necropsied, six had concurrent lesions. Two bulls had urinary tract infections and it is possible that vesiculitis was secondary in these animals. The other bulls had lesions outside of the genito-urinary tract and it would appear that infection of the seminal vesicles in these cases could have been metastatic. More detailed post mortem examinations are needed to help clarify the pathogenesis of the condition.

In the earlier reports *Br. abortus* was incriminated most frequently as the cause of vesiculitis in bulls; *C. pyogenes* was the number two offender. With the gradual elimination of brucellosis, *C. pyogenes* has become the principal agent involved. This organism is quite resistant to antibiotic therapy in vivo which may explain in part the therapeutic failures. The presence of abscesses in the vesicles renders them impervious to medicinal therapy.

**SUMMARY**

1. Seminal vesiculitis was observed in 16 (4.66 percent) of a series of 343 bulls examined.
2. One or both seminal vesicles were surgically removed from four bulls with vesiculitis.
3. *Corynebacterium pyogenes* was isolated from the vesicles of five bulls. *Proteus mirabilis* and *Streptococcus bovis* were isolated from single cases.
4. Six of 12 bulls which were necropsied had concurrent lesions in other parts of the body suggesting that the infection may be metastatic in many cases.
5. Massage of the seminal vesicles is a very useful test for aiding in the diagnosis of vesiculitis.
6. Bulls with vesiculitis should not be used for service.

**ACKNOWLEDGEMENTS**

The research was supported in part by a grant from the Wisconsin-Minnesota Cooperative Bull Stud Group. The surgery was performed by Drs. John F. Kavanaugh, Donald D. Delahanty and William C. Wagner. The
assistance of Dr. David A. Morrow in locating several of the cases and obtaining their histories is gratefully acknowledged. The cultural examinations were conducted by Drs. Herbert L. Gilman and John Bentinck-Smith.

REFERENCES

REPORT OF COMMITTEE ON INFECTIOUS DISEASES OF CATTLE


Mr. Chairman, members of the Association, and guests: In 1961, this Committee gave reports on (1) Possible Spread of Diseases by Bulls Used for Artificial Insemination, (2) Salmonellosis in Florida Cattle, and (3) Evaluation of Methods for Mastitis Control.

The Committee voted to study these problems further in order to make recommendations regarding each at the United States Livestock Sanitary Association meeting in Washington, D.C., in 1962. Because of the wide diversification of these problem areas, it was proposed that subcommittees, consisting of personnel with specific interest in the respective fields be selected to study and to make recommendations on specific subjects. The problem of mastitis was elevated to Committee status and its report has already been presented.

This year the Committee on Infectious Diseases of Cattle has concerned itself with the organization of the Subcommittee on Artificial Insemination and the Subcommittee on Contamination and Recontamination of Processed Fish, Poultry, and Animal By-products with Disease Producing Microorganisms. Both of these subcommittees have been appointed. They have conferred and have submitted reports which are attached and are presented herewith as the report of this committee.

1. Report of Subcommittee on Artificial Insemination


REGULATIONS FOR BULL STUD HEALTH AND INTERSTATE SHIPMENT OF SEMEN

INTRODUCTION

In the interest of promoting uniformity in regulations and reporting forms for interstate shipments of semen, we submit the following proposal for those states feeling a need for such regulations. It is our hope that this pattern will be followed by states in the future, thus keeping realistic and uniform regulations governing interstate shipments of semen.

168
I. Bovine semen may not be shipped or transported into ____________ (State) for the purpose of artificial insemination and bovine semen may not be used for artificial insemination unless it originates from bulls whose health status conforms to the requirements that follow.

A. All bulls must be interpreted to be free of tuberculosis by the State Disease Regulatory Officials on the basis of an official tuberculosis test within 60 days prior to the first collection of semen, destined for use in artificial insemination, and annually thereafter.

B. All bulls must be interpreted to be free of brucellosis by the State Disease Regulatory Officials on the basis of official tube agglutination blood test conducted by the State-Federal Laboratory and negative to the semen plasma test (tube agglutination - 1:25) for brucellosis within 60 days prior to the first collection of semen, destined for use in artificial insemination, and be interpreted to be brucellosis free by the State Regulatory Officials on the basis of the blood and semen plasma tests each six months thereafter.

C. All bulls must pass six negative examinations for six successive weeks for *Trichomonas foetus* following the last natural service performed and within 60 days prior to the first collection of semen, destined for artificial insemination, and one negative examination each six months thereafter.

D. All bulls must pass two approved negative blood tests 30 days apart, or the titre shall be shown to be stabilized, if present, for leptospirosis within 60 days prior to the first collection of semen, destined for use in artificial insemination, and each six months thereafter.

II. Bovine semen destined for use in artificial insemination in ____________ (State) must be extended a minimum of 1:25 in an extender treated by the addition of not less than 500 units of penecillin and 500 micrograms of streptomycin per cubic centimeter of extender and held a minimum of six hours before use, to prevent the transmission of *Vibrio fetus* and other bacterial pathogens.

III. All tests shall be conducted according to specifications adopted by the USLSA and approved by the Agricultural Research Service.

IV. All tests shall be reported on a uniform certificate as suggested on the following page:
UNIFORM CERTIFICATE FOR INTERSTATE OR INTRASTATE SHIPMENT OF BOVINE SEMEN FOR ARTIFICIAL INSEMINATION

ADOPTED BY THE
UNITED STATES LIVESTOCK SANITARY ASSOCIATION

<table>
<thead>
<tr>
<th>REGISTRATION NUMBER</th>
<th>BIRTH DATE</th>
</tr>
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</table>

<table>
<thead>
<tr>
<th>OWNER OF BULL OR NAME OF SEMEN PRODUCING BUSINESS</th>
<th>ADDRESS</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>LOCATION OF BULL</th>
<th>DATE OF ARRIVAL OF BULL AT A.I. STUD OR FARM</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>BULL, DATE RESTRICTED FROM NATURAL SERVICE</th>
<th>RENEWAL CERTIFICATE FOR RESIDENT BULL</th>
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</table>

<table>
<thead>
<tr>
<th>TESTS CONDUCTED UNDER SUPERVISION OF STATE-FEDERAL ANIMAL DISEASE CONTROL AUTHORITIES OF STATE WHERE BULL WAS LOCATED</th>
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### BOVINE TUBERCULOSIS - INTRADERMAL

<table>
<thead>
<tr>
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<th>DIAGNOSIS</th>
<th>BY</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIRST CERTIFICATE:</td>
<td>Test within 60 days prior to first shipment of semen</td>
<td>Test</td>
<td></td>
<td></td>
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<tr>
<td>RENEWAL CERTIFICATE:</td>
<td>Test</td>
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### BOVINE BRUCELLOSIS - BLOOD SERUM

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<tr>
<th>REGULATION</th>
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<tbody>
<tr>
<td>FIRST CERTIFICATE:</td>
<td>1st</td>
<td>Test</td>
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<tr>
<td>Test within 60 days prior to first shipment of semen.</td>
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<tr>
<td>RENEWAL CERTIFICATE:</td>
<td>2nd</td>
<td>Test</td>
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<tr>
<td>Two tests, semiannually</td>
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### BOVINE BRUCELLOSIS - SEMEN PLASMA

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<tr>
<td>FIRST CERTIFICATE:</td>
<td>1st</td>
<td>Test</td>
<td></td>
<td></td>
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<tr>
<td>Test within 60 days prior to first shipment of semen.</td>
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</tr>
<tr>
<td>RENEWAL CERTIFICATE:</td>
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<td></td>
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<tr>
<td>Two tests, semiannually</td>
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### BOVINE LEPTOSPIROSIS - BLOOD SERUM

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<tr>
<td>FIRST CERTIFICATE:</td>
<td>1st</td>
<td>Test</td>
<td></td>
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<tr>
<td>Test within 60 days prior to first shipment of semen.</td>
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<tr>
<td>RENEWAL CERTIFICATE:</td>
<td>2nd</td>
<td>Test</td>
<td></td>
<td></td>
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<tr>
<td>Two tests, semiannually</td>
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### BOVINE VENEREAL TRICHOMONIASIS-SMEGMA

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<tr>
<th>REGULATION</th>
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<th>DATE</th>
<th>DIAGNOSIS</th>
<th>BY</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIRST CERTIFICATE:</td>
<td>1st</td>
<td>Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Following last natural service, six weekly tests prior to first shipment of semen.</td>
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<td></td>
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<tr>
<td>RENEWAL CERTIFICATE:</td>
<td>3rd</td>
<td>Test</td>
<td></td>
<td></td>
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<tr>
<td>Two tests, semiannually</td>
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### STATEMENT OF ACCREDITED VETERINARIAN

1. The specimen necessary for each of the tests indicated was collected by me or under my supervision and submitted for diagnosis as shown. I test reports done by certificated laboratories or agencies are in my possession for the support of all data entered hereon.

2. This presentation is made on behalf of the producer of this bull and the producer has understood and agreed that all semen must be collected at a site not less than 1.25 and not more than 19.25 miles from the farm or ranch before not less than six hours in an interior containing not less per ml. than 300 units of penicillin and 100 micrograms for erythromycin.

3. The person or veterinarian shown on the reverse of this certificate is responsible for this certificate in the event of non-compliance with these regulations.

4. I hereby agree that in event additional tests are not carried out or if the results are other than negative I am obliged to notify the endorser officer of all facts concerned.

### ENDORSEMENT - STATE OF ORIGIN

### STATEMENT OF ACCREDITED VETERINARIAN

### APPROVAL - STATE OF DESTINATION
2. Report of Subcommittee on Contamination and Recontamination of Processed Fish, Poultry, and Animal By-Products with Disease Producing Microorganisms

E. M. Ellis, Ames, Iowa, Chairman; A. H. Frank, Ames, Iowa; L. C. Grumbles, College Station, Texas; C. Nivens, Jr., Chicago, Illinois; B. S. Pomeroy, St. Paul, Minnesota; J. Walker, Washington, D. C.

PROPOSED SANITARY CODE FOR PROCESSORS OF FISH, POULTRY AND ANIMAL BY-PRODUCTS*

INTRODUCTION

As a result of many individual and cooperative studies, domestic\textsuperscript{1-18} and foreign\textsuperscript{19-54} reports have documented that contamination or recontamination of processed fish, livestock, and poultry by-products with salmonella and other disease-producing microorganisms occurs. These studies indicate that this most likely occurs in handling or storage of the finished product.

WHY IS THIS CAUSE FOR CONCERN?

Animal by-products are used as a source of protein supplement and are commonly mixed with other ingredients to make up commercial animal and poultry foodstuffs. If processed animal by-products contain disease-producing microorganisms when mixed with other ingredients to form commercial foodstuffs, they may serve to transmit the microorganisms to healthy animal populations. Such microorganisms may cause widespread disease losses in poultry and other animal species or hamper established disease-control programs.

PURPOSE OF SANITARY CODE

The purpose of this code is to outline procedures to animal by-product processors for sound plant sanitation and product handling in order to supply a finished product that is not contaminated. This should assist in eliminating the possibility of disease organisms being present in animal feeds.

OFFICIAL RECOGNITION OF SANITARY CODE

A. The rendering industry or allied utilization industry groups should give special recognition to those animal by-product processors who operate within this or another officially recognized sanitary code.

B. It is recommended that this sanitary code be adopted by states, municipalities, or health districts subject to the appropriate legal authority.

*Recommendations concerned specifically with the genus \textit{Salmonella}. 
GENERAL APPLICATION

In applying the following code, by-product processors should strive for maintenance of forward product movement with adequate precautions during storage and shipping in order to prevent post-processing contamination or cross contamination from internal and external sources.

PLANT PREMISES

A. General

1. Plants should be separated from the surrounding area by a fence suitable for keeping out pets and stray animals.
2. Uncontrolled animals and birds should not be maintained on the plant premises.
3. Rodent, vermin, and insect control should be continuously maintained.
4. It is preferred that no other business be conducted on the same premises. In the event that this is not practical, all precautions should be taken to avoid the use of equipment, tools, vehicles, and personnel between businesses.
5. Buildings and surrounding grounds should be kept clean and free from refuse, trash, or accumulation of product or products of processing.  

**PURPOSE:** If humans, animals, or pests are allowed freedom in a processing plant and refuse, trash, and products are allowed to accumulate on the premises, it increases the possibility of disease-producing germs being carried from the raw to processed product. Inadequate trash and raw animal by-product disposal or handling can induce the breeding and feeding of flies and rodents which are considered capable of transmitting harmful germs.

B. Building construction and facilities

1. Facilities and equipment should be constructed so that they can be readily cleaned and disinfected after daily use.
2. Floors should be of impervious construction, leak proof, and properly pitched to allow drainage.
3. Inside walls should be of impervious construction that can be readily washed and scrubbed to remove accumulated material. Ceilings should be closed and of tight construction.  

**PURPOSE:** Floors, ceilings, and walls constructed of impervious material can be kept sanitary more easily than those constructed of wood, dirt, or similar material; hence, they are more apt to be kept clean.
4. Outside plant walls should be of such construction that will prevent entry of wild birds, rodents, and insects.
5. Doors should fit tightly. All doors should be equipped with self-closing devices. Screen doors or other devices designed to prevent fly entry should be used to protect areas handling or storing the finished product.
6. Windows should be equipped with screens that repel insects. **PURPOSE:** Doors, windows, and outside walls which are constructed so as to prevent entry of wild birds, rodents, and insects will lessen the possibility of cross contamination with harmful germs from raw to the processed product.

7. There should be an adequate supply of cleaning agents plus hot water or live steam. **PURPOSE:** An adequate supply of cleaning agents and hot water or live steam is a vital factor in keeping a plant and its equipment and tools in a clean, sanitary condition.

8. Plant drainage and sewage disposal systems should meet municipal, state, or national plumbing code requirements. Drains should be equipped with seal type traps.

C. Processing equipment

Processing equipment should be controlled and operated so that there is complete destruction of harmful bacteria or viruses during each operation. This may be accomplished by treating the raw product under one of the following processes with special attention to the recommended minimum time and processing temperature.

<table>
<thead>
<tr>
<th>Process</th>
<th>Temperature</th>
<th>Time</th>
<th>Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet rendering with steam injection</td>
<td>212 °F*</td>
<td>4 hours</td>
<td>none</td>
</tr>
<tr>
<td>Closed vat</td>
<td>287 °F*</td>
<td>1 hour</td>
<td>40 lbs.</td>
</tr>
</tbody>
</table>

**PURPOSE:** Thorough heating of animal by-products to the proper temperature for the recommended period of time gives the best assurance of killing all harmful germs or other agents.

D. Employee facilities and requirements

1. Adequate personnel showering, modern toilet facilities, dressing, and disinfecting facilities should be available for employee use. These facilities should be located so that personnel may not move from contaminated (unclean) to uncontaminated (clean) areas without using such facilities.

2. No person with any disease in a communicable form shall work in any capacity which brings him in contact with the production, handling, storage, or transportation of the processed product.

E. By-product processing and storage areas.

1. The processing area should be divided into clean (processed products) and unclean (raw products) areas. These areas should be separated by a solid impervious wall or floor. Loading and unloading facilities for raw or processed products should be separate.

*Temperature reading through the use of a recording thermometer should be representative of the entire lot of material being processed. This can be accomplished by making certain that the material is continuously agitated during processing.
2. Equipment and personnel used in the unclean area should not be used in the clean area. If different personnel are not available to both the clean and unclean areas, those personnel utilized in both areas should use different outer clothing, gloves, and head and footwear in each area.

3. Personnel who handle raw products and/or work in the unclean area should be provided with specially marked outer clothing, gloves, tools, and head and footwear. These specially marked tools and clothing should be used or worn only in the unclean area(s).

**PURPOSE:** It is foolhardy to process a product to kill the harmful germs it may contain and then not do the things necessary to prevent the finished or processed product from being seeded with germs again. Complete partitioning of the clean from the unclean areas and use of different personnel, equipment and tools in each area will greatly reduce the opportunities for seeding or recontaminating the finished product.

F. General Recommendation on storage

There should be continued effort to minimize the storage time of both raw and processed animal by-products.

**PURPOSE:** Minimizing storage time of both the raw and processed product cuts down the chances for cross contamination of harmful germs from one part of the plant to another.

G. Moisture control

1. Storage area, walls, floors, and ceilings should be kept free of all moisture.

2. The processed product should be kept dry at all times.*

**PURPOSE:** Germs require moisture to grow or multiply, so the finished products and containers or areas where it is stored should be kept dry to keep germ life down.

H. Dust control

Continuous attention should be given to controlling and removing accumulated processed-product dust which settles on shelves, window sills, equipment, etc. This may be accomplished best by vacuuming. Do not use water or steam for this because it will serve to establish pockets or pools of damp processed by-products in which germs may grow.

I. Doorway foot baths

A supply of disinfectant detergent solution should be placed at all points where contamination could be carried into a clean area. The footwear should be thoroughly cleaned with a stiff brush.

**PURPOSE:** To remove contaminating material from footwear to help prevent dissemination from one area to another.

*The use of overhead metal storage bins rather than putting the processed product on the floor will lessen the possibility of it being seeded with germs or getting wet.
INFECTIONOUS DISEASES OF CATTLE

VEHICLES

A. Truck-beds, tanks, and barrels used for transporting raw animal by-products should be covered, leak-proof, and so constructed that no drippings or seepage can escape.

B. Collection vehicles used for transporting dead stock or any product of dead stock should be thoroughly scrubbed out and disinfected after hauling each load or before proceeding for another load.

PURPOSE: Thorough cleaning and disinfecting of vehicles, which transport dead animals and poultry or their unprocessed products, will assist in preventing the spread of harmful germs from one premise to another.

C. Vehicles used for transporting processed bulk or packaged animal by-product material should be thoroughly cleaned and disinfected prior to use.

CONTAINERS

Only new sacking or other new containers should be used for shipping processed animal by-products.

BACTERIOLOGICAL CONTROL EXAMINATIONS

There should be bacteriological examination* of each lot of processed product consisting of not more than one day's production for the presence of salmonella organisms to assure that the sanitary code is functional. If a tank of processed products is found by bacteriological examination* to be contaminated, it should be reprocessed.

BACTERIOLOGICAL EXAMINATION OF OTHER FEED INGREDIENTS

It is recommended that each lot of products, such as grains, which are mixed with processed animal by-products to form a complete foodstuff, should also be subjected to frequent bacteriological examination by laboratory procedures.*

CLEANING AND DISINFECTING

Utensils and equipment such as shovels, trucks, rubber footwear, wheel barrows, carts, buckets, etc., can be disinfected by washing thoroughly with cleansing agent and water followed by application of a suitable disinfecting solution.

TRAINING

All plant employees should be thoroughly trained in plant procedures and need for strict adherence to the sanitary code.

*See USDA Handbook (ARS 91-36) "Recommended Procedure for the Laboratory Isolation of Salmonella Organisms from Animal Feeds and Meat By-Products" for sampling and laboratory culture techniques for isolating salmonella organisms.
REPORT OF COMMITTEE

RESPONSIBILITY FOR SANITARY CODE COMPLIANCE

Key plant personnel should be trained as a security or safety officer to ascertain that all aspects of the sanitary code are carried out.

GLOSSARY

A. Animal by-product

For the purpose of this code, animal by-product means fish, poultry, or livestock or any of their products.

B. Cleaning agents

Cleaning agents are applied in a hot-water solution in order to remove grease and all other material sticking to the surface of the object being cleaned. The following compounds are suitable cleaning agents when used in the recommended manner.

1. Sal Soda - Prepare a solution of 13-1/2 ozs. to one gallon of water.
2. Sodium hydroxide (Lye) - prepare a solution of 13-1/2 ozs. to five gallons of water. (Due to the extreme caustic nature of sodium hydroxide solution, precautionary measures such as the wearing of rubber gloves and boots to protect the hands and feet, and goggles to protect the eyes, should be taken by those engaged on the disinfection job. It is also advisable to have an acid solution such as vinegar, in readiness in case any of the sodium hydroxide solution should come in contact with any part of the body).
3. Live steam - The application of live steam can be helpful in removing deep accumulations of greasy or dried material. It should not be relied upon to sterilize the surface to which it is being applied.

NOTE: Generous applications of flowing hot water and brisk scrubbing along with and following the cleaning operation will assure the most satisfactory results. All surfaces should be free of grease and accumulated material following the scrubbing and cleaning operation prior to applying the disinfecting solution.

D. Clean area

Area used for transporting, handling, or storing the processed (cooked) animal by-product.

E. Unclean area

Area used for transporting, handling, and storing non-processed animal by-products.

F. Raw product

Animal by-product that has not been properly processed under provisions outlined by this code.

G. Processed product

Animal by-product that has been properly processed under provisions outlined by this code.
REFERENCES


MYCOBACTERIOSIS IN SWINE CAUSED BY ATYPICAL MYCOBACTERIA

W. L. Mallmann, Virginia H. Mallmann and James A. Ray*

East Lansing, Michigan

The isolation of Mycobacterium bovis from a lesion taken from a pig at slaughter prompted the current study of swine tuberculosis at Michigan State University. Because lesions were observed in swine from the University herd and M. bovis was cultured from one lesion examined, a routine tuberculin testing program was started in July 1961. The results of this testing program were reported by Beck et al.¹

Tissues have been collected from tuberculin-positive swine at slaughter over a period of a year. Lymph nodes were collected from both gross lesion (GL) and non gross lesion (NGL) animals. Intact lymph nodes were delivered to the laboratory shortly after collection. Each lymph node, upon delivery to the laboratory, was immediately placed in 1000 ppm hypochlorite solution to destroy contaminants during over-night storage at 4°C. The following day the lymph nodes were dissected from their fatty envelopes aseptically, and again rinsed repeatedly in 1000 ppm. hypochlorite solution. The lymph nodes were then sectioned for detection of gross lesions and selection of material for bacteriologic and histopathologic examinations. The lesion was ground by mortar and pestle. The ground tissue was mixed with a four percent NaOH solution, so the resulting mixture contained two percent NaOH. After 15 minute digestion, neutralization of the alkali, and centrifugation, the sediment was spread over the surfaces of slants of Lowenstein-Jensen, Middlebrook and Dubos media and incubated at 35°C.

With the exception of the M. bovis isolated in 1960, the other cultures have been classified as Runyon Group III acid-fast bacilli.² One isolant might be classified by some workers as M. bovis, but it is probably a highly virulent Group III mycobacterium.

The Group III organisms of human origin were originally classified by Runyon² as nonchromogenic mycobacteria characterized by slow growth. These organisms are generally antibiotic resistant, catalase positive, niacin negative, arylsulfatase positive and do not grow at 44°C. They are not pathogenic for the rabbit and chicken. When 0.1 mg is injected intradermally into the guinea pig, a lesion occurs at the injection site. In our laboratory, some isolants from cattle, swine, soil, feed etc. which were classified as Group III organisms, have deviated in one or more of the differentiating characteristics.

Approximately 100 strains of Group III mycobacteria have been isolated from both GL and NGL swine from the University herd. These isolants are characterized by close conformity and stability in their

*Department of Microbiology & Public Health, College of Veterinary Medicine, Michigan State University.
biochemical morphological and pathogenicity patterns. Some of the Group III isolants from bovine tissues are less stable. Passage through guinea pigs and calves has markedly increased pathogenicity of these isolants. No increase in pathogenicity of the swine isolants has resulted by passage through guinea pigs or chickens. Only a few of the swine isolants will grow at 44°C. These strains, grown at 44°C have been passaged through chickens. Although the organisms can be reisolated, no visible lesions are produced in chickens.

Three Group III isolants of swine origin produced an open, ulcerating lesion at the intradermal site of injection in guinea pigs which reached its maximum size at approximately four weeks. The animals reacted to both avian and mamalian tuberculin. No gross lesions were detected at autopsy.

Young pigs four to six weeks old were injected intradermally with two mg. (1 x 10^8) of Group III mycobacteria of swine origin. The intradermal route of infection was used. Previous experiments with laboratory animals and calves had demonstrated the intradermal route of inoculations to be the most favorable for producing infection.

Of the eight swine inoculated with Group III isolants, all produced skin lesions, three developed prescapular lymph node, two had prescapular lymph node lesions and three had submaxillary lymph nodes lesions. The organisms reisolated from all inoculated pigs.

All inoculated pigs gave positive mammalian and avian tuberculin positive tests at 60 and/or 120 days following inoculation. The degree of the tuberculin reactions between avian and mammalian tuberculin varied from 0.5 mm to five millimeters. In all but two instances, the avian tuberculin gave the greatest reaction.

Three pigs were inoculated intradermally with M. bovis with the same volume. These animals were slightly more sensitive to mammalian than to avian tuberculin. All had generalized infection with gross lesions. M. bovis was reisolated from the animals.

In one pen of pigs inoculated with Group III organisms, a fourth pig was uninoculated. At the end of 120 days contact, this pig was sensitive to both mammalian and avian tuberculin. On slaughter no gross lesions were detected, but the Group III organism was isolated. In another pen of Group III inoculated pigs, an uninoculated pig was tuberculin negative, on slaughter there were no gross lesions detected and no mycobacteria were isolated.

A control pen of three pigs were tuberculin negative at the completion of the experiment at 120 days, showed no visible lesion on slaughter, and no mycobacteria were isolated.

The studies on experimental infection in swine is being continued. Inasmuch as these studies are in progress, the histopathologic examinations in the above cited experiments have not been completed.

After the demonstration that the University swine herd was infected with Group III mycobacteria, and that the disease could be reproduced with Group III isolants, a survey was started to determine the extent of this disease in swine geographically. Lesions were obtained from swine at a Detroit slaughter house that came from Illinois, Missouri, Ohio, Indiana and Michigan. Eighty-five percent of the isolants were M. avium, the remainder were Group III mycobacteria.
These data demonstrate that the Group III disease is widespread. Undoubtedly, this disease is not new. Because animals with this disease react to both mammalian and avian tuberculin, the disease would be reported as either bovine or avian infection, depending upon the tuberculin test. Inasmuch as the tuberculin reaction with avian tuberculin was generally larger in most instances, the disease would be diagnosed in the field as avian tuberculosis.

At the present time, there is no way to differentiate bovine, avian and Group III infection by sensitivity reactions. There is no way to evaluate the extent of each type except by bacteriologic procedures. In the studies which are underway at our laboratories, an important phase is the development of laboratory and field procedures that may be used for determining the type of infection in the living animal.

Atypical mycobacteria are also producing disease in man. In the northern States, most of the cases are due to Group I (photochromogens) and to some extent to Group II (scotochromogens). In the southeastern area of the United States the Group III mycobacteria are causing a pulmonary infection. Data on the incidence of atypical mycobacterial infection in man is meagre. Not all hospitals employ procedures of typing atypical organisms at present. Procedures are still in the experimental stages on which there is little agreement among research workers. The diseases in man, caused by atypical mycobacteria, have been recognized primarily due to the fact that they are antibiotic resistant. At present, there is no known therapeutic agent.

A Group III mycobacterium causing disease in man has been named Battey as a means of identification. This organism was first isolated from a patient at the Battey Sanitarium in Georgia. By the use of a PPD sensitin (PPD-B) prepared from the original Battey organism, differentiation can be made from other Group III mycobacteria.

The relation of the Group III isolants from swine is not known. Some strains of Group III organisms from cattle sensitize guineapigs to PPD-B. Whether or not these Group III organisms would cause disease in man is unknown. Until such information is available, the Group III organisms in cattle and swine must be considered a health hazard to man.

We have also found mycobacteria in soil and feed which closely resemble the Group III organism. The frequency is such that it would appear that the organisms are wide-spread in Nature. These organisms are slow growing and are identical to those isolated from cattle and swine as measured by biochemical and morphological characteristics. However, they do not produce an intradermal lesion or disease in guinea pigs and do not cause sensitivity to tuberculin. None of the Group III organisms isolated from soil and feed which have been injected intradermally into cattle or swine have induced infection.

SUMMARY

We have demonstrated by field tests and by laboratory produced infection that swine are infected with Group III mycobacteria.
This disease cannot at present be distinguished from either bovine or avian tuberculosis except by isolation of the organism from tissues of tuberculin positive swine.

The means of transmission from animal to animal is not known. There is no reason to believe that it is different from that of the bovine and avian tubercle bacilli.

The public health significance of the disease is still unknown.

The disease is apparently wide spread in the United States and is indistinguishable in the live animal from the disease caused by bovine and avian tubercle bacilli.

REFERENCES


EXPERIENCES AND IMPLICATION OF AN OUTBREAK OF SWINE TUBERCULOSIS IN A UNIVERSITY SWINE HERD

Dr. C. C. Beck, D.V.M., M.S.,* Dr. D. J. Ellis, D.V.M., M.S.,* J. A. Hoefer, B.S., M.S., Ph.D.,** Dr. R. M. Scott, D.V.M.*** and Dr. J. M. Stewart, D.V.M., M.S.*

East Lansing, Michigan

INTRODUCTION

Tuberculosis-like lesions were found on routine slaughter. The finding of lesions prompted a bacteriological examination and typing of the acid fast organism. Isolation and typing of a *M. tuberculosis bovis* and Runyon Group three *Mycobacteria* from lesions found at slaughter stimulated a testing program in the swine herd. The test results are confusing because of the variations and inconsistencies that occur.

Little attention, and less significance, has been paid to the problem of swine tuberculosis for many years. The launching of the bovine tuberculosis eradication program in 1917 resulted in reduction of the percentage of bovine tuberculosis reactors from near five percent in 1917 to less than 0.5 percent in the early 1940's. A marked reduction in swine tuberculosis due to *Mycobacterium bovis* also resulted. Prior to and since that time most of the cases of swine tuberculosis have been attributed to *Mycobacterium avium*. Since this organism is of little significance in man or the bovine, little attention has been paid to the problem. Nevertheless, the 1960-1961 "Summary of Activities" report of the United States Department of Agriculture Research Service, records that of 64,209,639 animals inspected on post mortem, 6,147 carcasses were condemned in addition 1,581,791 carcasses were retained and later passed after trimming and removal of affected areas, and the condemnation of 342,773 portions (heads, hams, shoulders) of carcasses due to tuberculosis. This 64 million swine represents approximately 80-82 percent of the total number of swine slaughtered in the United States.

The virtual elimination of the small flock of chickens from most American farms (1950-1962) should have resulted in practically total elimination of swine tuberculosis due to the avian sources. This has not necessarily been the case. Actually, the percentage of lesions found on slaughter has remained quite consistent during this period of years.

Furthermore, since there is no regular program of tuberculosis testing in swine, the figures available are all based on microscopic findings on slaughter hogs rather than culture, typing, or test results using the

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intradermal tuberculin test. The cooking of garbage, in garbage feeding establishments, should drastically reduce the number of cases of swine tuberculosis due to *Mycobacterium tuberculosis* (human strain). It would not have any effect on contact with actual human carriers.

**LITERATURE**

Time does not permit an exhaustive review of the literature for this paper on the subject of tuberculosis in swine. Tuberculosis lesions of swine have been reported by Bankier (1946), Clapp (1956), Fledman (1938), Robinson (1955), Albiston (1954), VanEs (1925), Pullin (1946), Luke (1951, 1952, and 1953), Woodroffe (1950), Ginsberg and Fitzpatrick (1950), Tammernige (1953), and Francis (1958). These authors reported that avian, bovine, human and saprophytic mycobacteria were isolated from lesions found on slaughter. Also, they found a high percent of *Corynebacterium* in tuberculosis-like lesions.

Francis (1958) reported that several types of mycobacteria produce tuberculous lesions. He also states that there is little evidence to indicate that the infection spreads rapidly from pig to pig.

Bankier (1946) reported avian type in 87 percent, bovine in one percent, and non-infective agents in three percent of the swine tuberculosis lesions from Canadian abattoirs. Pullin (1946) reported 70.6 percent of the lesions were believed to be *Mycobacteria tuberculosis* by microscopic examination, culture, or animal inoculation. Of these, 104 cases were avian, two bovine and *Corynebacterium equi* was found in 21.1 percent.
Ginsberg and Fitzpatrick (1950), 9 reported one third of tuberculosis-like lesions in swine yielded C. pyogenes, while Tammernige (1953) 20 reported a high percentage of Corynebacterium equi from tuberculosis-like lesions of swine in Queensland. He also reported slightly more than half of the diseased lymph nodes yielded M. bovis when cultured and typed. Woodroffe (1950), 22 isolated Corynebacteria from tuberculosis-like lesions of the submaxillary nodes in pigs in South Australia. Robinson (1955), 18 isolated Corynebacteria from 50 percent of tuberculous submaxillary glands of swine. Clapp (1956), 3 reported that Corynebacterium equi produced a pseudotuberculous in the lymph glands of swine.

The avian and mammalian intradermal tuberculin test has been applied on the ear and evaluated in pigs by Luke (1951, 12 1952, 13 1953), 14 McDiarmid (1956), 15 and Pullar and Rushford (1954). 16 Luke (1953) 14 reported that in sensitized pigs, sensitivity wanes fairly rapidly in avian type infection. Avian infections have been overcome 112 days following infection as judged by negative cultured and biological tests. This author found bovine infection to be more progressive and sensitivity more prolonged. Many bovine type infections have negative tests 190 days after infection. Luke (1951), 12 found lesions at post mortem which were biologically and culturally negative also. Luke (1953), 14 found that tuberculin reactions were at their peak in 24 hours and were regressing in 48 hours in some pigs. In other pigs, the 24 hour reading was less than in 48 hours. In 1952, 13 Luke examined the possibility of old animals overcoming the infection as judged by low responses to tuberculin. Out of 100 sows, 17 gave tuberculin reactions of two mm. or more without showing any lesions at post mortem examination. In addition, eight sows showed lesions, but no skin reaction. No viable organisms were found in the lesions of the eight sows.

Pullar and Rushford (1954), 16 reported on the accuracy of the avian intradermal tuberculin test in pigs. Considering that 100 percent increase in skin thickness as positive, this author found the test to be 95.5 percent accurate in 511 tested animals. He used both avian and mammalian tuberculin. Feldman (1960), 7 pointed out the significance of unclassified Mycobacteria as these organisms were isolated from approximately 25 percent of swine tonsils studied. These organisms produce sensitivity in test animals.

Daines (1938), 5 produced tuberculin sensitivity in cattle from acid fast bacteria that were not tubercle bacilli. Crawford (1926), 4 produced mammalian tuberculin sensitivity with a variety of saprophytic acid fast bacteria. Karlson and Feldman (1940), 11 found an acid fast microorganism in 25.5 percent of 94 tonsils from the same number of swine which produced no recognizable disease, but did sensitize guinea-pigs to avian tuberculin. It produced no sensitivity to mammalian tuberculin.

MATERIALS AND METHODS

An arrangement was made in 1960 with the Meat Inspection Division of the United States Department of Agriculture to assign to the College of Veterinary Medicine at Michigan State University a veterinary inspector.
The veterinary inspector has two major functions at the College: (1) to teach the course in meat inspection to the veterinary students, and (2) to apply the principles of meat hygiene during processing operations at the meats laboratory of the College of Agriculture.

During the post mortem inspection, small opaque or large irregularly-shaped lesions were observed in the lymph nodes of swine which varied in size from 0.5 mm. to 10 mm. in diameter. The lesions contained a yellowish-white to white caseous material usually with calcium salts to give it a rough, hard texture when palpated, or a gritty texture when incised. The lesions were restricted to the mandibular and mesenteric lymph glands. The lesions were submitted to the Tuberculosis Laboratory at the College of Veterinary Medicine for bacteriological examination and typing of the acid fast organisms.

A program of routine testing of all Michigan State University swine was undertaken in July 1961 following verification of the presence of tuberculosis due to *Mycobacterium bovis* and other acid fast *Mycobacterium*.

Various methods of testing are discussed in Dunne's *Disease of Swine*. Testing methods consisted of intradermal injections of tuberculin (3/8" - 26 guage intradermal needles) using 0.1 ml. avian tuberculin in the right ear and 0.1 ml. mammalian tuberculin in the left ear. The right and left sides of vulva can likewise be used. Readings were made at 48 and 72 hours. The usual instructions call for injection at the base of the ear. In testing adult breeding stock in a chute with a standard head squeeze, it is essential to move out on the ear to areas not subject to injury, contusion and subsequent swelling as a result of struggling in the chuts. Likewise, when identifying hogs by notching, tattoos, ear tags, buttons and other devices, it is imperative that this procedure does not interfere with reading of the test at 48 and 72 hours. Similarly, fight wounds, etc. must be considered.

**DISCUSSION**

It is evident from these charts that a tuberculosis problem exists. The testing of purchased boars at the Boar Stud and purchased animals from twelve other sources indicated an incidence ranging from 0-88 percent. The problem obviously is not one peculiar, nor confined, to Michigan State University's swine herd.

Charts II and III illustrate many variations and apparent inconsistencies in test results. Animals changing from N - R - N etc., as shown in Chart III, are not too uncommon. Luke (1952, 1953) reported on the waning of sensitivity on both avian and mammalian infection. The exact significance of these changes in sensitivity are not fully understood. Chart IV illustrates some of the variations experienced within one given litter farrowed by a sow that was negative at the time of farrowing and has remained so to date. An attempt was made to uncover the possible sources of infection such as:
CHART I
Summary of Estimated Results 7/1/61 - 7/1/62

<table>
<thead>
<tr>
<th></th>
<th>Number of Tests</th>
<th>Number of Negative</th>
<th>Number of Positive</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michigan State University Breast-</td>
<td>1177</td>
<td>859</td>
<td>318</td>
<td>27.0</td>
</tr>
<tr>
<td>ing Herd and Offspring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swine Evaluation Station</td>
<td>189</td>
<td>123</td>
<td>66</td>
<td>33.6</td>
</tr>
<tr>
<td>Swine Disease Research (S.P.F.)</td>
<td>57</td>
<td>46</td>
<td>11</td>
<td>19.3</td>
</tr>
<tr>
<td>Endocrine Research</td>
<td>61</td>
<td>26</td>
<td>35</td>
<td>57.4</td>
</tr>
<tr>
<td>Purchased Experimental Stock</td>
<td>152</td>
<td>76</td>
<td>76</td>
<td>50.0</td>
</tr>
<tr>
<td>(12 sources)</td>
<td>1636</td>
<td>1130</td>
<td>506</td>
<td>30.9</td>
</tr>
</tbody>
</table>

Regular testing at 60 day intervals has been continued, but for purposes of this Chart, only those tests for the year 7/1/61 - 7/1/62 are included.

CHART II
Test Reactions of Some Selected Individual Sows

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Y 37-1-60</td>
<td>298</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Y 37-2-60</td>
<td>256</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>R</td>
</tr>
<tr>
<td>Y 37-6-60</td>
<td>276</td>
<td>N</td>
<td>R</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>R</td>
</tr>
<tr>
<td>Y 6-2-60</td>
<td>297</td>
<td>N</td>
<td>N</td>
<td>S</td>
<td>S</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Y 22-3-60</td>
<td>259</td>
<td>N</td>
<td>N</td>
<td>R</td>
<td>D</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>X 82-1-60</td>
<td>15</td>
<td>N</td>
<td>N</td>
<td>S</td>
<td>N</td>
<td>R</td>
<td>N</td>
</tr>
<tr>
<td>X 83-4-60</td>
<td>3</td>
<td>N</td>
<td>R</td>
<td>R</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H 6-2-60</td>
<td>243</td>
<td>N</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>H 9-3-60</td>
<td>244</td>
<td>N</td>
<td>N</td>
<td>S</td>
<td>N</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>H 18-1-60</td>
<td>251</td>
<td>N</td>
<td>R</td>
<td>N</td>
<td>R</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Y 20-0-59</td>
<td>277</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

1. Skin testing of all personnel having contact with swine.
2. Testing of feeds and feed substance for presence of acid fast organisms.
3. Trapping, testing and culturing rats, pigeons, and cats on piggery premise.
4. Culturing of boar semen from boars used in artificial insemination. Acid fast organisms mainly of Group three type were isolated from three different boars.
5. Culturing of embryonated ascarid eggs collected from reactor animals upon slaughter.

These investigations were negative or inconclusive; the source of infection was unknown. There were no previous tuberculosis tests and it
SWINE TUBERCULOSIS

CHART III

Summary of T.B. Tests on Females Retained in the MSU Swine Breeding Herd

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Tested</td>
<td>83</td>
<td>74</td>
<td>58</td>
<td>96</td>
<td>89</td>
<td>56</td>
<td>91</td>
</tr>
<tr>
<td>Number Reactors</td>
<td>14</td>
<td>19</td>
<td>6</td>
<td>20</td>
<td>14</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Number Passed</td>
<td>69</td>
<td>55</td>
<td>52</td>
<td>76</td>
<td>75</td>
<td>56</td>
<td>70</td>
</tr>
<tr>
<td>Percent Reactors</td>
<td>16.9</td>
<td>25.7</td>
<td>10.3</td>
<td>20.8</td>
<td>15.7</td>
<td>0</td>
<td>23.1</td>
</tr>
<tr>
<td>Tested first time</td>
<td>83</td>
<td>21</td>
<td>6</td>
<td>20</td>
<td>12</td>
<td>33</td>
<td>42</td>
</tr>
<tr>
<td>Repeat tests</td>
<td>0</td>
<td>53</td>
<td>32</td>
<td>76</td>
<td>77</td>
<td>23</td>
<td>49</td>
</tr>
<tr>
<td>Repeat tests, no change</td>
<td>0</td>
<td>38</td>
<td>40</td>
<td>64</td>
<td>60</td>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td>Repeat tests with change</td>
<td>0</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>17</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Repeat tests with change N to R</td>
<td>14</td>
<td>3</td>
<td>9</td>
<td>7</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Repeat tests with change R to N</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>10</td>
<td>4</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

CHART IV

Tuberculin Test Results on One Litter of Pigs

Pigs Farrowed 4/7/62 By Sow 16-10-61

<table>
<thead>
<tr>
<th>Pig Identification</th>
<th>5/8/62</th>
<th>8/14/62</th>
</tr>
</thead>
<tbody>
<tr>
<td>58-3</td>
<td>R</td>
<td>N</td>
</tr>
<tr>
<td>58-11</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>58-4</td>
<td>R</td>
<td>N</td>
</tr>
<tr>
<td>58-7</td>
<td>R</td>
<td>N</td>
</tr>
<tr>
<td>58-5</td>
<td>R</td>
<td>N</td>
</tr>
<tr>
<td>58-12</td>
<td>R</td>
<td>N</td>
</tr>
<tr>
<td>58-8</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>58-6</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

would be difficult to determine when the infection initially entered the swine herd.

Both negative and reactor animals were followed to slaughter and lymph nodes were collected for culturing and typing by the Tuberculosis Laboratory. The report of these findings on this phase of the project will be presented by Mallman, et al.

Very little is actually known about swine tuberculosis. Much that is written is based on survey rather than research. Little is known regarding infectivity, transmission, pathogenicity amongst swine, role of such factors as breed, age, sex, possible transfer from sow to pig, role of feeds, housing, management practices and the like.
Some items of interest can be gleaned from testing and observations.

1. The manure from the swine farm had been spread on fields later used for grazing cattle. The cattle revealed no evidence that infection or sensitization of cattle had occurred as a result of this practice.

2. A former piggery site on campus was immediately adjacent to the old Sheep Barn. A test of 123 adult breeding ewes and rams revealed only one minor response and this was in a purchased ewe.

3. Even though the poultry plant is separate and distant from the swine plant—the old hens used on breeding projects, etc. were tested, but no tuberculosis problem was disclosed.

4. Limited transmission studies have been and are being conducted. Using organisms isolated from early cases, it was possible to reproduce infection and recover the organisms from the experimental cases.

There is limited evidence based on test results that a fairly high percentage of pigs farrowed by and nursing reactor sows will become reactors. This would undoubtedly vary with site and activity of lesions in the particular sow. Limited tests using pens of pigs half of which were on animal protein and half on vegetable protein, failed to be of significance.

There is a definite need for an extensive research program on swine tuberculosis. What is the public health significance of this swine situation in view of the increased isolations of atypical Mycobacteria associated with pulmonary disease in humans? Is it endangering the lives and well being of the individuals handling, feeding, slaughtering and otherwise involved with the swine herd?

How significant are the avian and Group three or so-called atypical Mycobacteria in swine?

What role does feed play in the swine tuberculosis situation? Of what significance are the animal sources of protein used in swine rations if they contain material from slaughtered reactors or infected animals?

What is the economic effect as far as rate of gain and feed efficiency on swine? What role do newer management factors such as slats, expanded metal, paved feeding areas, and lagoon, etc. play in this problem? Example: Does contusion from slats serve as portal entry for infection? What role do such disease conditions as Atrophic Rhinitis, Virus Pneumonia and the like, play in susceptibility to infection?

It has been shown that the swine ascarid egg can harbor and serve as a vector of the Hog Cholera virus. Can or does it serve a similar role in the Tuberculosis problem?

The diagnostic test used is designed for the bovine with sites and dosages not necessarily proved to be optimum for swine. Tuberculin of type and concentration designed for species other than swine is used. This entire area needs study. Culture methods apparently leave much to be desired.
CONCLUSION

The results of repeated tests (tuberculin) of swine from a herd with positive reactors have been reported. The experience we have had with these repeated tests and the high incidence of reactors found in herds other than M.S.U.'s suggests that major problems exist with respect to diagnostics and the economic and public health significance of tuberculosis in swine. The fact that animals positive to the conventional tuberculin test have been found in a very high percentage of outside herds tested further suggests that the "swine tuberculosis problems" may be widespread rather than limited in scope.

This is a problem about which actually little factual material is known. The entire problem of tuberculosis as it affects swine and its interrelationship with other species is in need of research. Testing procedures, test reagents or allergies, and the potential public health significance of this entire problem are in dire need of evaluation.

REFERENCES

THE STATUS OF STATE-FEDERAL BOVINE TUBERCULOSIS ERADICATION

A. F. Ranney*

Washington, D. C.

We are moving forward toward our goal of complete elimination of tuberculosis. One of the most important factors for encouragement is the increasing willingness on the part of those engaged in tuberculosis eradication to roll up their sleeves, dig in, and stamp out the infection when and wherever it may be found.

Dr. J. Arthur Myers,1 an untiring supporter of tuberculosis eradication whether in man or animals, has made the following comment relative to follow-through on important projects:

"For numerous activities of life, it has long been observed that many persons work enthusiastically and untiringly on health and other projects until the glamour is gone and the drudgery of the final clean-up begins. The number willing to carry through to complete accomplishment is greatly limited, which is apparently why so many worthwhile projects are never completed."

With the increased interest in bovine tuberculosis eradication during recent years and the added efforts to reach our goal, there is every reason to believe that we will reach that goal.

Committees on tuberculosis, of this Association and others, have frequently called attention to the importance of proper program procedures. The 1948 United States Livestock Sanitary Association Committee report2 contains an example: "The Committee cannot stress too strongly that every effort be made by those in charge of tuberculin testing in the various States to insist that tests be performed according to the best procedures at one's command, and that they should instill in those making the test a sense of individual responsibility for work well and honestly done."

The increased use of the suspect classification with proper recording of tuberculin reactions is a tightening up of program procedures. In the past, many reactions to tuberculin have gone unrecorded. All known facts, including tuberculin reactions must be available to those supervising the program so that complete epidemiological studies can be made. Without the recording of all reactions, we are stumbling in the dark unable to compare results in different areas. While this is being overcome to some degree, we are a long way from the degree of uniformity that this phase of the program deserves.

Statistical data for the past years indicate a definite trend toward the increased use of the suspect classification. For the Fiscal Year 1960, 39 States reported suspects. For 1962, the number had increased to 43. With comparable numbers of cattle tested each year (between nine and ten million), the suspect classification has increased in the three year period, from 7,763 to 20,350.

*Dr. A. F. Ranney, Chief Staff Officer, Tuberculosis Eradication, Animal Disease Eradication Division, Agricultural Research Service, Washington 25, D. C.
During the same time there was a reduction in the number of reactors branded from 14,149 to 10,940. See Figures 1 and 2.

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Total cattle tested</th>
<th>Lots &amp; cattle tested showing suspects but no reactors</th>
<th>Suspects in lots containing reactors</th>
<th>Total reactors</th>
<th>Total suspects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960</td>
<td>9,439,706</td>
<td>3845 164,592 6,267</td>
<td>1,496 14,149</td>
<td>7,763</td>
<td></td>
</tr>
<tr>
<td>1961</td>
<td>9,778,386</td>
<td>5447 322,708 10,123</td>
<td>3,477 14,579</td>
<td>13,600</td>
<td></td>
</tr>
<tr>
<td>1962</td>
<td>9,219,298</td>
<td>7279 383,943 15,426</td>
<td>4,924 10,940</td>
<td>20,350</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>No. tested to find 1 reactor</th>
<th>No. tested to find 1 lesion reactor</th>
<th>No. tested to find 1 suspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960</td>
<td>667</td>
<td>3,090</td>
<td>1,215</td>
</tr>
<tr>
<td>1961</td>
<td>671</td>
<td>3,437</td>
<td>720</td>
</tr>
<tr>
<td>1962</td>
<td>843</td>
<td>4,731</td>
<td>453</td>
</tr>
</tbody>
</table>

Figure 1

**Tuberculosis Eradication**

**INCREASED USE OF SUSPECT CLASSIFICATION**

A decrease of about 3,600 reactors during the past year and an increase of 6,750 suspects support our contention that a major share of the suspects reported in 1962 resulted from tests that formerly would be included in the summaries as negative.
Twenty-two of the 52 reporting units (50 States, Puerto Rico, and Virgin Islands) reported more suspects than reactors.

Adequate data to determine the portion of cattle branded as reactors that were suspects on prior tests should be obtained as an aid in evaluating the program, and is therefore now included in our program planning.

It is interesting to make a comparison between two populous cattle States. During Fiscal Year 1962, each reported more than 800 reactors. One tested approximately 600,000 cattle and reported approximately 7,000 suspects in addition to the reactors. The other tested approximately 400,000 cattle and reported no suspects in addition to the reactors. Does this mean that one State was recording a large portion of the responses found in addition to the reactors branded and that the other State found no significant responses beyond those declared reactors, or did the second State ignore them? Are we keeping our cards above the table?

The Committee on Tuberculosis in 1946 stated, "This Committee has repeatedly called attention to the fact that we need tests other than tuberculin or perhaps a better tuberculin to complete the job of eradicating tuberculosis from our cattle. Although the tuberculin test is as accurate as any biological test of which we know, it has some shortcomings. The shortcomings of the test have long been known but they were not particularly serious so long as we had a considerable amount of tuberculosis to deal with. Now the incidence of the disease is very low throughout the country, the NVL (no visible lesion) cases are a cause for real concern."

While this subject has had a great amount of attention and study over the years, a special field project was recently designed to compare the results of injecting cattle with 0.1 cc. of standard tuberculin in one caudal fold and an equal amount of diluted tuberculin (1/100) in the opposite fold. The data on this project accumulated through July 1962, show that about 60 percent of the animals that responded to standard tuberculin were negative to the dilute tuberculin. About 40 percent of those responding to the standard tuberculin also had responses to the dilute tuberculin but for the most part to a lesser degree.

Of the herds in which reactors were branded, only three would have been classified negative on the basis of the results of the dilute tuberculin alone. These three herds each had one NGL (no gross lesion) reactor.

On the other hand, there were four herds with lesion reactors. The lesions in three of the four herds were sent to the laboratory where a diagnosis of tuberculosis was supported. These three herds containing ten reactors (of which four carcasses were condemned on meat inspection) did not have any responses greater than pp or x to the dilute tuberculin.

The data accumulated to date on this project suggest that the changes in the Uniform Methods and Rules approved April 24, 1962, with respect to increased use of the suspect classification are a much more realistic approach to the non-specific sensitivity problem than the use of dilute tuberculin (1/100). This conclusion as a result of limited field data is compatible with findings of W. Meyer, a German investigator, who concluded, "That although diluted tuberculins helped to eliminate non-specific reactions, this was at the expense of accuracy, as proved P.M." (post mortem).
The tuberculosis diagnostic work at the National Animal Disease Laboratory, Ames, Iowa, is providing disease eradication officials with valuable scientific information. All suspected tuberculous lesions found on meat inspection can now be examined. The definitive examination of tuberculous lesions provides us with the opportunity to evaluate the tools that we have been using. Broad investigative projects are now possible to sharpen our eradication tools.

During the Fiscal Year 1962, 927 suspected tuberculous cases were submitted for pathological examination. A diagnosis of tuberculosis was supported in 492 cases while 435 were considered negative. Acid-fast organisms were found as a result of examinations of 40 skin lesion specimens.

Culturing attempts were made on 191 specimens of bovine origin. These specimens produced 34 positive cultures of which two were Mycobacterium avium, two were M. bovis, and 12 were Runyon Type IV Mycobacteria. The other 18 isolates are being typed.

A graphic breakdown of results of examinations made on 513 suspected tuberculous specimens examined during the period January to July 1962, is shown in Figure 3.

This Association last year (1961) adopted a list of program priorities to strengthen the Tuberculosis Eradication Program and to gain maximum benefits from funds expended. The number one item on this list called for concentration on the elimination of tuberculosis from "Red Flag" herds and other herds known to be affected with tuberculosis. A "Red Flag" herd is one that has revealed reactors with lesions of tuberculosis on repeated
tests and is still under quarantine. Some of these herds have been infected for many years. It naturally followed that the next four priority items would call for epidemiological studies of the cattle and herds exposed to known tuberculosis. Then number six on the list called for maintaining modified accredited status.

Too frequently, in the past the modified accredited status has held the number one position with the other phases relegated to a place of secondary or even minor importance. Fourteen years ago your Committee on Tuberculosis stated, "All those in charge of this work should be more concerned with tuberculosis eradication than with the reaccreditation of areas as such."

It has been demonstrated that the program priorities recommended by last year's Committee on Tuberculosis have been effective. Concentrated efforts in "Red Flag" herds has produced encouraging results. There were 239 such herds in 1960. This was reduced to 101 herds in 1961, and down to 50 herds in 1962. The reduction in "Red Flag" herds and the location of herds presently in this category are illustrated in Figures 4 and 5 respectively.

In reviewing the Committee reports on tuberculosis that were adopted by the Association one cannot help but be impressed with the frequent recommendations for adjusting our methods to provide for better identification and supervision of movements of cattle as an aid to eradication. I believe it would be appropriate to review some of these at this time—thus I quote:

\[
\text{Tuberculosis Eradication}
\]

\[
\text{RED FLAG HERD PROGRESS}
\]

\[
\begin{array}{c}
\text{239 Herds} \\
1960 \\
1961 \\
1962 \\
\text{JULY}
\end{array}
\]

\[
\begin{array}{c}
\text{101} \\
\text{50}
\end{array}
\]

U.S. DEPARTMENT OF AGRICULTURE  AGRICULTURAL RESEARCH SERVICE

Figure 4
1940⁵ "That records kept of tuberculin tests of herds should be such that when animals shipped interstate react and show lesions, notification should be sent to the livestock sanitary official of the State of origin.

"That in each case, when at slaughter animals reveal lesions of tuberculosis, a report be sent to the livestock sanitary official giving the tag number, recorded brand, description of animal, and name of consignee so that the herd from which it came could be retested.

"That more adequate control of truck movement of cattle and closer supervision over traffic of animals through livestock markets should be exercised."

1946³ "The finding of tuberculous herds through the tracing of diseased animals discovered in the slaughter houses of the country has contributed substantially to the solving of the tuberculous problem. This method should be perfected in the establishments where Federal inspection is maintained, and it should be used as fully as possible in the local slaughter houses under State and municipal inspection."

1954⁶ "Broaden the service for tracing straight slaughter cattle revealing lesions of tuberculosis and inaugurate a tag, tattoo or brand recording system in all States so that cattle of this nature may be readily traced to the herd of origin."

1956⁷ "That the Agricultural Research Service make a special study of the problems encountered in identifying and tracing to herds
of origin animals that show lesions of tuberculosis on regular kill and to place into effect adequate measures for advancing this phase of the program to maximum efficiency.

"That every effort be made to determine the origin of each reacting animal and to follow up on animals that have been exposed to infection in order to help locate other foci of infection."

19584 "We recommend that every effort be made to trace all non-reactors disclosing lesions at slaughtering establishments to the herds of origin, and promptly test all cattle directly or remotely exposed to such animals."

A lot of recommending and talking about identification of market cattle to assist in traceback for tuberculosis eradication has taken place. It is imperative that we have more down-to-earth action relating to supervision of animal movements and the development of better animal identification with complete carcass examination. In many instances a diligent search is made for the herd of origin of a poorly identified tuberculous animal. Field men have reason to be disheartened with the meager information presently available on many diseased animals.

In the Uniform Methods and Rules adopted April 1962, accreditation of areas may be based on animal identification through slaughter. For example, in Paragraph 12 it is stated that accredited status may be continued for three additional years provided that: Eighty (80) percent of animals marketed for slaughter are slaughtered at accredited establishments; and procedures have been developed to regularly trace animals with lesions of tuberculosis to herds of origin. Also, Paragraph 13 states that: Reaccreditation may be extended for a six year period if - during each year at least five percent of cattle over two years of age in the area, or a total of 30 percent during the six year period, have been subjected to a meat inspection examination at an accredited establishment and can be identified with the herds of origin. Successful operation of this plan of action is dependent upon three fundamental conditions for all animals included in the statistics for reaccreditation:

1. Adequate meat inspection examination.
2. Adequate identification of each animal.
3. Records and information that will permit identifying the diseased animal with those with which it may have been associated.

During Fiscal Year 1962, there were 24 lesion cases reported by veterinary meat inspectors for cattle that had moved to slaughter from feed lots in 11 States. The diagnosis of tuberculosis in each case was supported by laboratory findings. Due to the lack of identification, the source of infection was not determined in any of those cases which included nine cattle with extensive lesions of tuberculosis. This shows a serious need for maintaining the identify of individual animals from the parent herd through the feed lot to slaughter.

Much progress has been made in the individual identification of cattle by use of the uniformly-numbered ear tags and back tags. Some States have developed, or are developing, effective record systems that will make
it possible to associate tag numbers with specific herds and related health records.

However, high-speed, mechanized operations in many slaughtering plants have been adopted without adequate provisions being made for maintaining the identity of the hide with the cattle carcass until examination of the carcass has been made. Thus, when disease conditions are revealed, the ear tag or animal description is not in evidence and it becomes difficult or impossible to locate the cattle previously associated with the diseased animal. This problem is particularly acute in certain plants.

Under Federal inspection in the continental United States, there are about 494 cattle slaughtering plants. The trend is toward higher speed, mechanized cattle slaughter. At least 61 plants use "on the rail" dressing methods where the hide is taken away before viscera inspection. There is a total slaughtering capacity in these plants of approximately 4,000 cattle per hour. It is conservatively estimated that this fast-moving operation includes at least 28 percent of the cattle subject to Federal meat inspection.

In order to obtain better animal description, a simple direct method for identifying the hide with head and carcass is being tried in a few "key" locations so that when an infectious disease is reported, the descriptive identity of the affected animal can be determined. The method is referred to as the three-part tag system which supplements the two-part tag now generally used to identify heads and carcasses in plants operating under Federal meat inspection. The three-part tag is applied in a manner that does not require additional labor in the plant.

The value and need for use of the three-part tag is apparent when we consider the results obtained during the Fiscal Year ending June 30, 1962. Investigative studies were conducted following 366 case reports that included 395 cattle with lesions on regular kill. State and Federal officials could not identify the herds of origin of at least 64 or 17.4 percent of the cases reported. The laboratory examination of lesion specimens supported the diagnosis of tuberculosis in 50 of those 64 cases; while no determination was reported on the other 14 cases.

Considerable time and expense were directed to the 64 cases which resulted in unsuccessful field investigations. When the diseased animal is not adequately described or identified at the slaughtering plant, many problems are encountered before the herd of origin can be located. Figure 6 clearly depicts the movement from the farm to slaughter of cattle in 220 cases reported with lesions of tuberculosis on regular kill. Intermediate movements of these animals through dealers and markets could not be included because the maze of travel routes would have obscured the overall lines of travel.

Figure 7 identifies 26 States where reactors were disclosed in the herds of origin after tuberculous lesions were reported on regular kill. This supports the information depicted in Figure 6 that tuberculosis infection is still dispersed country-wide.

The results of the 366 case studies in 1962 justify an all-out effort to identify individual cattle and to use that identification whenever it is necessary to locate originating herds. As Figure 8 shows, during 1962, testing
TB TRACEBACK-ADE 6-35
Animal Movements Reported

- Slaughter Plant
- Point of Origin
ADE 6-35 (Lesions, Regular Kill)

Fiscal Year 1962

Figure 6

TB TRACEBACK-ADE 6-35
States With Reactor Herds

ADE 6-35 (Lesions, Regular Kill)

Fiscal Year 1962

Figure 7
Tuberculosis Eradication

RESULTS OF ADE 6-35 INVESTIGATION

CATTLE TESTED

| 0.34% |

REACTORS FOUND

| 8.6% |

LESION REACTORS DISCLOSED

| 22.5% |

% OF U. S. TOTAL
FISCAL YEAR 1962

U. S. DEPARTMENT OF AGRICULTURE AGRICULTURAL RESEARCH SERVICE

Figure 8

as a result of traceback on lesion cattle regular kill amounted to only .34 of one percent of the total cattle tested. However, in those tests, 8.6 percent of the reactors were found and 22.5 percent of all lesion reactors were revealed.

Comparative results of all tuberculin testing versus testing after lesions of tuberculosis are reported on regular kill (ADE 6-35 cases) is illustrated in Figure 9. This shows a marked contrast of 33 cattle tested to find one reactor after 6-35 traceback, as compared to 919 cattle tested to find one reactor under other program tests. Again, one lesion reactor was found among 71 cattle tested after 6-35 traceback and one lesion reactor among 6,088 cattle tested under other tests.

Our goal is: Complete description and identification of each animal found infected on regular kill and prompt action to locate and eliminate the source of the infection. Our motto should be "Hunt—Don't just find T.B."

This goal will be realized when the livestock and meat industry become active participants. This will require the exchange of information concerning the responsibilities and necessary action for providing effective programmed service. Each of these participants must be kept informed concerning current problems in maintaining identification and history of diseased or exposed animals. Consequently, frequent contacts and organized discussion programs are essential. This will make it possible for the producer to cooperate by having historical data for the animals he owns, the dealer for animals he buys and sells, the marketer for animals consigned and sold, and the slaughterer for maintaining descriptive identity of diseased animals reported.
Lesion reactors

<table>
<thead>
<tr>
<th>Fiscal Year 1962</th>
<th>Total Testing</th>
<th>6-35 Traceback testing</th>
<th>All other testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cattle</td>
<td>Cattle</td>
<td>Percent total</td>
</tr>
<tr>
<td>Cattle Tested</td>
<td>9,219,298</td>
<td>31,444</td>
<td>9,187,854</td>
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<tr>
<td>Reactors found</td>
<td>10,940</td>
<td>945</td>
<td>9,995</td>
</tr>
<tr>
<td>Tested to find 1 reactor</td>
<td>842</td>
<td>33</td>
<td>919</td>
</tr>
<tr>
<td>Reactors Slaughtered</td>
<td>10,744</td>
<td>945</td>
<td>8.8</td>
</tr>
<tr>
<td>Lesion reactors</td>
<td>1,949</td>
<td>440</td>
<td>1,509</td>
</tr>
<tr>
<td>Percent - reactors with lesions</td>
<td>18</td>
<td>46.5</td>
<td>15.4</td>
</tr>
<tr>
<td>Reactors slaughtered to find 1 lesion case</td>
<td>5.5</td>
<td>2.1</td>
<td>6.4</td>
</tr>
<tr>
<td>Cattle Tested to Find a Lesion Reactor</td>
<td>4,730</td>
<td>71</td>
<td>6,088</td>
</tr>
</tbody>
</table>

Figure 9

Some people say that we should relax; that there is very little tuberculosis; that we should wait for someone to develop a more specific diagnostic test; or that we should let the disease die out spontaneously. But we must squarely face the facts that:

1. There is a widely-dispersed population of tubercle bacilli in our domestic livestock.
2. These bacilli are capable of multiplying rapidly.
3. The incidence is low; we have the means to stamp it out.

These conditions make it imperative that we promptly locate and eradicate tuberculosis and thus meet our obligation to the livestock industry in protecting the great number of tuberculosis-free herds and cattle.

REFERENCES

REPORT OF THE COMMITTEE ON TUBERCULOSIS


In March 1962, the Tuberculosis Committee met in Chicago to consider recommended changes in the Uniform Methods and Rules for tuberculosis eradication. The basic philosophy of objective testing had been presented at previous meetings, in subsequent correspondence and other contacts. The many activities at the annual meeting that demanded the attention of Committee members dictated the need for the meeting which was held in Chicago. The resulting revision of the Uniform Methods and Rules was then circulated to all State officials and with their approval was presented to the United States Department of Agriculture for final approval and adoption in April 1962.

Again during the coming year the Committee intends to develop further Uniform Methods and Rules to apply toward a bovine tuberculosis free area. During the year this proposal will be circulated to all members of the Executive Committee for their evaluation. A final revision can then be made and put into the hands of all interested individuals at least several months before the next Committee meeting. This again makes it possible for all to be fully informed before they are required to make a final decision.

This report has been before the members of this Association for better than six months and any revisions at this time are based on actual experiences. The changes which will be discussed later are all relatively minor in nature and essentially are for clarification.

We are pleased to report marked progress in the total eradication program. Virtually all lesions indicative of tuberculosis are now being examined by the Diagnostic Services, National Animal Disease Laboratory. The number of "Red Flag" herds has been cut in half during the past year. Unfortunately, however, not all available tools are being used in this area. Only 50 percent of herds in this category have been tested with the cervical test. With more individual attention being given to problem herds utilizing all available tools, it is believed that infection in the "Red Flag" herds can be eliminated at an early date. There is every indication too, that we are now receiving far more accurate reports of the reactions to tuberculin test than have been received in many years. Many States report that the new procedures have eliminated many of the problems that had been facing them. A few States, however, are concerned over the definition of a "Commercial Dairy Herd" and indicated that this requires an
appreciably larger number of herd tests than had been necessary under the old rules.

The Committee thoroughly reviewed the need to test all commercial dairy herds and believe that any relaxation would be a step backwards. The small commercial herds producing milk for canneries or other processing has been virtually uncontrolled for many years and public health officials and sanitarians have questioned this neglect. Much of the raw milk consumed on the farms originate in such herds and with the general lack of management the public health factor to the farm family can be a serious one. It must be recalled that a large number of individual tests have been eliminated by requiring only the testing of cattle over 24 months of age in routine area work.

With the trend toward utilizing slaughter reports in lieu of specific tests the problem presented by these States can be rapidly reduced by adopting the Market Cattle identification including the backtag system.

The trend in all disease control must continue to be directed away from farm to farm testing and must utilize information received at the slaughtering plants. In fact, this trend has such tremendous potential every effort possible must be made to perfect it not only for the brucellosis and tuberculosis eradication programs, but for all disease programs now in operation and anticipated in the future. It's use in surveys of diseases common to both man and animal alone would justify every effort.

The cooperation of all marketing agents, breed associations, and packing industries must be vigorously pursued. If answers are found to many of the problems now a part of such a system the reward will be beyond that received from any specific program now in operation.

We are very pleased to note the research advancement at Michigan State University and at the National Animal Disease Laboratory at Ames, Iowa. Specifically, to further these efforts it is absolutely essential that a tuberculosis infected herd be established and maintained for the use of researchers on tuberculosis.

Encouraging progress being made in the development of serological and other diagnostic measures dictate that these studies be continued as rapidly as possible.

A sub-committee on paratuberculosis has been recommended and Drs. A. B. Larsen, J. G. Flint, and E. L. Brower have been appointed.

We recommend the following changes in the Uniform Methods and Rules:

1. In Part I, definitions, Paragraph 5, following the word milk insert the words, "that is marketed."
2. The provisions of Paragraph 11 (c), (d), (e), (f), and (g), shall apply equally to Paragraphs 12 and 13.
3. Place in parenthesis following 11 (b) the following: "If a record is maintained indicating that an annual average of at least five percent of the cattle over two years of age in a herd have been slaughtered at accredited establishments and identified to the originating herd, such a herd record may be substituted for the testing required in 11 (a) and 11 (b) of this paragraph. This substitution may be made if the record is continuous and has been in effect in excess of one year."
PART I: Definitions

1. "Accredited Herd" is one in which no reactors have been found on at least two consecutive annual tuberculin tests.

2. "Advanced tuberculosis" refers to carcasses of animals in which a lesion of tuberculosis (other than tuberculoid skin lesion) is found as a result of post-mortem examination.

3. "Annual tests" for purposes of herd accreditation means tests made in not less than 11 nor more than 15 months.

4. An "Accredited establishment" is one at which supervised meat inspection is maintained at all hours when slaughter is in progress and post-mortem procedures meet recognized standards for the disclosure of lesions of tuberculosis.

5. A "Commercial dairy herd" is one composed of cattle of the recognized dairy breeds which produces milk that is marketed for human consumption in any form.

6. "Modified Accredited Area" is a State or portion thereof in which the degree of infection does not exceed one-half of one percent.

7. A "Registered purebred herd" is one that has a minimum of 10 registered purebred cattle during all parts of the year and from which registered animals may be sold for breeding purposes.

PART II: Individual Accredited Herd Plan

1. (a) Classification of animals tested

   (1) Reactors - "R"
   Animals showing a P1 - X2 or greater response to the tuberculin on routine test should be classed as reactors in the professional judgment of the testing veterinarian a suspect classification is justified. In herds with known tuberculosis all animals that respond to the tuberculin test will be classified as reactors.

   (2) Suspects - "S"
   This classification is to be used for animals showing any response at point of injection not classified as reactors, with the exception noted below.

   (3) Negatives - "N"
   Animals showing no tissue disturbance at site of injection will be classed as negatives. Animals showing a minimal
3. The entire herd, or any cattle in the herd, shall be tuberculin tested or retested at such times as are deemed advisable by the Cooperating State and Federal authorities.

4. No animals that has been designated as a reactor at any time shall be retested.

5. (a) Reactors to the tuberculin test shall be removed from the farm in accordance with State and Federal laws and regulations. After their removal, the infected premises shall be thoroughly cleaned and disinfected with a disinfectant permitted by the Animal Disease Eradication Division, ARS, USDA, and in a manner satisfactory to the cooperating State and Federal authorities.
   (b) A complete epidemiological survey shall be made on all herds in which reactors are disclosed, by appropriate State or Federal personnel.

6. Herd additions must originate directly from tuberculosis-accredited herds, or herds (not under quarantine) in a modified accredited area that were tested and found negative within a 12-month period immediately prior to being added to the herd. Other additions to an accredited herd shall originate directly from herds not under quarantine in modified accredited areas; pass a negative test not more than 30 days prior to entry; and be segregated from the remainder of the herd until retested and found negative at least 60 days after entering the premises.

7. To qualify for accredited status all animals must be bona fide members of the herd as attested by a certificate issued jointly by the local State and Federal officials. The accredited herd status may be valid for not more than 1 year (365 days) from the date of the qualifying herd test. To qualify for reaccreditation the herd must pass a negative test within a period of 15 months from the last previous accreditation test.

8. Owners of accredited herds shall be required to maintain such environmental conditions as are consistent with the generally accepted standards of good sanitation and herd management. The use of milk or other dairy products for feeding is prohibited unless such products are from a known safe supply to have been pasteurized or sterilized. Identity shall be provided by ear-tag or other satisfactory means for all animals and complete records must be kept of all additions to the herd. Only properly cleaned and disinfected vehicles may be used for transporting cattle into accredited herds.

9. Failure on the part of an owner to comply with these methods and rules shall constitute sufficient cause for revocation of the accredited herd certificate.
PART III: Modified Accredited Area Plan

10. The provisions of the individual accredited herd plan that relate to testing, quarantine, removal of reactors, cleaning, disinfecting, sanitation, and epidemiology shall apply to the modified accredited area plan, except that in routine area testing all cattle twenty-four (24) months of age or over shall be tested. In herds with advanced tuberculosis all cattle in the herd shall be tested.

11. An area may be accredited or reaccredited for a period of six years provided that:

(a) All commercial dairy herds are tested.
(b) All registered purebred herds that are maintained under confinement at any period of the year are tested. (If a record is maintained indicating that an annual average of at least five percent of the cattle over two years of age in a herd have been slaughtered at accredited establishments and identified to the originating herd, such a herd record may be substituted for the testing required in 11(a) and 11(b) of this paragraph. This substitution may be made if the record is continuous and has been in effect in excess of one year.)
(c) All cattle in herds of origin or cattle associated with those showing evidence of tuberculosis at time of slaughter are immediately tested.
(d) All herds containing reactors with advanced tuberculosis within the past 12 years are tested.
(e) Other cattle as may be considered necessary by the State and Federal cooperating officials are tested.
(f) The testing schedule of all reactor and suspect herds is current at the time of accreditation or reaccreditation.
(g) The total number of reactors found in the area on the last test of each herd during the reaccreditation period does not exceed 0.5 percent of the cattle tested.

12. An area that has met the minimum requirements of paragraph 11, may be continued in an accredited status for an additional three (3) year period provided that:

(a) Eighty (80) percent of animals marketed for slaughter from the area are slaughtered at accredited establishments; satisfactory procedures have been developed to regularly trace animals with lesions of tuberculosis to herds of origin; and there is no increase in incidence of tuberculosis in the area.
(b) All cattle in herds of origin or cattle associated with those showing evidence of tuberculosis at time of slaughter are immediately tested.
(c) All herds containing reactors with advanced tuberculosis within the past 12 years are tested.
(d) Other cattle as may be considered necessary by the State and Federal cooperating officials are tested.
(e) The testing schedule of all reactor and suspect herds is current at the time of accreditation or reaccreditation.

(f) The total number of reactors found in the area on the last test of each herd during the reaccreditation period does not exceed 0.5 percent of the cattle tested.

13. An area may be reaccredited for a period of six years provided that:

(a) Reports are produced showing that during each year at least five percent of the cattle over two years of age in the area as determined by statistics of the A.M.S., or a total of 30 percent during the six-year period, have been subjected to a meat inspection examination at an accredited establishment and can be identified with the herd of origin.

(b) All cattle in herds of origin, or cattle associated with those showing evidence of tuberculosis at time of slaughter, are immediately tuberculin tested.

(c) All herds containing reactors with advanced tuberculosis within the past 12 years are tested.

(d) Other cattle as may be considered necessary by the State and Federal cooperating officials are tested.

(e) The testing schedule of all reactor and suspect herds is current at the time of accreditation or reaccreditation.

(f) The total number of reactors found in the area on the last test of each herd during the reaccreditation period does not exceed 0.5 percent of the cattle tested.
Recent developments in the field of anthelmintics for large animals

A. C. Todd, Ph.D.*

Madison, Wisconsin

The objective of applied veterinary parasitology must be to increase efficiency of livestock production as a result of prevention and control of parasitisms.

With respect to a given animal economy, the concept of control of infectious disease has to vary from suppression of animal mortality to extermination of the parasites, that is, control programs in a country the size of the United States has to reflect our geographic concepts of animal production and market schedules. Control objectives in a cattle rangeland economy, for example, are more limited than control objectives in a geographic area of feedlot cattle, or in a dairy area. In a rangeland economy chief attention is given to treatment to limit mortalities. In a feedlot area effects of parasitisms have to be measured in terms of feed conversion.

The quality and quantity of nutrition afforded a herd is related to its capacity to withstand infections. Rangeland parasitisms are aggravated by a lack of—and the quality of—nutritional intake and are, therefore, more severe than in other animal economies. Such parasitisms have their origins in our failure to recognize that our present ranges are overcrowded where, as a consequence, movement of the herds has become restricted severely. Opportunity for exposure is immeasurably greater on overcrowded range than it is on properly managed ranges. No real progress has been made in management—and sanitation—of the rangelands.

Conversely, the average age of feedlot animals has declined steadily in the past two decades, and these herds lack the natural age resistance to parasitisms characteristic of breeding herds. Mortalities and other types of losses in herds in the feedlots usually occur following a breakdown of sanitation, despite the better quality of nutritional intake.

The dominant problems of large animals parasitisms continue to lie in management of the livestock, in lack of sanitation, together with the feed available for the herds and flocks. Still other problems are inherent in our concepts of medicinal treatment.

The concept that a single treatment should destroy the entire parasitic population in an infected host is at once an inspiration and a frustration in practical attempts to control parasitisms. If our entire efforts are to be expended in the development of the magic "full curative" treatment necessary for reducing the gravity of infections at the clinical level then we should admit defeat as parasitologists, because there is a substantial body of evidence that the principal effects of parasites upon their hosts are produced by the activities of the immature stages as they establish themselves within, and become adjusted to life in their hosts. Moreover, if we use

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medicinal treatment only to relieve physical distress in the individual animal, then we have failed to treat parasitisms as herd problems. In herds and flocks we cannot selectively treat single individuals on the basis of their signs of infection, if we are to destroy or appreciably reduce the parasite populations in the groups as a whole.

The employment of treatment for parasitisms as a device to increase herd production is not new as a concept of anthelmintic efficacy, but it now is gaining greater acceptance by livestock producers. Simply stated, preventive treatment rests upon recognition of the universal prevalence of parasitic infections and attempts to remove as large a portion of the parasitic population from a herd as possible, prior to breeding, after calving, lambing or farrowing, prior to herd release to pasture, or prior to, or immediately after confinement of animals in the feedlot, and, preventive treatment also attempts to minimize re-exposure. It can be observed that this concept reflects the production schedule of livestock, and is based upon geographical and climatic situations in that farmers in areas where feed supply is not a problem can use extra feed to sustain higher producing herds with greater reproductive capacity. In some areas of the country the reproductive capacity of herds has to be governed by the amount of feed available.

It is interesting at this point to reflect on the growing realization on the part of parasitologists themselves that full, curative treatments, and preventive, full curative treatments are not abstract concepts, but that, indeed, millions of host animals actually receive treatment for parasitic infections.

In the past two decades we have observed a still further expansion of the anthelmintic concept from a defensive weapon to an offensive one. Strange as it may seem, when anthelmintics changed from the simple status of "worm-killers" to that which affected the reproductive potential of a geographical parasite population, they became even more closely linked to herd production programs, and resulted in discriminate selection of the host populations which were to be treated. These so-called low-level anthelmintics are directly related to the quality of the livestock.

Low-level anthelmintics are designed for ease of administration to entire herds, to obviate handling of animals, and lessen labor costs of treatment. Their cummulative effect is expressed as suppression of parasite reproduction, and reduction of the future exposure of the herd to infective stages of the parasites. The low-level anthelmintics contradict the primitive concept that individual diagnosis is the basis of anthelmintic treatment. From the standpoint of control of parasitisms, herd diagnosis is incomparably more important.

An immediate problem in the control of internal parasitisms lies in the recent development of an extensive series of new anthelmintics. It is clearly evident that parasitologists consider that the manufacturers of new products have a responsibility to control parasitisms on a broad scale. This is to say, that a new product brought out simply for economic advantage of the manufacturer will not thereby increase the efficiency of livestock production. Each new anthelmintic must be developed in such a manner that it will add an advantage to the older preparations and expand control concepts.
tissue response (pp or x) in herds which contain no reactors on the current test, and in which advanced tuberculosis has not been demonstrated on previous tests may be classed as negatives.

Decisions will be based upon the professional judgment of the testing veterinarian in accordance with the policy established in a given area by the cooperating State and Federal officials.

(b) Herds in which reactors occur shall be quarantined and must pass a negative tuberculin test after a period of at least 60 days to be released from quarantine. If there is indication of advanced tuberculosis in one or more reactors, or if lesions of tuberculosis are found at slaughter in animals directly traceable to the herd, two negative tests at intervals of no less than 60 days will be required for release of quarantine.

(c) All herds in which reactors with advanced tuberculosis occur shall be retested in approximately 12 months but not more than 15 months following the first negative test after disclosure of reactors. At this time, the herd may be accredited or reaccredited if it otherwise qualifies. These tests are to be followed by at least two annual herd tests.

(d) When suspects to the tuberculin test are disclosed in herds not containing reactors, such animals shall be restricted to the premises when disclosed. The accredited herd status shall be suspended, and no movement of animals from the herd permitted except under permit issued by the cooperating State authorities until the status of the herd is determined.

(e) Suspects may be sold by permit only for immediate slaughter at an accredited establishment or retained under restriction for a 60 to 120 day retest. If the suspects are removed for slaughter without retest, or are not slaughtered at an accredited establishment, the herd shall be retested in not less than two nor more than six (6) months from the date the suspects were disclosed.

(f) Tuberculin tests shall be applied by a veterinarian employed in a full-time capacity by the State, the Animal Disease Eradication Division, ARS, or by an accredited veterinarian. All tuberculin tests are official tests. A report of all tuberculin tests, including a record of all responses, shall be submitted in accordance with the requirements of the cooperating State and Federal authorities. These officials reserve the right to supervise any test conducted by an accredited veterinarian.

2. The official tuberculin test shall be the intradermic test. The intradermic injection of tuberculin in the cervical area is indicated for use only under the direct supervision of full-time employed State or Federal veterinarians in herds where advanced tuberculosis has been disclosed.
The present emphasis upon broad-spectrum anthelmintics is in part a reflection of basic researches into the pathology of a greater number of parasites, and is also a reflection of the observed response of the mixed populations of helminths to earlier anthelmintics. The basic response of helminths to the effective anthelmintics is a relative increase in number of the least susceptible species, because the species most susceptible to a given anthelmintic are selectively eliminated from the natural parasite population. A response within the natural population of a single parasite species is isolation of strains naturally most resistant to the anthelmintic.

Study of the reaction of parasitic populations to the addition of anthelmintics to the environments they encounter in hosts has developed a useful tool for the research parasitologist. A shift of parasite populations in ruminants from the predominant Haemonchus-Oesophagostomum to predominant Trichostrongylus-Cooperia groups for example, indicates that selective management of the populations is possible in situations where the host populations can be controlled on the basis of their greater economic value. New anthelmintics should accentuate and continue the shift in the composition of the parasite population.

The introduction of new anthelmintics to reflect greater knowledge of the damage produced by the diverse worm parasites and the relationship of the control of parasitisms to management of the hosts, and livestock production, can be traced clearly in a review of the swine anthelmintics. The drug of choice in the 1920's for the removal of Ascaris lumbricoides was Oil of Chenopoidium, the use of which was followed by administration of a purgative to remove the dead worms. Such single curative treatments actually had little relation to control in that they did not serve to reduce appreciably the total number of infective eggs already present in the environment. Moreover this full curative treatment was effective against a single type of worm infection, Ascaris.

The earliest anthelmintics required their administration directly to the individual pig. By standards of present day swine production, the cost of labor involved in treating pigs individually restricts control programs based upon treatment of pigs as individuals.

The first successful drug for mass treatment of herds of swine infected by ascarids was sodium fluoride. When properly mixed into the feed at the rate of 0.75-1.00 percent, this anthelmintic proved amazingly efficient against the larger and mature specimens. Its efficiency is somewhat less against specimens of the worm less than two inches long. With the development of sodium fluoride as an anthelmintic, parasitologists had achieved ease of administration and treatment of an entire herd; it had been established long before that it was impossible to diagnose this infection through visual examination of the exterior of the individual pig.

The safety margin of swine anthelmintics was increased greatly by the development of cadmium products in the late 1940's and early 50's. The efficiency of the cadmians as ascaricides is somewhat less than that of sodium fluoride, but the safety margin is much greater. The cadmiums also were designed for mass treatment of the herd population of worms within the digestive tracts of the swine.
If the internal parasite fauna of swine had consisted solely of the large roundworm, *Ascaris suum*, the problem might have met a fair solution. From its first discovery the very size of the worm had drawn to its presence the label of "important infection." It is true that in pure infection, in sufficient numbers, the worm is known to kill individual pigs, to severely stunt the growth of others and to interfere markedly with the feed conversion ability of all infected swine. Unhappily, worm infections are comprised of a single species only on extremely rare occasions. On the average, a worm infection is comprised of an aggregate number of species, some of which, at least, clearly have been established to be severe pathogens in their own right. None of the older swine anthelmintics had a spectrum of activity much greater than action against the ascarids.

It is possible to direct anthelmintics in a system of periodic full curative treatment to move toward over-all control of worm infection and away from the concept that such treatments relieve only the distress of individual animals. When full curative treatment is employed with relation to the life cycle of ascarids, then it is possible to time administration of the anthelmintic so that worms in the digestive tract are removed just prior to or at the beginning of their egg production. This concept moves anthelmintics from the simple status as worm killers to a second approach to the control of worm infections, an attack upon the reproductive capacity of the parasites.

In 1954 two piperazines were brought to the United States from England. These two piperazines were introduced primarily as ascaricides but they also possessed extremely useful activity against other parasites in swine, principally the nodular worms. The piperazines can be used to attack internal parasites on a herd basis. Because both soluble and insoluble preparations are available, administration of these drugs does not involve much extra labor; they have been administered effectively in drinking water and mixed in the feed. The piperazines were not designed as worm killers, but rather they function to drug the worm so that it cannot maintain itself against peristalsis within the host digestive tract. The safety margin of the piperazines has been notable.

Because of their ease of administration the piperazines can be incorporated readily into systematic periodic treatment of the herd. The disadvantage attached to the piperazines is that they can be used in full curative dosage only. This fact restricts their usefulness more to specific "treatment" of infected herds. It prevents their use in overall control which requires more continuous subtherapeutic treatment.

From a parasitological standpoint the piperazines no doubt have their greatest usefulness in removing the bulk of ascarid and nodular worm infection from feeder pigs as such animals go into the feedlot. The immediate advantage of their use in overcoming heavy infections in severely parasitized pigs is self-evident.

Anthelmintics should serve at least three objectives when employed in control of worm parasites, in relation to the life cycles of the parasites. A drug can function to destroy mature worms within the digestive tract. It might also destroy infective larvae upon their first entrance into the host. It should, and this may be the most important of all, affect the
reproductive capacity of the parasites to prevent future re-exposure of the same or of succeeding crops of pigs at a given farm.

From the standpoint again of control of worm parasites as distinct from treatment of specific infection, the newest swine anthelmintic, hygromycin, incorporates more desirable features of an anthelmintic than the other yet produced.

The anthelmintic hygromycin can be used in a continuous system of attack in subtherapeutic amounts. In fact, there is no "full curative" dosage of hygromycin for an individual pig. This anthelmintic supports the proposition that worm infections must be attacked primarily on a herd basis. Without a "curative dosage" the safety margin certainly is less critical. Daily administration of the drug to attack parasites emphasizes the fact that exposure to infection occurs daily and continually throughout the entire herd.

In addition, hydromycin has increased the spectrum of anthelmintic activity of drugs useful against parasites in swine from the ascarids and a portion of the nodular worm population to swine ascarids, the full nodular worm population and the whipworm. Evidence has been obtained at the Wisconsin Veterinary Parasitology laboratory that hygromycin exerts an exact action against the red stomach worm and the swine lungworm.

A first action of hygromycin against internal parasites evidently is directed at the capacity of the females to produce eggs. The drug apparently is fatal to the worms themselves, but this action seems to be a cumulative one. Rapid expulsion of mature ascarids, so common following piperazine therapy, is not characteristic of hygromycin. Fairly rapid expulsion of worms has been observed of pigs on complete feed when the amount of hygromycin consumed has reached levels of 100 to 150 mg. per animal per day.

The introduction of an anthelmintic into the ration of swine on a continuous daily subtherapeutic system has enabled parasitologists to move more directly toward the measurement of production loss due to parasitic infection. Customarily parasitic infections are described either as clinical, evident from pronounced growth failure and even mortality, or subclinical, that is, with no determinate effect of the presence of the infection recorded except when production is measured in terms of feed conversion. The absence of exact production studies with respect to subclinical parasitism has made it difficult for parasitologists to direct attention to the fact that the subclinical infections are more costly in the long run and constitute the continuing reservoirs which maintain the infections in every herd of swine in the country. It is hoped that widespread usage and investigation of an anthelmintic such as hydromycin now being conducted throughout the country will establish this latter fact.

The situation with respect to anthelmintics for ruminants reflects the entire recent development in the field. One anthelmintic, phenothiazine, is at once the oldest, the most widely used, the standard of efficiency and the newest in that it can be used to meet every requirement of a modern anthelmintic. It is, in fact, the prototype of the modern anthelmintics, so much so that it is widely used for basic study of the impact of anthelmintics upon parasitic populations.
The efficiency of phenothiazine is related to amount of the drug used, and its particle size and purity. Its spectrum of activity is expanded when its particle size is decreased. At the same time the drug can be used both in the so-called full-curative doses, and in small daily doses.

When spectrum is considered, that is, the number of genera and species against which a drug is effective, then it is possible to understand the present main area of interest for ruminant anthelmintics.

Phenothiazine is the first of the broadspectrum anthelmintics. In sheep and cattle it is superbly effective against the nodular worm, and the Eastern stomach worm. In varying degree, it also is effective against the bankrupt worms, hookworms, the cooperids, the Western stomach worms, and still other species. It is not effective against whipworms, the capillarids, the tapeworms and the flukes.

The second broad spectrum anthelmintic to reach the market in the United States is thiabendazole. In evaluations conducted by the manufacturer and by federal parasitologists and the agricultural experiment station parasitologists this new drug has been found superbly efficient against both Eastern and Western stomach worms, nodular worms, bankrupt worms, hookworms, etc. It appears not to be effective against whipworms, capillarids, tapeworms and flukes. In short, this second successful anthelmintic for ruminants has a broad spectrum of extremely efficient action.

The question of greater efficiency of thiabendazole over phenothiazine, or vice versa, at this writing, appears a matter of formulation and the type of "problem herd" in which the drugs are used. Neither drug is most efficient in poorly-fed animals, and in problem herds and flocks parasitism invariably is linked to quality and quantity of nutrition available.

A third ruminant anthelmintic, bephenium, (bephenium hydroxynaphthoate) has a broad spectrum of action and its effectiveness against the thread-necked worms, Nematodirus sp., is noteworthy.

Of the materials presently receiving evaluation as ruminant anthelmintics, those collectively known as organophosphates are most prominent. In tests throughout the country, extreme efficiency has been found against a broad spectrum of worms. Characteristically the organophosphates have a lesser margin of safety in the host, and tissue residues are of some concern. It appears that useful preparations will be found among the organophosphates and that their spectrum of activity may be even greater than those of phenothiazine and thiabendazole.

It is interesting that the organophosphates as anthelmintics are derived from insecticides. The broad principles of insect control by chemicals are the same as those control principles in livestock parasitology and a union of effort by entomologists and parasitologists is obvious in the future.

REFERENCES


REPORT OF THE COMMITTEE ON PARASITIC DISEASES


At no time in the history of treatment of internal and external parasites of livestock have so many new drugs been on the market or in the researcher's hand. It becomes impossible for the livestock man to evaluate them or even to know which one should be used. When a perusal of the literature is made it is bewildering to find so much variation in the research reports on control of parasites. Some investigators will report excellent results from one anthelmintic while another investigator will give adverse results with the same drug.

The most important factor still in the control of parasites is sanitation. Parasite control is first of all a sanitation project. Heavily contaminated feeding grounds, parasite contaminated surroundings and inadequate pasture rotation undermine the parasite control program. When sanitation is not possible the livestock man seeks a cure-all for all "worms."

There is no "cure-all" for all internal parasites. All drugs or chemicals used for treatment of parasite conditions are selective in their action. Some anthelmintics are specific for one species of parasite and have little affect on other species. Some will destroy adult parasites but have little affect on the immature. Others may have definite action against parasites in the stomach but not against those in the intestinal tract. The major problem that has been pointed out is the lack of an exact diagnosis of the specific parasite present, or the condition of the animal, before "worm" treatment. Too many believe that parasites are all the same and any chemical will give the desired results. This, of course, if not true.

NEWER ANTHELMINTICS

Chemical antiparasitic measures are the most powerful aids presently available against parasites and parasitic diseases; yet, specific treatments generally have a comparatively short period of usefulness. Indeed, many of the currently preferred treatments were unknown a decade or so ago and, in all probability few, if any, of those in use today will be primary choices a decade or so hence. Furthermore, the growing concern with respect to residues in food animals necessitates consideration of alternative methods to chemical control, or, at least, methods and measures that minimize reliance on foreign chemicals. These include investigations of immunological procedures, management practices which minimize exposure of animals to parasitic infections, and natural control measures such as parasites, pathogens, and predators of economically important livestock pests.
Without reliable alternatives to extrinsic chemicals for parasite control, prudence dictates at least provisional acceptance of them; and every effort should be made to develop safe, efficient agents that do not result in noxious residues in treated animals nor in the evolvement of drug-resistant parasite strains. In this connection, the status of specific antiparasitic agents requires constant review; and new treatments, which appear with ever-increasing frequency in publications and advertising, should be comparatively evaluated in all respects concerning the usual criteria of safety, efficacy, simplicity, and cost.

It may be noted that most would-be successors to established antiparasitics are hopefully promoted but fall by the wayside, unequal to the demanding tests of unbiased, critical evaluation and field experience. The eventual development of chemicals with an overall usefulness paralleling, or exceeding, that of present day antiparasitics is inevitable; but one must not be unduly influenced by promotional enthuasiams nor mislead by the popular notion that something new is, ipso facto, superior.

Among the newer anthelmintics that should be given careful study and consideration are thiabendazole, methyridine, bephenium hydroxynaphthoate, and tetrachlorodifluoroethane. Thiabendazole, known chemically as 2-(4'-thiazolyl) benzimidazole, represents a new class of potentially useful anthelmintics and apparently possesses highly significant activity against a variety of economically important parasites of livestock. Early developmental trials indicate that:

1. The chemical is unusually effective.
2. It has a wide margin of safety.
3. It is rapidly absorbed and promptly eliminated from the animal body.
4. It is highly palatable and therefore amenable to free-choice as well as individual dosing in feed or mineral supplements.
5. It is non-staining, and
6. It is not hazardous to the user.

Furthermore, in addition to activity against adult parasites, the chemical appears to be exceedingly effective against the immature stages—a circumstance leading some workers to suggest that the chemical may have an unfavorable influence on the development of immunity.

Despite unusual promise, it is emphasized that thiabendazole is still in the developmental stages insofar as its broad applications in livestock are concerned. There is much to be learned regarding specific efficacy, optimum dosage and methods of administration, antiparasitic actions, detoxication, and elimination. There are moreover, certain problems relating to physical and chemical stability of prepared suspensions of the chemical.

Methyridine, 2-(beta-methoxyethyl) pyridine, appears to be especially effective against Trichostrongylus in the abomasum, Trichostrongylus, Cooperia, and Nematodirus in the small intestine, and Trichuris in the cecum and large intestine. Action against other gastrointestinal nematodes is more variable. Initial reports indicate that the chemical is effective against immature as well as mature worms and that it is active when given
either orally or by subcutaneous injection. The oral route is said to be somewhat safer although subcutaneous injection seems to be the preferred method.

The suggested dosage is about 200 mg./kg. of body weight; but methyridine may cause death in both sheep and cattle, irrespective of the route of administration, when given at the rate of 400 mg./kg. Fatal doses produce a neuromuscular block resulting in respiratory depression. Tissue irritation, characterized by edema and focal necrosis of the subcutaneous connective tissue and superficial skeletal muscle, occurs at the site of injection; these reactions occur more frequently and are generally more severe in cattle than in sheep. Healing of the damaged tissues is apparently well advanced, however, in about a week. It should be noted also that lameness develops when the injection is given too near articulations such as the shoulder and hip joints.

Although methyridine possesses a broad spectrum of anthelmintic action, is active against immature as well as mature stages of susceptible species, and has the novel advantage of subcutaneous administration, a definitive evaluation of its potential usefulness in parasite control programs cannot be made on the basis of available information. It is rather evident, however, that the potential toxicity of the chemical may militate against its general use.

Bephenium hydroxynaphthoate appears to be the most effective of a new series of quaternary ammonium compounds that exhibit anthelmintic activity against a variety of gastrointestinal nematodes of man and animals. These compounds, designated by the generic name of bephenium, are especially effective against Nematodirus spp. They are reported to have significant action also against Haemonchus, Trichostrongylus axei, Cooperia, Ostertagia, and Oesophagostomum. It is particularly noteworthy that the chemicals are effective against larval as well as adult stages of Nematodirus, and there is some evidence also that they are effective against immature stages of Haemonchus and Ostertagia as well.

The bephenium compounds are given at the rate of approximately 250 mg. per kilogram of body weight, either by drench or capsule. Maximum tolerable limits have not been ascertained, but no signs of intoxication were evidenced in lambs that were given oral dosages up to 2000 mg./kg.; no information is available on the tolerance of cattle for dosages exceeding 250 mg./kg.

In short, bephenium has been shown to have considerable activity against Nematodirus spp. in both sheep and cattle, but its efficiency against other parasites has not been adequately determined. The efficacy of the compound against immature stages of Nematodirus suggests its use in the prophylaxis and treatment of this infection. The cost of bephenium, however, may militate against its wide-scale use in livestock, especially in cattle.

Tetrachlorodifluoroethane, C₂Cl₂F₂, known commercially as Freon 112, has been shown to be effective against Fasciola hepatica in sheep. In preliminary trials a dosage of 0.15 gram per pound of body weight removed nearly all mature flukes but was not uniformly effective against immature parasites, a circumstance also obtained with such established fasciolicidal
agents as carbon tetrachloride and hexachlorethane. Significantly, however, tetrachlorodifluoroethane elicited no toxic reactions in sheep that were given dosages up to 1.0 gram per pound of body weight, and no histopathological changes were found in liver, kidney, or myocardium from animals that were given maximum dosages. The chemical is among the more promising of the newer fasciolicides for ruminants; other compounds in this category that warrant further consideration are bithionol \([2,2'\text{-thiobis (4,6-dichlorophenol)}]\), hexachlorophene [bis(2-hydroxy-3,5,6-trichlorophenyl) methane], and 1,4-bis-trichloromethyl benzene.

**ORGANOPHOSPHATE INSECTICIDES**

The organic phosphates are another group of chemicals that have been showing promise against a number of parasites in various animals. This was noted in the Committee's report last year. The organic phosphates are very efficient against a variety of gastrointestinal parasites, but in some instances may cause toxic effects. The margin of safety between high anthelmintic efficiency and toxicity is sometimes very narrow and all organic phosphates cannot be recommended as safe and efficient anthelmintics. Under the proper dosage some of the organic phosphates are showing promise as anthelmintics in cattle. Low-level feeding of some organic phosphates in cattle along with mineral mixtures have shown promise in controlling parasites along with controlling horn flies, face flies, lice, ticks and cattle grubs.

**PHENOTHIAZINE**

Phenothiazine is still used in treatment by many livestock men for sheep, cattle and horses. But phenothiazine is very selective in its effectiveness against the gastro-intestinal parasites of these animals. The low-level feeding of finely ground phenothiazine in a palatable mineral, vitamin, meat scrap, molasses mixture is still recommended by some workers. Combinations of phenothiazine with other anthelmintics have been effective and in some research work resulted in significantly greater gains in sheep.

**RECOMMENDATIONS**

1. The Committee recommends an extensive educational program to awaken the livestock industry to the tremendous need for more effective parasite control.

**PSOROPTIC SHEEP SCABIES ERADICATION**

*Inspection and Dipping Activities*

Psoroptic scabies was reported in 767 flocks of 62,251 sheep in 316 counties in 24 States compared to the report of 872 infected flocks in 296 counties in 24 States the previous year. 121 infected lots were found at
public stockyards compared to 187 during 1961. 12,770,000 sheep were inspected during 1962 and 590,000 dipped—an increase over 1961 when 12,030,000 were inspected and 507,000 dipped.

Outbreaks in the Sheep Scabies Free Area

Mississippi and California each reported 3 infected flocks, and New Mexico reported 13 infected flocks.

Eradication Program Makes Progress

The entire States of Arkansas, North Dakota, Wisconsin, and South Dakota, considered infected in August 1960, have reached scabies-free status. A breakdown of progress by counties is as follows: In August 1960 there were 1,444 counties considered scabies-free, an active eradication program was underway in 44 counties in one State and 1,666 infected counties in 23 States and territories failed to qualify as Sheep Scabies Eradication Areas.

By October 1962 the number of scabies-free counties had increased to 1,772 (a net increase of 328 counties), 553 counties (an increase of 1,200 percent) were official Sheep Scabies Eradication Areas, and the number of infected counties in which an eradication program had not been established was reduced to 829, a reduction of 50 percent.

Psoroptic cattle scabies was reported in 4 herds during fiscal year 1962—one each in Wisconsin and New Mexico and two in Texas. The two Texas outbreaks, on adjacent premises in Hansford and Ochiltree counties, were found as a result of tracing movements from the infected herd in Quay County, New Mexico. The latter herd was probably the source of the March 1961 outbreak in Illinois. The Wisconsin outbreak, in an Iowa County feedlot, was found by Federal inspectors at the Chicago Union Stock Yards.

During fiscal year 1962, increased efforts were made to locate any additional evidence of the disease and one-half million more cattle were inspected than the previous year—8,160,000 head compared to 7,660,000. Due to fewer infected herds reported, the number of official dippings was reduced from 234,300 to 123,500.

Field trials with acaricides continued and were conducted in Texas, New Mexico, Arizona, Colorado, California, and Wyoming. The trials involved dips against both mites and ticks and were designed to establish accurate vat management and replenishment procedures to maintain the proper concentration of dips using chemicals for which no vatside test is available.

CATTLE FEVER TICK ERADICATION

Further Work Indicates Boophilus Microplus Eradicated from Florida

Additional work in Florida indicates that cattle fever ticks have been eradicated from the State where 16 premises in Martin, Palm Beach,
Osceola, Indian River, and Hillsborough Counties had been infested the previous fiscal year. Systematic inspections and dippings of cattle and horses were completed but surveys to detect any additional infestations continued. 55,970 herds of 838,285 animals were inspected and/or dipped in fiscal year 1962 compared to 5,092 herds of 122,244 animals the previous year.

**African Ticks Eradicated**

*Rhipicephalus evertsi* and *R. Pulchellus* ticks found on imported animals at Catskill, New York, and Tampa and Boca Raton, Florida, have been eradicated by treating both the host animals and the premises involved.

**Active Program Continued in Texas**

During fiscal year 1961, in the buffer area in Texas, 201 livestock illegally crossing the border were caught; twenty-one tick-infested United States herds were found; and 57,177 lots of 1,224,176 livestock were inspected for ticks and 11,065 lots of 68,839 livestock were dipped.

**Tick Surveys Begun**

Florida began a tick survey in 1960. The survey was extended to all States during fiscal year 1962. Much useful information is expected from the survey which is continuing.

**STATUS OF SCREWWORM ERADICATION PROGRAM**

The Southwest screwworm eradication program was begun in mid-February of 1962, following a period of unusually severe cold weather. Limited numbers of screwworm flies were reared and made sexually sterile in facilities near Kerrville, Texas. The permanent facility near Mission, Texas, designed to produce up to 75 million sterile flies per week, was dedicated in mid-June. Efforts to prevent spread from the overwintering areas were partially successful. Screwworms did spread throughout Texas, but livestock producers reported fewer screwworm cases than usually experienced during spring and summer.

Only two cases of screwworms have been reported east of Texas, Oklahoma, and Kansas—one in June in northeastern Louisiana and one in early October in a Parish bordering Texas in southwestern Louisiana. Screwworms have migrated, or were spread by shipments of infested animals, throughout New Mexico and Oklahoma.

The eradication efforts during spring and summer have been concentrated on reducing to a minimum the number of screwworms in areas of Texas and New Mexico where the insect is likely to overwinter during the 1962-1963 winter season. It is anticipated that sterile flies, with constant surveillance and control of animal movements, will prevent migrations from areas of recurring infestations in the United States and Mexico into the areas freed of screwworms.

The inspection line along the eastern boundaries of Arkansas and Louisiana unloaded, inspected, and treated nearly one-half million
in-transit animals going into the southeastern States this year. Many ani-
mals infested with screwworms have been intercepted at the inspection
stations along this line, principally those animals afflicted with cancer
eye.

In view of the finding of exotic ticks in Florida and New York, and in
other States, be it resolved that the United States Livestock Sanitary As-
sociation urge that the Secretary of Agriculture take necessary action to
provide that the importation of all wild and domestic animals, regardless
of species, be included in the regulations administered by the United States
Department of Agriculture. All animals offered for importation, regard-
less of species, be held in the country of origin and an import permit be
withheld until such animals are freed of ectoparasites and certified as
such. All animals, regardless of species or place of origin, be inspected
at the port of embarkation, found free from ectoparasites, and again
treated with approved pesticides and by approved methods for the eradica-
tion of ectoparasites. Be it further resolved that insofar as possible,
anthelmintics be administered for the control of endoparasites and that
feed and bedding and crates unloaded at the port of debarkation, or used
during transportation of animals from port of debarkation, be immediately
destroyed or appropriately treated.

Be it further resolved that the United States Livestock Sanitary As-
sociation urge that the Secretary of Agriculture request, and that the Con-
gress appropriate adequate funds in the amount of $1,450,000 for Fiscal
Years 1964, 1965, and 1966 to provide for the final drive to eradicate
psoroptic sheep and cattle scabies from the United States.
SOME FORMULATION PROBLEMS ASSOCIATED WITH THE USE OF ORGANIC PARASITICIDES

R. D. Radeleff*
Kerrville, Texas

Rules and regulations are developed from time to time to control the movement of livestock infested with ectoparasites that either produce or transmit disease. Such rules and regulations generally require that infested animals be cleared of the parasites by treatment with approved dips, sprays, or other types of medicaments prior to their movement between states or nations, or within states.

In prescribing the approved substances and the manner of their use the responsible official realizes that two prime objectives must be accomplished: first, that the prescribed procedure must be as near absolutely effective as can be obtained and, second, that the procedure should not cause a loss or damage of the livestock. To insure this performance, the operator must know that his materials are, and will continue to be, of uniform quality and concentration.

As in the promulgation of any law, those actively participating in its enactment are familiar with most all of the available data. Once the law or rule is established, it must be enforced by persons who were not so thoroughly informed. Being human, they follow the prescribed routine and are not usually in position to judge deviations from the expected normal performance. As time passes, new employees are involved, resulting in an even greater isolation from the original facts.

I believe that the people attending this meeting, and those who may read the proceedings, can profit by a review of some of the fundamentals involved in the use of chemicals for ectoparasite control. These fundamentals have not been generally emphasized, to my knowledge, since the period of 1948-1952. There are many problems that need discussion, but I will limit the present material to the most fundamental of the problems.

What I have to say does not constitute an indictment of current regulatory operations. Rather, I wish to offer explanation for some of the unexpected intoxications of livestock and failures to control ectoparasites.

Arsenical dips were used to bring piroplasmosis under control in the United States by eliminating the vector—a tick. Those who handled the program were fortunate that the ranchers at that time were more tolerant of damage to their animals than is true today. True, many a "tick man" had to argue his program from the disadvantageous point just in front of the muzzle of a loaded rifle—and many good vats were removed between dippings with carefully placed dynamite. One could hardly say that the program was unanimously supported! It is most unlikely, I believe, that

*United States Department of Agriculture, Agricultural Research Service, Animal Disease and Parasite Research Division.
such a program could be introduced and carried to completion with arsenic today. Losses due to skin "burning" and outright poisoning were frequent and large numbers of cattle were affected.

In spite of the losses that occurred with arsenic, due primarily to the necessity of using it at concentrations that did not permit a margin of safety worth mentioning, arsenical dips offered several advantages over modern pesticides. First, a solution that was, by and large, stable, avoided the problems we now have with emulsions and wettable powders. Second, an accurate and rapid vat-side test was available, simple enough to be run by the average cowboy.

Lime-sulphur and nicotine sulfate dips offered similar advantages, but occasionally produced undesirable results.

If these two advantages could be had with a modern pesticide, a number of problems could be eliminated.

The development of the chlorinated hydrocarbon insecticides was welcomed by ranchers because they seemed capable of resolving the toxicity problem—and would, I am sure, in every respect save the nagging problem that appeared almost at once with undesirable residues of the compounds in meat and milk. The safest material, DDT, was one of the worst offenders in residues.

Let us examine two of the major problems that continue to exist, assuming for the moment that we are concerned with the use of chlorinated hydrocarbon or organic phosphorus parasiticides that have been approved so far as residues in tissues and reasonable safety are concerned. These two major problems are the development of stable formulations capable of uniform action, and the need for a rapid test to determine the concentration of parasiticide during treatment.

**Rapid Testing**

Modern compounds do not lend themselves to rapid testing methods. The only reasonably effective rapid method has been that based on extraction of the dip with a suitable solvent followed by a determination of the specific gravity of the extract. The method is reasonably accurate when the change in specific gravity is appreciable. The method is not always easy to apply. In suspensions or emulsions used for dipping of sheep the presence of suint and other foreign matter from the sheep sometimes makes extraction impossible—and if possible, of questionable accuracy. In some areas the quality of the water and soil tends to defeat easy extraction. When the concentration of active ingredient drops below 0.1 percent the specific gravity/extraction technique is useless.

We must, therefore, admit that present methods are very limited in application.

Much research is needed to develop truly useful, rapid methods of analysis. At present we must depend heavily upon studies in which typical formulations are studied in a limited number of experiments, with sampling being followed by laboratory chemical analysis. We must then assume that such results can be applied to most situations—a hazardous assumption at best.
Because of these severe restrictions on our ability to determine concentrations at the point of action, we must depend almost entirely upon having a uniform product capable of uniform performance under a wide variety of conditions—which brings us to the second problem.

Formulations

Most, but not all, of the newly developed parasiticides are insoluble in water, or soluble only to the extent of a few parts per million. This means that simple solutions are not possible. These water-insoluble compounds can be used in water only as suspensions or emulsions.

A. Suspensions—Suspensions are, as the name implies, composed of particles of insoluble material dispersed in a vehicle such as water. Most water-insoluble parasiticides, if ground into extremely fine particles, will float or sink rather quickly, and the particles will tend to unite to form a single mass. To prevent this sort of action, the compound may be impregnated on diatomaceous earths or fine-particle clays, which aids in dispersing the chemical and restraining it from its natural tendency to aggregate. If the resultant particles are of extremely small dimensions they will tend to remain suspended in the dispersing medium for long periods, held there by the physical forces that create the phenomenon we call Brownian movement. In addition, if the particles are nearly equal to water in specific gravity, they will remain suspended for longer times. Water muddied by clay has been observed by all of us—we know that large particles soon settle out but that, sometimes for days or weeks, the water stays colored by the extremely fine particles. Suspensions prepared from wettable powders of parasiticides are like the muddy water—except that we can control the physical and chemical properties of the "mud."

While a few wettable powders are made up of parasiticide and the carrier earth and nothing more, many also contain chemicals that aid in dispersing the powder or in maintaining the suspension. In plant formulations chemicals are often added to cause the suspended particles to adhere to foliage.

Wettable powders might, by elaborate screening, be produced in uniform particle size. The cost would be prohibitive, so the commercially available materials always contain particles of a great variety of sizes. If we remember our muddy water we should realize that the finer the particle size the longer the water will remain muddy—and the longer it will retain a reasonably uniform concentration of suspended particles.

Another factor must be considered. All of us realize that it is easier to filter large particles from a mixture than it is to remove extremely fine particles. Exactly the same process must be considered with animals since their hair or wool acts as a filter toward suspensions flowing through from sprays or dips and the natural result is that large particles are retained in the hair while the smallest tend to remain in suspension. In a dipping vat this occurs—the first animals through remove all the suspended large particles. Each additional animal takes its share of progressively smaller particles until the very fine particles are reached, from which the deposit on the hair or wool is almost exactly equivalent to the volume of
dip vehicle retained on the animal—in other words, the deposit approaches that which we might expect from a true solution. The equivalency is never reached, but is approached so closely that the disparity is negligible from the standpoint of practical applications.

Powders containing "stickers" can be very dangerous when used in place of regular powders. They cause the particles to adhere like a gum to almost any introduced surface.

Not all manufacturers have come to appreciate these characteristic behaviors of wettable powders. A part of this is due, unfortunately, to the contention of a few scientists in control of recommendations who have for many years considered wettable powders, even of rather toxic compounds, as being without hazard. True wettable powders tend to "fail safe," in that the settling out process removes many large particles and in that the greater weight of chemical in the large, filtered particles is offset in some measure by a decrease in surface area that can contact the skin and be available for absorption. What these authorities have neglected to emphasize is that the "fail safe" principle applies also to the parasite they are intending to eliminate—allowing it to survive.

Failure to kill the parasite is an inconvenience at best—but when control of a disease vector or agent is involved, a failure endangers our livestock industry through a sense of false security.

It is my opinion that we must learn to evaluate wettable powders before we use them and to use only those that can assure uniformity of treatment.

The slides which follow illustrate acceptable powders whose uniformity of performance has been rather carefully studied and powders whose performance has been much less than desirable. Notice the range of particle sizes and the average size in each case. (Slides 1, 2, 3, 4). The next slides depict what happens to the concentration in a dipping vat during a typical dipping operation. (Slides 5, 6). The implications of the poor performance of the unsatisfactory powder should be clear to all regulatory people.

Standards have not been prepared for particle size or steadiness of suspensibility. Standards are sorely needed, particularly for regulatory activities.

I hope I have made clear how little we have been concerned with a fundamental problem and the need for standardization in the use of wettable powders.

B. Emulsions—Emulsions are composed of insoluble droplets of one liquid suspended in another liquid. Milk is one of our most common emulsions. We are all familiar with the slow rise of the fat droplets to form cream. We also know that we can churn the cream and obtain a coalescence of the droplets to form butter. Anyone who has tried to re-suspend a thick cream in milk is aware that the cream sticks to the spoon and that violent shaking only partially redistributes the particles.

Emulsifiable concentrates of parasiticides are usually composed of the active compound, a solvent, and an emulsifier. Other additives to increase dispersibility and emulsification may be added. When such a concentrate is added to water it breaks into small droplets. In a properly fashioned emulsion for livestock treatment these droplets are virtually
colloidal in dimension. The poorest concentrates behave almost like oil when mixed with water, forming large droplets that soon get back together. Mediocre formulations produce droplets too small to be readily seen by the naked eye, but which soon cream upward or downward to form a layer of high concentration. The best formulations disperse into such fine particles that they create an opalescent appearance.

Mediocre emulsions may be successfully used in spray equipment having adequate agitation. If the agitation stops for any reason, creaming takes place, creating a volume of low concentration and one of high. The high concentration layer can kill animals while the low concentration layer allows the parasite to go unharmed. The poor and mediocre emulsions have no place in dipping operations. Creaming must be avoided. Very few emulsifiable concentrates are available for dipping because of the stringent requirement for high quality. Standards have been prepared for high quality emulsifiable concentrates.

The Effect of Formulation

Take a suspension of a wettable powder being thoroughly stirred by the magnetic stirrer. Dip pledgets of cotton, a dozen in this case, into the swirling mixture, drain them briefly and put them aside. From the remaining suspension we remove 15 ml. of the suspension to a centrifuge tube and spin it with another tube containing 15 ml. of the original suspension. Note the result. About half the powder has been removed by the cotton due to filtration. Keep in mind that this was very loose cotton; wool would be more tightly packed.

It is easy to see that only half the original parasiticidal activity could remain.

Take two beakers of emulsifiable concentrates containing equal amounts of parasiticide and equal amounts of red dye. Make emulsions of equal concentration, then dip cotton-tipped applicators into each for equal times. The applicators are then immersed in equal quantities of acetone which extracts the color and the parasiticide. It is obvious, I believe, that the one beaker (from the poor emulsion) contains much more dye than the other. This is exactly what happens when animals are dipped in such emulsions.

Again, may I reiterate, this extra deposit may bring death to part of the animals and yet not kill parasites on many of the remainder of the herd. This is true because the excessive deposits on the first animals leave a decreased concentration in the dip for the next ones, and so on. In the case of the wettable powder illustrated, dip concentration of lindane used to treat sheep declined from 0.061 percent on charge to 0.012 percent after 200 sheep and to 0.002 percent after 600, and so while an additional 300 were dipped. The vat was recharged with fresh suspension periodically to replace the volume of dip removed by the sheep, but this could not raise the vat concentration appreciably.

My discussion may have seemed elementary to you. If so, then there has been gross laziness in applying these principles, especially to wettable powders. If it has not seemed elementary, then I hope we may look forward to improvement in our judgement in selecting formulations for livestock treatment.
REPORT OF THE COMMITTEE ON PHARMACEUTICALS

S. F. Scheidy, Philadelphia, Pennsylvania, Chairman; G. D. Cloyd, Ashland, Ohio; P. C. Enge, Davis, California; O. D. Grace, Lincoln, Nebraska; H. E. Schaden, Frederick, Maryland, H. Richer, Dover Delaware

This Committee is of the opinion that pharmaceuticals and chemicals are vital to the control of many diseases of livestock and poultry. In the past several decades numerous chemical compounds have been synthesized, studied and developed. These compounds, when used in accordance with their indications have materially promoted economy in livestock and poultry production. However the current requirements to develop a pharmaceutical or a chemical and to make it available to the livestock industry are so costly that some manufacturers have reduced their efforts or abandoned entirely research and development operations for this market. Increased demands for information regarding drug substances by the regulatory agencies, have during the past few years caused some confusion and unusual delays in obtaining clearance for such agents to be used by the livestock industry and veterinarians.

Anticipated legislation and regulations undoubtedly will further depress the drug and chemical industry's interest and effort of developing new compounds for use in food producing animals. Fortunately, many useful compounds were made available for use in livestock production prior to the enactment of the Food additives Amendment of 1958 and subsequent regulations. But subsequent regulations on the Federal, and in some cases on the State level, seem to many people appropriate and justified. However, those who are familiar with the regulations are concerned about their effects on the animal industry. Indeed, the animal industry, as well as this association, which is so vitally interested in animal disease control work, could be well advised about these new regulations and how they may affect their work and the industry as a whole.

The major activity in the field of anthelmintics and pesticides continues to be directed toward development of safe and efficacious products. Innumerable permutations of organic phosphate compounds are being investigated as potential insecticides (internal and external) and anthelmintics. Some of these phosphates have demonstrated low toxicity and high efficacy, but their toxicity varies unpredictably from species to species; for example, a phosphate that may be almost non-toxic for rats will be highly toxic for dogs, or visa-versa. In general, the organic phosphates have a broad spectrum against nematodes in the alimentary tract but almost no effect on helminths in other organs. Organic phosphates in currently recommended dosage levels have proved safe for most healthy animals. But caution to avoid excess exposure must be exercised by the person administering them.
Thiabendazole, (4-thiazolyl)-benzimidazole, is a compound that has attracted much attention for its low toxicity and high efficacy for ruminant helminths. It appears to have a broader spectrum and better efficacy than phenothiazine. It also appears to have some effect on migratory stages of nematodes.

Methyridine, 2-(B-methoxyethyl) pyridine, developed in England, is being used parentally in ruminants against trichostrongylids. Efficacy is good, but some investigators have reported reaction at the site of injection.

A newly developed injectable, disophenol 2, 6-diido-4-nitrophenol, is highly efficacious against dog hookworm. Its specificity for this common parasite, however, limits its overall usefulness.

Bephenium hydroxynaphthoate (Benzylidimethyl-2-phenoxyethyl ammonium-3-hydroxy-2-naphthoate) has been found to be effective in the oral treatment of hookworm infections in dogs. It also may have value as an anthelmintic agent in other species of animals, however at this time data regarding such uses are very limited.

The United States Department of Agriculture has prepared Agricultural Handbook No. 120 "Insecticide Recommendations of the Entomology Research Division for the Control of Insects attacking Crops and Livestock for 1962." Formulations have been designated as "approved" or "permitted" with those in the "permitted" category being used in official eradication programs. This is an excellent reference for those engaged in animal disease and parasite control work.

Tylosine, a new antibiotic substance produced by Streptomyces fradiae now is available in several different dosage forms for use in animals. This agent is active against both Gram positive and Gram negative organisms. Favorable claims also have been made for its use in the control of P.P.L.O. infections in poultry. Experimental studies in laboratory animals (swine and chickens) indicate that it is essentially non-toxic when given prophylactically or in therapeutic doses. In addition when added to feed, it stimulates growth in swine and poultry.

A new and interesting antibiotic substance is griseofulvin, produced by several species of Penicillia, namely, P. griseofulvum, P. janczeweki and P. raistrickii. It possesses antifungal activity following oral administration in animals and humans. This substance is particularly active in overcoming trichophyton infections without producing serious toxic effects.

During the past year many new mixtures of antibiotic substances and coccidiostats have been made available, chiefly for use in medicated feed. Most of these are used by the poultry industry. Because of the feed additive amendment of 1958, these mixtures prior to their release for general use were more extensively studied than similar preparations that were available before the enactment of the amendment.

In recent years a great deal of work has been done to synthesize, study and develop new and safer compounds that are effective as diuretic agents in humans. Such compounds also have been studied in animals, especially in those suffering with edematous conditions. The compounds generally referred to are carbonic anhydrase inhibitors and include
chlorothiazide, hydrochlorothiazide, and acetazolamide. In adequate dosage, these compounds appear to be useful in the management of mammary edema frequently observed in high milk producing, pre- and post-parturient, heifers and cows. This means of therapy appears to be quite popular with many dairymen, however, data are lacking to establish objectively the merits of such therapy.
The poultry industry in the United States constitutes a significant part of our agricultural economy. In 1960 the gross income from poultry and poultry products amounted to approximately 3.3 billion dollars; whereas, the cattle, sheep, and swine industries yielded 12.6, .3, and 3 billion dollars, respectively. In terms of the number of producers concerned with animal production, the breakdown is as follows: cattle, 2.6 million; poultry, 2.1 million; sheep, .3 million; and swine, 1.8 million. Aside from sheep, the economic growth and evaluation has essentially tripled for the cattle, poultry, and swine industries in the last 20 years. The poultry industry has made phenomenal advances during the past three decades and its products have become highly competitive with those from other animal sources.

During the rapid expansion in poultry production, avian diseases have also increased in importance. A profitable poultry industry today cannot survive unless greater attention be given to the prevention, control, and eradication of poultry diseases. Three decades ago only a few avian pathologists shouldered the responsibility of safeguarding the health of our poultry. During the last 20 years the rapid expansion of the industry has attracted more professional men to specialize in avian diseases. Outstanding advances in coping with certain diseases have been observed, which in turn has made it possible for the industry to expand at the rate that it has. New drugs, vaccines, and control programs have been developed to reduce disease losses. Nutrition, breeding, and management also have contributed markedly to the growth of the poultry industry.

However, in practically all areas of our nation we are still confronted with devastating disease problems that constitute a real challenge to the veterinary profession. The demand for poultry disease specialists at the present time is most critical. More veterinary personnel are needed in poultry disease practice, research, instruction, regulatory work, and extension education to meet the problems that presently exist and that may arise in the future.

Another important aspect of the disease problems that confront the poultry industry is the monetary sacrifice that is made by breeders, producers, and hatcherymen for medication, testing, and vaccination costs as well as losses suffered from mortality and decreased production.

Present poultry operations cannot avoid these disease control costs unless improved changes are instituted in several areas.
In this paper it is the intent to call your attention to the serious gaps that exist between the United States Livestock Sanitary Association and the poultry industry relative to disease problems. The United States Livestock Sanitary Association from its inception has served the livestock industry most effectively in improving the health status of the domestic food-producing animals. Also, through the years it has recognized poultry health problems through its Committee on Transmissible Diseases of Poultry, as well as including in its formal program, papers on significant disease entities. However, opinions and desires have been expressed that the United States Livestock Sanitary Association should be called upon to play a greater role in poultry disease control and eradication. This is particularly true regarding the control and eradication of pullorum disease and fowl typhoid. Both of these diseases have plagued the poultry industry for more than six decades. These two diseases have been extensively investigated and our knowledge is adequate to eliminate them successfully from our breeding flocks.

A national voluntary control program for pullorum disease was instituted in 1936 and later specifically included fowl typhoid. The voluntary program known as the National Poultry Improvement Plan and sponsored by the Poultry Division, Agricultural Research Service, United States Department of Agriculture, has been successful in reducing the percentage of pullorum infection from 3.66 in 1936 to .013 in 1961 among flocks participating in the Plan. During this 26-year period 700,166,569 birds have been tested for pullorum disease and fowl typhoid. It is estimated that approximately 100 million dollars have been expended for pullorum disease testing by Federal and State governments and the poultry producers during the past 26 years. In addition to this cost, one must recognize also that there have been expenditures to control these diseases by flock owners and hatcheries not participating in the Plan. It is estimated in 1961 that a total of 3,513 hatcheries with a capacity of 509 million eggs were operative in this country. However, only 59 percent of these hatcheries participated in the Plan, representing 71 percent of the total hatching capacity. Furthermore, money expended for medication, losses through mortality, retarded growth, and decrease in production should all be taken into account in estimating the loss resulting from these two diseases. It is apparent that a reassessment of present conditions should be made and that appropriate action should be instituted and implemented by regulatory agencies properly qualified to undertake this task.

PRESENT STATUS OF THE PROBLEM

It has been stated that outstanding progress has been made in reducing pullorum infection from our breeding flocks (Table I). For the most part this has been accomplished on a voluntary basis through the National Plan sponsored by our Federal government. It should be recognized, however, that the National Plan has limitations in its effectiveness in eradicating pullorum disease from our breeding flocks since less than 75 percent of the hatching capacity is under the Plan. It may be stated further that there are indications that more hatcheries may drop out of the Plan.


PULLORUM DISEASE CONTROL

TABLE I
Progress in Pullorum Disease Eradication for a Twelve-Year Period

<table>
<thead>
<tr>
<th>Northeastern States</th>
<th>1950</th>
<th>1956</th>
<th>1961</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of flocks</td>
<td>14,330</td>
<td>9,712</td>
<td>5,086</td>
</tr>
<tr>
<td>No. of birds</td>
<td>11,818,661</td>
<td>12,078,156</td>
<td>11,233,849</td>
</tr>
<tr>
<td>Percent reactors</td>
<td>0.20</td>
<td>0.03</td>
<td>0.007</td>
</tr>
<tr>
<td>Birds in clean flocks</td>
<td>9,466,328</td>
<td>11,564,274</td>
<td>10,897,348</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>United States</th>
<th>111,422</th>
<th>70,468</th>
<th>33,334</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of flocks</td>
<td>37,237,674</td>
<td>36,112,781</td>
<td>37,124,838</td>
</tr>
<tr>
<td>No. of birds</td>
<td>13,302,642</td>
<td>31,273,701</td>
<td>35,892,899</td>
</tr>
<tr>
<td>Percent reactors</td>
<td>0.72</td>
<td>0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>Birds in clean flocks</td>
<td>13,302,642</td>
<td>31,273,701</td>
<td>35,892,899</td>
</tr>
</tbody>
</table>

TABLE II
A Seven-Year Turkey Pullorum Testing Summary for Fourteen States (1954-60)

<table>
<thead>
<tr>
<th>State</th>
<th>Total flocks tested</th>
<th>Total birds tested</th>
<th>Infection detected</th>
<th>No. years</th>
<th>Maximum percent per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conn.</td>
<td>184</td>
<td>97,524</td>
<td>6</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Del.</td>
<td>94</td>
<td>18,327</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Me.</td>
<td>52</td>
<td>42,899</td>
<td>1</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Md.</td>
<td>289</td>
<td>110,599</td>
<td>6</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Mass.</td>
<td>264</td>
<td>151,503</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>N. H.</td>
<td>82</td>
<td>69,059</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>N. J.</td>
<td>170</td>
<td>50,392</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>N. Y.</td>
<td>251</td>
<td>225,357</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>N. C.</td>
<td>414</td>
<td>375,230</td>
<td>7</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Pa.</td>
<td>599</td>
<td>318,280</td>
<td>6</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>R. I.</td>
<td>36</td>
<td>6,588</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Vt.</td>
<td>90</td>
<td>34,705</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Va.</td>
<td>3,435</td>
<td>1,813,070</td>
<td>7</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>W. Va.</td>
<td>115</td>
<td>49,690</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

This trend may be stimulated by integrated changes in the poultry industry, by placing undue emphasis on the cost of testing and by a lack of appreciation of adequate measures designed for eradication.

It has been intimated by some that eradication of pullorum disease is not feasible and practical. No concrete evidence has been presented that this is the case. On the contrary, evidence is accumulating in certain areas that the disease can be eradicated completely from our commercial breeding flocks (Table III).

Furthermore, the annual number of "breaks" in negative flocks reveals a downward trend and some States have not had a "break" for several successive years (Tables IV and V). Also, the number of pullorum disease diagnoses among diagnostic cases has declined markedly in many States and in a few instances, no pullorum infection had been detected in chickens for successive years (Tables V and VI). It is of interest to note
TABLE III
Eight-Year Summary of the Incidence of Pullorum Reactors in the New England States

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Connecticut</td>
<td>0.001</td>
<td>0.038</td>
<td>0.003</td>
<td>0.005</td>
<td>0.004</td>
<td>0.006</td>
<td>0.004</td>
<td>0.00</td>
</tr>
<tr>
<td>Maine</td>
<td>0.017</td>
<td>0.00</td>
<td>0.002</td>
<td>0.001</td>
<td>0.002</td>
<td>0.00</td>
<td>0.00</td>
<td>0.002</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>0.10</td>
<td>0.022</td>
<td>0.009</td>
<td>0.002</td>
<td>0.001</td>
<td>0.001</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>New Hampshire</td>
<td>0.004</td>
<td>0.00</td>
<td>0.002</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>0.008</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Vermont</td>
<td>0.00</td>
<td>0.0008</td>
<td>0.001</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

TABLE IV
Range of Infection Among Pullorum Breaks in Massachusetts Chicken Flocks for Seventeen-Year Period

<table>
<thead>
<tr>
<th>Season</th>
<th>No. of flocks 100% negative</th>
<th>Breaks</th>
<th>Range of infection (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>% .05</td>
</tr>
<tr>
<td>1945-46</td>
<td>375</td>
<td>20</td>
<td>5.33</td>
</tr>
<tr>
<td>1948-49</td>
<td>379</td>
<td>6</td>
<td>1.58</td>
</tr>
<tr>
<td>1951-52</td>
<td>350</td>
<td>8</td>
<td>2.29</td>
</tr>
<tr>
<td>1954-55</td>
<td>258</td>
<td>5</td>
<td>1.94</td>
</tr>
<tr>
<td>1956-57</td>
<td>220</td>
<td>1</td>
<td>0.45</td>
</tr>
<tr>
<td>1958-59</td>
<td>182</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>1961-62</td>
<td>118</td>
<td>1</td>
<td>0.85</td>
</tr>
<tr>
<td>Totals</td>
<td>1,882</td>
<td>41</td>
<td>2.18</td>
</tr>
</tbody>
</table>

TABLE V
Incidence of Pullorum Infection as Detected Among Tested Flocks and Diagnostic Consignments in Fourteen Northeastern States

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Conn.</td>
<td>3</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Del.</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>16</td>
<td>5</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Maine</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Md.</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Mass.</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>N. H.</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>N. J.</td>
<td>16</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td>3</td>
<td>15</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>N. Y.</td>
<td>3</td>
<td>11</td>
<td>0</td>
<td>28</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>N. C.</td>
<td>5</td>
<td>34</td>
<td>9</td>
<td>24</td>
<td>3</td>
<td>21</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Pa.</td>
<td>12</td>
<td>21</td>
<td>7</td>
<td>16</td>
<td>4</td>
<td>21</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>R. I.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vt.</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Va.</td>
<td>10</td>
<td>21</td>
<td>22</td>
<td>9</td>
<td>18</td>
<td>12</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>W. Va.</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

*Infected tested flocks.
**Number of positive diagnoses among diagnostic consignments.
TABLE VI
Incidence of Pullorum Disease Diagnoses Among Consignments Submitted to the Diagnostic Services in Eight States

<table>
<thead>
<tr>
<th>State</th>
<th>1945</th>
<th>1950</th>
<th>1954</th>
<th>1958</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>2,495</td>
<td>3,282</td>
<td>3,670</td>
<td>10,390</td>
</tr>
<tr>
<td>Delaware</td>
<td>4,445</td>
<td>3,241</td>
<td>3,855</td>
<td>1,053</td>
</tr>
<tr>
<td>Indiana</td>
<td>--</td>
<td>1,917</td>
<td>1,383</td>
<td>1,094</td>
</tr>
<tr>
<td>Maryland</td>
<td>1,107</td>
<td>2,447</td>
<td>1,997</td>
<td>1,918</td>
</tr>
<tr>
<td>Minnesota*</td>
<td>375</td>
<td>470</td>
<td>200</td>
<td>76</td>
</tr>
<tr>
<td>New Hampshire</td>
<td>928</td>
<td>1,701</td>
<td>2,549</td>
<td>1,893</td>
</tr>
<tr>
<td>North Carolina</td>
<td>1,131</td>
<td>1,278</td>
<td>1,595</td>
<td>5,106</td>
</tr>
<tr>
<td>Oregon</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>2,422</td>
</tr>
</tbody>
</table>

C - Consignments examined.
D - Diagnoses of pullorum disease.
*Chick cases 0-6 weeks.

TABLE VII
Percentage of Chicken Population Tested for Pullorum Disease in New England States

<table>
<thead>
<tr>
<th>State</th>
<th>Chickens on hand 1/1/61</th>
<th>Birds tested 1961</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conn.</td>
<td>3,463,000</td>
<td>466,720</td>
<td>13</td>
</tr>
<tr>
<td>Maine</td>
<td>4,680,000</td>
<td>1,628,311</td>
<td>35</td>
</tr>
<tr>
<td>Mass.</td>
<td>3,485,000</td>
<td>685,905</td>
<td>20</td>
</tr>
<tr>
<td>N. H.</td>
<td>2,029,000</td>
<td>586,282</td>
<td>29</td>
</tr>
<tr>
<td>R. I.</td>
<td>432,000</td>
<td>75,070</td>
<td>17</td>
</tr>
<tr>
<td>Vt.</td>
<td>812,000</td>
<td>137,266</td>
<td>17</td>
</tr>
<tr>
<td>Totals</td>
<td>14,901,000</td>
<td>3,579,554</td>
<td>24</td>
</tr>
</tbody>
</table>

*Does not include commercial broilers.

that the lowest incidence of the disease is observed in the New England States where the ratio between the number of birds tested and the number of chickens on hand is greatest (Table VII). However, there are exceptions to this situation as revealed in other States (Table VIII). For the country as a whole, approximately 10 percent of the chickens on farms were tested in 1961. In addition to greater participation in the annual testing of flocks, other efforts such as greater vigilance against the introduction of the disease, more effective sanitation, and a closer working
### TABLE VIII

Percentage of Chicken Population Tested for Pullorum Disease in Selected State

<table>
<thead>
<tr>
<th>State</th>
<th>Chickens on hand 1/1/61*</th>
<th>Birds tested 1961</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del.</td>
<td>846,000</td>
<td>622,495</td>
<td>74</td>
</tr>
<tr>
<td>Md.</td>
<td>1,848,000</td>
<td>287,901</td>
<td>16</td>
</tr>
<tr>
<td>N. J.</td>
<td>11,492,000</td>
<td>450,337</td>
<td>4</td>
</tr>
<tr>
<td>N. Y.</td>
<td>10,128,000</td>
<td>529,551</td>
<td>5</td>
</tr>
<tr>
<td>N. C.</td>
<td>14,072,000</td>
<td>4,162,110</td>
<td>30</td>
</tr>
<tr>
<td>Pa.</td>
<td>20,063,000</td>
<td>1,420,925</td>
<td>7</td>
</tr>
<tr>
<td>Va.</td>
<td>6,758,000</td>
<td>1,106,957</td>
<td>16</td>
</tr>
<tr>
<td>W. Va.</td>
<td>2,218,000</td>
<td>97,010</td>
<td>4</td>
</tr>
<tr>
<td>Totals</td>
<td>67,425,000</td>
<td>8,677,286</td>
<td>13</td>
</tr>
</tbody>
</table>

*Does not include commercial broilers.

relationship with the flock owner to maintain his interest in the true objective of this program, have contributed to the reduction of the infection.

**EVALUATION OF "BREAKS"**

In Massachusetts, the number of "breaks" in general has declined as the percentage of reactors was reduced and the number of infected flocks diminished. The percentage of "breaks" at no time exceeded 12.96 percent which occurred in 1932. Since the war years there has been a gradual decline in "breaks." Also, the majority of "breaks" revealed a small percentage of reactors (0.5 percent or less) on first test (Table IV). Most flocks with "breaks" were retested and obtained one or more negative tests in the season during which the "break" was detected. It should be emphasized that "breaks" as a rule may be expensive to the breeder and hatcheryman. The source of the infection causing the "breaks" could not always be determined. However, an insight into the behavior of the disease suggests that the origin of the infection stems from an infected breeding flock either through direct or indirect egg transmission through hatchery channels. Circumstantial evidence suggests that the presence of such infections may be identified in flocks several months after the stock has become infected. If "breaks" are identified one should go back to the flock from which the stock originated and the channels through which the stock has passed including the hatchery, the chick handler, and the grower. It is advocated that the incidence of "breaks" can be eliminated if the regulatory agent will prevail upon the industry members to exercise effective preventive measures and constant vigilance against the introduction of infection.

**ANNUAL TESTING OF BREEDING FLOCKS ESSENTIAL**

It has been advocated that a relaxation on annual testing of breeding flocks be instituted in order to reduce the costly burden of testing. While
this policy may appear to be admirable on the surface, it can be considered only to exert, at best, a tempering effect in eliminating the burden inflicted by these diseases. Further, it is contrary to sound disease control principles that are employed to cope with other animal diseases. Pullorum disease and fowl typhoid are not unique to the extent that they can be treated in a different manner from other infectious diseases. A relaxation in our present testing requirements should not be endorsed until certain specific criteria have been met that will insure against a resurgence of these two diseases among our breeding flocks.

It appears essential that a more inclusive control and eradication program should be adopted and relegated to the animal disease regulatory agencies in the various States and the Federal government. Both pullorum disease and fowl typhoid should be made reportable diseases in order that a proper agency can follow through on their control and eradication. All breeding flocks should be tested annually and only those that can qualify as pullorum-clean or its equivalent should be used for hatching egg production. An effective and reliable testing program should be instituted in all States. A Federal regulation for the interstate traffic of poultry should be designed and adopted for the control and eradication of these diseases. Greater cooperation should be obtained between the industry and disease control agencies.

It is apparent that the poultry industry needs the assistance of the United States Livestock Sanitary Association in order to continue the progress in pullorum disease eradication. In conclusion, it is recommended that the United States Livestock Sanitary Association sponsor a Committee with the express purpose to further extend control and eventually to eradicate pullorum disease and fowl typhoid from the United States.

REFERENCES

AVIAN PLEUROPNEUMONIA-LIKE ORGANISMS: RESEARCH
BASIS FOR A CONTROL AND ERADICATION
PROGRAM FOR THE POULTRY INDUSTRY

O. L. Osteen, D.V.M.*
Beltsville, Maryland

This report results from research started in 1952 by public and private organizations in a concerted effort to solve the disease problem that is caused by pleuropneumonia-like organisms (PPLO). I consider this problem to be the most economically important of all poultry disease problems, although some of my colleagues dealing with avian leukosis may dispute that statement.

First, I want to say that I do not intend to burden you with lengthy references. More than 500 reports on the subject have been published since the PPLO work began. As I do not want to offend some authors by naming "important references," I will not list any. These references are all listed in the scientific journals of the past 12 years. Also, printing costs of this Association's Proceedings must be considered.

State regulatory officials need to be reminded that their past response to the poultry disease problem has been less than enthusiastic. Last year's proceedings** relative to "proposed rule-making publication by the Department of Agriculture involving the interstate movement of birds to prevent the dissemination of fowl typhoid" stated that "a considerable response was received from industry, most of it opposed to certain provisions of the proposed regulation, it is true, but not one State livestock sanitary official responded, either for or against."

Among the officials from the 50 states represented at this meeting are those who recognize that the poultry industry in individual states exceeds, equals, or is a close second to all other agricultural efforts. This report should particularly appeal to these representatives.

In the interest of present information and proper nomenclature concerning PPLO, the following terms are defined and used throughout this report:

*Mycoplasma* is the scientific name for a group of micro-organisms, smaller than the common bacteria but larger than viruses, that occur in man, livestock, and poultry.

*Mycoplasma gallisepticum* is the scientific name for the specific type of *Mycoplasma* that causes Chronic Respiratory Disease in chickens and Infectious Sinusitis of turkeys.

*Assistant to the Director, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.*

Air Sac Disease is the disease complex that occurs when Mycoplasma gallisepticum infection is complicated by secondary infections. This disease complex should more properly be designated complicated CRD. CRD (Chronic Respiratory Disease) is the name given to the respiratory disease produced by Mycoplasma gallisepticum in chickens. Infectious Sinusitis is the name given to the respiratory disease produced by Mycoplasma gallisepticum in turkeys. Airsacculitis is any inflammatory reaction of the air sacs. This may be due to a wide variety of causes, including Mycoplasma gallisepticum. PPLO (pleuropneumonia-like organism) is the name formerly used for the group of organisms now known as Mycoplasma.

A work conference was called by the Animal Disease and Parasite Research Division of the Agricultural Research Service, May 28 through May 30, 1962 in Washington, D. C. on Air Sac Disease of chickens and Infectious Sinusitis and Airsacculitis of turkeys. The purpose of the conference was to prepare for the poultry industry a report of facts relative to Mycoplasma gallisepticum infection in poultry, and to recommend procedures for the control and/or eradication of this infection.

Research has progressed to the point where definite recommendations can be made to the poultry industry. This research began in June, 1962, when the Animal Disease and Parasite Research Division of ARS initiated a national cooperative research program with several state experiment stations. Other states engaged in broiler production initiated their own research programs. Private organizations interested in the poultry industry have contributed greatly in this research directly or through grants to research institutions and individuals.

Mycoplasma gallisepticum infection in poultry causes, or is associated with, the diseases and conditions previously defined in this paper. It is undoubtedly the poultry industry's foremost problem. Losses directly or indirectly attributable to it have certainly run as high as $125 million a year.

Avian Mycoplasma vary in their susceptibility to drugs and antibiotics. All known Mycoplasma are resistant to penicillin, thallium acetate, and most sulfonamide drugs. Many antibiotics (for example, streptomycin, tetracycline, erythromycin, and tylosin) inhibit Mycoplasma in test tubes. These antibiotics are also effective in live birds if extremely large doses are administered. The use of antibiotics, therefore, to control M. gallisepticum infection in very large market or breeder flocks is not economical and is not recommended. They should be used, however, when small flocks free of M. gallisepticum infection are being established.

TRANSMISSION

Research furnishes clear evidence that the transmission of M. gallisepticum from the hen through the egg to the chick or poult is the principal manner in which the disease is spread. The rate of transmission is greatest during acute infection and decreases with time. Contact transmission within a flock is recognized to be second in importance.
Experimental infection under controlled conditions demonstrates that birds will show signs from one to several weeks after becoming infected depending on (1) virulence of the strain, (2) the dose, and (3) route of exposure. Spread of the disease within a naturally infected flock may vary from days to months. Transmission of the disease may also occur within a flock without apparent clinical signs of the disease and may be accelerated by other infections. Recorded evidence of incubator transmission of *M. gallisepticum* is scant. However, this method of spread should be given serious consideration. Where birds from various sources and unknown histories and of different ages are housed in the same hatchery or on the same farm, excellent conditions exist for outbreaks of *M. gallisepticum* infection. Contact with contaminated equipment or personnel is recognized as a possible means of spread. Drinking water, crates, chick boxes, footwear, and other mechanical carriers may be possible sources of spread from infected to clean flocks. The possibility of transmitting *M. gallisepticum* through live poultry vaccines has not been established, but this potential hazard should be considered. *M. gallisepticum* has been recovered from various wild fowl and its transmission from turkeys to pheasants has been recorded. The role of various wild birds in transmitting the infection to domestic poultry has not been fully established, but wild birds are not suspected as being important carriers.

Although vaccination as a disease-control measure is widely accepted and is effective against several viral diseases of poultry, few vaccines against infections caused by bacteria and bacteria-like organisms, such as *M. gallisepticum*, have any value. There is, therefore, no effective procedure currently available to immunize poultry against *M. gallisepticum* infection.

**HOST RANGE**

Many species of animals harbor *Mycoplasma*. Avian species include chickens, turkeys, ducks, pheasants, pigeons, guinea fowl, peafowl, parakeets, sparrows, quail, and partridges. Nine different serotypes of avian *Mycoplasma* have been reported, but the information on the host range of the pathogenic *M. gallisepticum* other than in chickens and turkeys is limited. While *Mycoplasma* have been isolated from air sac lesions in many avian species other than chickens and turkeys, their pathogenicity in domestic poultry has not been determined. At the present time it is the general consensus that the various species of *M. gallisepticum* are host-specific.

**COMPLICATING FACTORS**

Virus respiratory diseases are a part of air sac infection—the viruses of Newcastle disease (ND) and infectious bronchitis (IB) commonly occur in cases of this disease. Experimentally, ND and IB viruses have greatly increased the severity and rate of spread of *M. gallisepticum* infection. Because live viruses used to vaccinate against ND and IB have frequently
been incriminated as stress factors in air sac disease, their use must be carefully considered. The decision to use them should be based on local conditions. Further, the eggs used to grow the ND and IB vaccine virus may harbor *M. gallisepticum* and this infection may be spread when the ND or IB virus is used.

The respiratory tracts of normal birds have gram-positive flora; those affected with air sac disease have high concentrations of gram-negative flora that are mainly coliforms. As a complicating agent, one of the coliforms, *Escherichia coli*, contributes most to clinical air sac infection. Pathogenic types of *E. coli* have been found in the intestinal tracts of normal birds, in litter, feed, and rodent feces, but the importance of these sources of bacteria in clinical air sac infection has not been determined. Bacteria causing coryza, fowl cholera, and other poultry diseases also complicate *M. gallisepticum* infection.

Extreme of widely varying environmental conditions can predispose to or increase the severity of chronic respiratory disease (CRD). Low or fluctuating brooder temperatures, for example, have increased the severity and spread of *M. gallisepticum* infection and the severity of *E. coli* infection. Environmental stress can be minimized by good management.

**DIAGNOSIS**

In chickens, signs of *M. gallisepticum* infection may be like those of Newcastle disease, infectious bronchitis, laryngotracheitis, fungus infection, and others. The usual signs are a nasal discharge, and a slight swelling below the eye. Coughing, sneezing, and a hoarse throat rattle may accompany these signs. Turkeys often have swollen sinuses with gelatinous exudate, watery eyes, and coughing with cheesy or cloudy air sacs. Since these signs are not specific, and since birds may be infected and therefore carriers without showing signs, specific tests for diagnosing the infection must be used. These specific tests usually require the services of a veterinary diagnostician. They are described here to show the weapons available in the fight against *M. gallisepticum* infection.

It has been demonstrated unequivocally that chickens and turkeys infected with pathogenic *M. gallisepticum* develop blood components (antibodies) that will agglutinate a preparation containing the organism. There are three serological tests that are useful in determining whether infection has taken place. These tests are the whole blood plate, serum plate, and tube agglutination. The hemagglutination inhibition test (H-I test), though highly reliable in the laboratory, is not practical for routine testing of flocks.

Broth smears contain small, round (coccoid) bodies when stained with Giemsa method, whereas other staining methods may fail to reveal the organism. On solid culture medium (agar) the colonies are small and circular with a central dense papillae and can be differentiated by the Dienes staining method. Colonies not exceeding 0.5 mm. in diameter (in circumference) seem to grow into the agar and do not change on subculture.
Differentiation of *M. gallisepticum* from other serotypes can be accomplished by a summation of cultural and serological characteristics and pathogenicity. Many serotypes have been described but *M. gallisepticum* appears to be the prevailing pathogenic type in chickens and turkeys.

**HISTOPATHOLOGY**

The microscopic picture produced by *M. gallisepticum* in chickens and turkeys provides satisfactory and valuable supporting evidence of infection. Lymphofollicular proliferation, although commonly found, is not pathogenic (decisively characteristic) for the infection.

Researchers are supposed to read the literature—a description would mean nothing to a poultryman!

Methods for the diagnosis of *M. gallisepticum* in poultry other than serological are available and well understood. The laboratory diagnostician also relies on bird inoculation, embryo inoculation, cultural isolation in either artificial culture media or tissue culture systems. Histological procedures are also helpful.

**CONTROL**

The basic essentials for control or eradication of the disease may be presented in the following manner:

1. *M. gallisepticum* infection is primarily transmitted through the egg. Dipping hatching eggs in suitable drugs or antibiotics reduces the incidence of egg transmission. The dipping, however, will not entirely eliminate egg transmission because no remedy, immunizing procedure, or other preventive or therapeutic practice in man, animal, or poultry has ever been 100-percent effective.

2. Similarly, certain drugs or antibiotic agents administered to adult breeders reduce the incidence of, but, again, will not entirely eliminate, egg transmission of *M. gallisepticum* infection.

3. Immunizing agents cannot be relied upon to eliminate egg transmission.

4. Serological tests satisfactorily detect infected flocks. But if a flock is tested and the tests are negative, the infection-free assumption is only valid for a short time. Periodic testing is therefore necessary to find out if the infection-free status is being maintained. When making test, at least 10 percent of the flock with a minimum of 100 birds should be tested.

Feeding antibiotics along with a reduced calcium level in the feed is beneficial but can be used for only a limited period. Potentiation of antibiotics with terephthalic acid has been outlawed by the Food and Drug Administration. *Mycoplasma* organisms do develop a tolerance for antibiotics.

Continued progress against *M. gallisepticum* infection depends on more than expanded and intensified research and the dissemination of research findings. Research workers and regulatory officials within an area
or State must maintain close liaison so the incidence of the disease can be known at all times and mutual problems can be handled together. Similarly, flock owners are asked to cooperate with research workers and regulatory officials so that the control of *M. gallisepticum* infection will become a reality instead of an unresolved problem.

The several disciplines of biology, nutrition, and environmental control have contributed in past research on this problem, and intend to join in a tighter group in future research aimed at a solution to this problem in the United States.

A final word—some commercial chicken producers are already following these research results with pronounced success. This fact alone places upon regulatory officials from the states where poultry production is foremost, or nearly so, the responsibility of protecting the commercial producer in his effort to help the industry free itself of this disease. Too, with your cooperation and guidance the entire industry will join in the effort to make the poultry industry of the United States produce the finest quality of meat and eggs in the world.
REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF POULTRY

H. E. Goldstein, Columbus, Ohio, Chairman; Dr. S. E. Schmittle, Athens, Georgia; Dr. R. A. Bankowski, Davis, California; Dr. B. S. Pomeroy, St. Paul 1, Minnesota; Dr. R. H. Singer, Ames, Iowa; Mr. Robert Hogue, Lafayette, Indiana; Dr. John W. Walker, Washington D. C.; Dr. Charles H. Cunningham, East Lansing, Michigan; Dr. Henry Van Roekel, Amhurst, Massachusetts; Dr. C. L. Vickers, Columbia 1, South Carolina; Dr. W. F. Lamoreux, Fremont, California

Your Committee on Transmissible Diseases of Poultry for the past year has centered its activities along the lines of disease control and eradication programs. Members of the Committee have participated in regional poultry meetings throughout the country in discussing salmonella reporting, control and eradication of pullorum disease and fowl typhoid, as well as discussing mycoplasmosis control programs.

This year's Committee received more correspondence from regulatory officials and industry representatives relative to such programs than in past years. It was indeed most gratifying to have a few state veterinarians interested in the poultry programing and take time to submit suggestions.

The response to field problems was great enough this year that your Committee held an open session for industry members as well as non-committee members to express themselves and then a closed session for Committee members.

The Committee was presented with two resolutions which were adopted at the 34th Annual Meeting of the Northeastern Conference on Avian Diseases, Ontario Veterinary College, Guelph, Canada, June 11 to 13, 1962. These resolutions are as follows:

WHEREAS, the Poultry Research Branch of the Animal Husbandry Research Division of the Agricultural Research Service, United States Department of Agriculture, has been administering the research investigations on fowl leukosis, a function which more logically fits into the duties of the Animal Disease and Parasite Research Division of the Agricultural Research Service, and

WHEREAS, the Animal Disease and Parasite Research Division is the division charged with research investigations on the federal level on livestock and poultry diseases, now

WHEREFORE BE IT RESOLVED, that the Northeastern Conference on Avian Diseases recommends, and urges, that fowl leukosis research activities be transferred to the Animal Disease and Parasite Research Division.

WHEREAS, pullorum and typhoid control in the National Poultry Improvement Plan and National Turkey Improvement Plan is now under the administration of the Poultry Research Branch of the Animal Husbandry
Research Division, Agricultural Research Service, United States Department of Agriculture, the duties of which do not include any other disease control function.

WHEREAS, the original federal authority for the control and eradication of diseases of livestock and poultry was vested in the former bureau of Animal Industry, and

WHEREAS, the present Animal Disease Eradication Division of the Agricultural Research Service, United States Department of Agriculture, is the agency charged with these functions and has the organizational structure and professional manpower to control and eradicate disease outbreaks, and

WHEREAS, poultry diseases in addition to pullorum and typhoid are under consideration for inclusion in the National Poultry Improvement Plan and National Turkey Improvement Plan, and

WHEREFORE BE IT RESOLVED, that this Northeastern Conference on Avian Diseases recommends and urges that the poultry disease control portion of the National Poultry Improvement Plan and National Turkey Improvement Plan be placed under the jurisdiction of the Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture.

Your Committee gave serious consideration to these two resolutions and after careful deliberation feel that no action should be taken at the present time.

The Committee discussed the Poultry Disease Resolution adopted by the National Association of State Departments of Agriculture in their convention at Grand Rapids, Michigan, September 23 - 27, 1962, sent to the Secretary of Agriculture as follows:

(1) In cooperation with the several states involved survey the disease problems of the nation's poultry industry, and

(2) Encourage and support within the Agricultural Research Service the necessary research to provide the nation with the essentials, of both knowledge and tools, to begin an organized cooperative attack on the most serious poultry diseases, and

(3) Set up and establish within the Animal Disease Eradication Division of Agricultural Research Service a section specifically designed to combat such diseases as Newcastle, bronchitis, airsacculitis, and PPLO infection, and others as they may appear, in cooperation with the several states, and

(4) To provide specific financing for this function annually. The first year of operation it is requested that an amount of not less than $200,000 be provided, and that steps be taken to provide sufficient standardized antigen for flock testing for PPLO infection, and that the development of a cooperative program for such testing be speedily implemented.
Your Committee has endorsed this resolution and suggests that the executive committee of the United States Livestock Sanitary Association instruct the executive secretary to submit the same resolution to the Secretary of Agriculture from the United States Livestock Sanitary Association.

Your Committee emphasizes that successful, widespread application of serological testing programs for *Mycoplasmosis gallisepticum* depend upon the availability of suitable antigens. Immediate steps must be taken to provide antigens to those states that do not now have a source. These antigens should meet certain standards as directed by a central agency.

The Committee is of the opinion that the Animal Disease Eradication Division of Agricultural Research Service should undertake the manufacturing and standardization of diagnostic antigen for *M. gallisepticum* infection.

Last year's Committee recommended that a National Poultry Disease Advisory Committee be formed. This Committee could not be formed due to administrative changes, and the United States Department of Agriculture advised each member of the proposed Committee that they would be used as collaborators as needed.

This Committee recommends that each state regulatory official become familiar with the problems and have a trained staff member assigned to poultry programs in each respective state.

The Committee suggests most strenuously that each chief livestock official of each respective state promulgate legislation making pullorum disease and fowl typhoid reportable diseases. This will strengthen the cooperative State-Federal reporting program initiated this year by the Animal Disease Eradication Division of Agricultural Research Service, as requested by the poultry industry.

When the proper reporting of these two diseases is a reality then the problem can adequately be recognized. The poultry industry has requested this definition prior to considering eradication. As was pointed out in last year's Committee report, a national eradication program cannot be initiated until the poultry industry so desires. The Committee encourages individual states having personnel statuatory programs, as well as budgets to carry out their own programs. This will lend impetus to the stimulation necessary for national programming.

The Committee is continuing its study of the proposed uniform rules and methods for eradication of pullorum disease and fowl typhoid. Avian leukosis is still a most serious economic problem for the poultry industry. This Committee recommends:

1. That an all out support from all phases of the poultry industry be exerted to implement the existing research program, and
2. Epidemiological studies be directed toward transmission and control.
DIETARY LEVELS OF RADIONUCLIDES IN FOODS OF ANIMAL ORIGIN

A. H. Wolff, D.V.M.
Washington, D. C.

Gentlemen, I am happy to have this opportunity to discuss the subject of Dietary Levels of Radionuclides in foods of animal origin. This title only tangentially describes the actual subject which I will primarily discuss, namely the levels of iodine 131 in fresh milk supplies. I thought it would be appropriate, however, to leave this title on the paper because I would like to discuss the current situation with respect to iodine 131 in the framework of the over-all picture that the title portrays.

As many of you know, during the early years of weapons testing, almost exclusive attention was given to strontium 90 as the important fallout contaminant. There are probably many in this audience who think that iodine 131 is a new problem. Actually, this is not the situation and I would like to develop a little of the background of how the current level of concern about iodine 131 has developed. Early in 1957, the Public Health Service, in response to growing awareness that nuclear weapons were discharging increasing amounts of various radioactive contamination into the environment, undertook an investigation to determine the amount and kind of fission products that conceivably reaches the human through his diet. With the knowledge available at that time concerning the important fission products and with the knowledge available concerning the chemical and physical characteristics of these fission products and how this information was relevant to its entrance into the food chain, milk stood out as the most important and critical food stuff to consider. Therefore, milk was chosen as the first item of the diet to be extensively and most intensively studied. I might add that it remains today as the most important single food to consider in surveillance of the diet for radioactivity. The reasons for our particular interest in milk are several. First, milk is the major contributor to strontium 90 in the total diet. It has also been determined in the last several years that milk is equally important as the major source of iodine 131 in the diet. Another reason for our primary interest in milk is that it is consumed by all segments of the population, but especially by infants and children who, based on generally accepted radiobiological considerations, are assumed to be the most radio-sensitive segment of the population. The fourth reason that we have selected milk is that it is produced on a year-around basis and produced in almost every area of the United States; consequently, it serves as a good index of the levels of environmental contamination. Another reason is that analytical methods for milk could be developed and could be applied or extended to many other environmental media.
As pointed out, prior to 1957 almost exclusive attention was directed to strontium 90 and indeed the primary efforts of our first investigations with milk also were directed towards strontium 90. However, in setting up the Public Health Service surveillance studies there was reason to believe that there were various other radionuclides including radio-iodine in particular that warranted surveillance. The rationale of our particular interest in iodine is actually quite straight-forward. Earlier investigations had revealed that iodine 131 was ubiquitously present in the thyroid glands of grazing animals. These data, together with good data from experiments on the metabolism of iodine 131 in dairy cattle indicated that there probably were appreciable levels of iodine 131 being secreted in the milk as the result of tropospheric or early fallout during and immediately following periods of active testing, if these periods coincided with the pasture season of dairy herds. In our preliminary examination of several market milk supplies, we showed that in addition to strontium 90 and iodine 131 there were three other radionuclides that were occurring in readily detectable concentrations and perhaps warranted surveillance. These were cesium 137, another isotope of strontium—strontium 89, and barium 140. So the decision was made to analyze for all five of these radionuclides. We developed methods of analysis for these fission products and our early attention was given primarily to the development of simple methods which were suitable for analysis of large numbers of samples; a methodology that could be successfully carried out by well-trained but not necessarily professional personnel.

In our initial sampling programs we collected only samples of raw milk from well-defined geographical areas. We confined the collection of samples to milksheds which served large urban populations in selective regions of the United States and we had several criteria for delineating the sections upon which we settled. One, the milk was to be representative of a group of farms that were milking at least a thousand dairy cows. Secondly, the number of individual farms was to be small enough so that the collection of collateral field data from these farms was feasible, and third, the milk samples were to be from a milkshed, as previously mentioned, that supplied a metropolitan area, and finally each milk sample was to be representative of the same group of farms in the production area of interest on a continuing basis. Our analysis of samples in this manner provided information necessary for research on the factors influencing the levels of radionuclides in milk as well as basic data on radionuclide exposure of a portion of the population in the various metropolitan areas who were consuming milk from these particular milksheds.

During the first year of operation, five areas were selected for the raw milk surveillance programs: New York, Cincinnati, St. Louis, Salt Lake City, and Sacramento. Before the first year of operation was over we had added several other cities—Atlanta, Chicago, Austin and Spokane and we have since conducted a continuous monthly sampling from each of these stations collecting one-gallon raw milk samples monthly from each of these stations.
I might just briefly summarize the levels of activity that we have found during the first seventeen months of operation from June 1957 through the Fall of 1958. The iodine data from the first five cities resulted in an average daily concentration of about 125 micromicrocuries per liter. The high was St. Louis with an average of about 160 micromicrocuries per liter and Sacramento was the low city with about 40 micromicrocuries per liter. Perhaps these figures, themselves, are not too meaningful for many of you. The only reason that I mention them is to stress the point that these levels are comparable to the levels we are finding since the resumption of Russian and United States testing and these levels are comparable to what we have been finding throughout the country in a much more detailed surveillance program which I will describe in a few moments.

There was very little concern directed toward these early findings for several reasons. First, there hadn't been established, and I might add there still are not established, standards or permissible levels for the general population. So, arbitrarily, when we would report these data, just so we would have a handle to which we could relate these data, we related them to "permissible levels" that were one-tenth of the occupational levels or the permissible levels that were stipulated for occupational workers; and this meant that we were comparing it to a daily average of 3,000 micromicrocuries per liter. The finding of levels in the order of 100 micromicrocuries as an average and comparing it to this arbitrary standard indicated that there was no need to be concerned over iodine 131. Then you will recall that in the Fall of 1958 a moratorium on nuclear weapons testing was established by the United States and the Russians followed suit. The data of our early efforts to analyze iodine 131, you might say, went into the archives.

The original purpose of this milk work was really to develop methodology and radiochemical analytical proficiencies. During the operation of this limited program it became apparent that a broader sampling program, one more directly related and more representative of the milk actually being consumed by the population of the United States was necessary. So, consequently there was a transition of this early program of sampling raw milk from milksheds of very limited size to a sampling program representative of the pasteurized milk consumed in various selected municipalities.

Perhaps I should take a moment to explain why when there is no nuclear weapons testing going on there will be little or no iodine 131 found in the milk in contrast to strontium 90: Iodine has a very short half-life in the order of eight days, and therefore, with the cessation of weapons tests the iodine will rapidly disappear from the environment so that after two or three months the levels become undetectable. Consequently, the short-lived isotopes are environmental or contamination problems only during or shortly after periods of active testing as opposed to the long-lived isotopes such as strontium 90 and cesium 137 which have half-lives of many years and are continually being intruded into the environment from a stratospheric reservoir.
In 1960 we set out to establish a pasteurized milk surveillance program in about 60 stations. This was established in cooperation with state and local health and milk sanitation agencies. In contrast to our raw milk network, this later program emphasizes measurement of the concentrations of radioactivity in pasteurized milk consumed by the public in various regions of the country; and it provides for at least one sampling point in virtually all the states and some additional points dictated by the widely varying conditions of the milk supply and the need to cover unusually large population groups. Each sample was composited in proportion to the volume of milk sold by a group of milk plants supplying not less than 90 percent of a city's milk supply. Prior to September 16, 1961, the composite sample was taken from one day's sales per month and was as representative of a community's total supply as could be feasibly achieved. The network essentially was formed during a lull in weapons testing and we were collecting samples on a monthly basis which is not too infrequent insofar as the surveillance of the long-lived isotopes, particularly strontium 90, is concerned. But since September, 1961, when radioactivity from the U.S.S.R. test series was detected in our network, most stations have been taking weekly and in some instances bi-weekly samples in order to more accurately evaluate the short-lived radionuclides—barium 140, strontium 89 and particularly iodine 131.

Coincident with the resumption of testing by the Russians, the FRC (the Federal Radiation Council) released a report that contained background material for the development of radiation protection standards. The FRC is an interdepartmental council within the Federal Government that concerns itself with problems that might be related to radioactivity that is generated, one might say, by the Federal Government or its contractors. As I pointed out, that in September, 1961, this Federal Radiation Council Report came out with background material for the development of radiation protection standards. Considerable public and scientific concern resulted, because in several areas the levels of iodine 131 in milk exceeded the standards stipulated by the FRC. There has been some confusion over the interpretation of the FRC guides as related to fallout. The FRC has recently issued a statement emphasizing that the standards presented in this report were intended to apply only to normal peacetime development of the nuclear industry and that guidance more specifically relevant to fallout is in the progress of development. Regardless of the standards or guides that eventually will be applicable to iodine 131 it appears that during periods of weapons testing, iodine 131 in the milk supply will probably stand out as being a problem of major importance.

I might again mention that the samples from our pasteurized milk network are collected with the assistance of state and local health and milk sanitation agencies. Analyses are done at our three laboratories: the Southeastern Radiological Health Laboratory in Montgomery, Alabama, Southwestern Laboratory in Las Vegas, Nevada, and a newer laboratory—the Northeast Laboratory at Winchester, Massachusetts.

I think at this point I will show a few slides to provide a better idea of what the levels have been over the past few months. The first slide shows the monthly network averages and in addition to iodine 131 it also
includes levels of strontium 89 and strontium 90, which probably during periods of testing would rank in second and third order of importance. All the values are presented in micromicrocuries per liter. We can see that both iodine 131 and strontium 89 (being short-half-lived isotopes) during the moratorium in testing had activity levels close to zero. In September, 1961, we can see the rise in I-131 beginning shortly after the Russians resumed testing. The iodine started to rise rather sharply and these are the average figures for all of the 60 stations. During December and January when most of the cattle are being taken off of pasture, they no longer have access to the major source of contamination and the levels start dropping to below the limits of detectability again. To a lesser extent the same thing happens with strontium 89. Strontium 89 has a longer half-life so that hay or silage made during the Summer or Fall would still carry some radioactivity over into the winter feeding and the strontium 89 levels do not drop down as sharply as iodine.

The next slide shows a graph of the milk levels obtained from the St. Louis pasteurized milk sample. From September—again this is the point of the resumption of testing—through December we can see a characteristic pattern of high peaks that rapidly drop off. Apparently peaks are reached in two to four days and generally fall off with about a five-to-eight-day half-life. I mentioned that Iodine 131 has an eight-day physical half-life; However, in the field the milk levels will fall off more rapidly due to rain and perhaps cropping off from grazing so that the effective half-life will be shorter than the physical half-life. Usually a pattern of a single insertion into the environment and a half-life of five to eight days prevails. Then when the cattle are off pasture, levels markedly subside.

The next slide presents a similar graph for the State of Washington. I believe this is a Seattle milk sample. In general the Western States do not show levels as high as the Eastern and Southeastern States because of the peculiar weather patterns a year ago in the Fall. Each geographical area, of course, has its own characteristic pattern and it is very difficult to predict what the pattern is going to be. You will note that this graph also provides data on the levels of radioactivity in air. There appears to be little correlation between the air levels and the iodine levels in the milk. Much of the iodine in milk and results from rain-out on pasture.

This next slide shows the environmental levels of iodine 131 in the milk of Palmer, Alaska. I might add that Palmer, Alaska and Salt Lake City have the dubious distinction of having the highest cumulative levels, and they're in a vulnerable spot for Russian and Nevada weapons tests fallout respectively.

The next slide shows the pattern for New Orleans; this graph shows that a different pattern prevails for the southern and southeast part of the United States. Cattle stay on pasture a little longer and we have less of an abrupt drop during the end of November and December.

In concluding, I would like to make a few remarks about countermeasures or public health preventative measures related to iodine 131 in milk. There are several effective countermeasures that could be taken
in the event the levels reach unacceptable concentrations, and it might be well to mention some of these. There are a few measures that could be taken which actually depend on the storage or shelf-time of the milk product. For example, it's been recommended by our National Advisory Committee on Radiation that in the event that levels become extremely high that all children—certainly children below the age of five, lactating mothers and pregnant women be put on evaporated milk. Because the usual shelf-life of these products is weeks or months the iodine 131 will decay prior to consumption. There are other milk products that meet this requirement, for example frozen fluid milk, frozen whole milk concentrate or canned sterile whole milk. There are some experiments going on for the treatment of milk for the removal of iodine by an ion exchange process but it is still in the experimental stages.

The addition of stable iodine or the medicinal administration of thyroid extract to humans would reduce the level of iodine 131 deposited in the thyroid by an appreciable factor if given in sufficient quantities.

Perhaps the most feasible measure and one that has been successfully tried in several states consists of feeding cows with uncontaminated food—that is, feed that has been stored long enough for the radioactivity to decay. In other words, the animals are removed from pasture and placed on feed that has been stored for at least three or four weeks. This results in remarkable reduction of the iodine 131 levels in milk as compared to the levels resulting from milk produced from cows remaining on pasture.
REPORT OF THE COMMITTEE ON PUBLIC HEALTH
AND RADIOLOGICAL FALLOUT

Robert J. Schroeder, Los Angeles, California, Chairman; G. H. Collacutt, Toronto, Ontario, Canada; Robert D. Courter, Atlanta, Georgia; Luther Frederickson, Nashville, Tennessee; R. H. Huffaker, Ann Arbor, Michigan; H. J. Rollins, Raleigh, North Carolina; James H. Steele, Atlanta, Georgia; Frank A. Todd, Washington, D. C.; E. E. Wedman, Ames, Iowa; R. D. Wenger, Washington, D. C.

The primary efforts of the Public Health Committee this year have been directed toward the development of data on radio-active fallout and its effect upon animals and food products of animal origin. However, we also wish to make some brief comments concerning other problems of public health significance.

SALMONELLOSION

Considerable effort is being expended by the United States Department of Agriculture and the United States Department of Health, Education and Welfare to determine the scope and importance of Salmonellosis in animals and its transmission to man.

The Communicable Disease Center, United States Public Health Service in Atlanta, Georgia, is continuing to expand activities on Salmonella investigations. During the past year a special Salmonella investigations unit was created for the purpose of providing assistance in tracing outbreaks of Salmonellosis. Efforts to achieve better reporting of human cases have been successful. As a result, monthly summaries covering reported cases from a number of states are published and disseminated to epidemiologists in each respective state.

More emphasis is being placed on tracing the origin of exposure back to the animal host. The rise in the total number of reported human cases in the past ten years is attributed to increased exposures to contaminated food, particularly poultry, eggs, and pork products. Unless further action is taken in reducing the level of infection in animal host reservoirs, the human disease incidence probably will not change. The Veterinary Public Health Laboratory has investigated the environmental contamination of animal and poultry slaughterhouses, animal rendering plants, and fish meal plants with Salmonella organisms and found many contaminated to a serious degree.1

The Animal Disease Eradication Division, Agricultural Research Service, has distributed almost 4,000 reprints of the report entitled "Salmonella and Other Disease Producing Organisms in Animal By-Products - A Survey." A few copies are still available upon request.

One of the conclusions of the survey was "the need for development of a uniform method for the isolation of Salmonella from animal by-products." A Committee of specialists is preparing standard procedures which they hope will soon be available for distribution to all diagnostic laboratories.
Another conclusion of the survey was the "development and adoption of a sanitary health code by processors of animal products and allied utilization industries to aid them in offering products free from disease." A United States Department of Agriculture Committee is drafting a proposed code for study by industry and state regulatory officials.2

SWINE BRUCELLOSIS

National Institute of Health Investigators report that a part of the largest epidemic of brucellosis ever reported in humans can be attributed to airborne infection.3 One Hundred twenty-eight (128) workers in a swine slaughtering plant were involved.

Isolations of Brucella suis were made from the air in the "kill room" during the outbreak. This is apparently the first time that the organisms have been recovered from the air. Knowledge of the fact that Brucella suis infection can be spread to humans through aerosols adds impetus to the campaign to control and eradicate the disease.

The Public Health Committee recommends strong support of the program.

Veterinary Public Health problems which are receiving considerable attention in addition to those previously mentioned are cancer, heart disease, and listeriosis. These shall be reported upon by the Public Health Committee in the future.

RADIOACTIVE FALLOUT

The task of developing data on radioactive fallout and its effect on animals and food products of animal origin is a tremendous one.

Our investigation revealed a large amount of written material. Several agencies and institutions are involved. The following have the major responsibility and are conducting, financing, and supervising most of the research:

United States Department of Agriculture - Agricultural Research Service.


Department of Defense - Departments of the Army, Navy and Air Force.

United States Atomic Energy Commission.

Many Universities and Colleges and their agricultural experiment stations.

This is a very general list but to be more specific would require lengthy tabulation.

After considerable discussion and correspondence, the Public Health Committee agreed that the formation of a bibliography listing current and appropriate references on the subject would be of great value to those
interested in obtaining accurate, scientific data. For classification purposes the bibliography is divided into the following sections: General, Livestock, Strontium-Calcium, Iodine 131, Food, Dairy Cattle and Milk, Swine, Burros, and Eggs and Chickens. Sixty-nine references are listed.

It is the intent of the Public Health Committee to keep the material up-to-date each year by deleting obsolete references and adding new published reports from the research and testing groups. (The Committee is particularly indebted to Dr. Frank A. Todd, Assistant Administrator for Emergency Programs, Agricultural Research Service, United States Department of Agriculture; and Dr. Arthur H. Wolff, Chief, Research Branch, Radiological Health Laboratory, Department of Health, Education and Welfare, for their help in the development of the bibliography.)

Radioactivity in food has recently caused considerable concern to public health officials and to various parts of the food industry in several areas throughout the United States. Particularly important is the interpretation of the federal radiation standards.

There has been widespread misunderstanding of the Radiation Protection Guides of the Federal Radiation Council. These guides were intended for evaluating effects of radiation on people under continuing industrial conditions.

As indicated by the Chairman of the Council, the Guides are not intended to be a dividing line between safety and danger in general radiation exposure. Nor are they intended to be the sole basis for protection measures.

While there may be some slight risk from any level of radiation exposure, the Council does not believe there is any major risk of health hazard until exposure is many times the Guide levels.

At the present time, iodine-131 readings are very low. In some areas, where readings had caused some local concern, there is now little or no evidence of iodine-131. The Council recognizes a continuing need for guidance in this field and is continuing its studies.

It is apparent from our committee study that a great deal of emotionalism is entering into the picture of radiation exposure both from the general public and from many officials in the public health field. We strongly recommend that a scientific approach be taken by everyone in positions of authority and that great care be exercised in the release of information to the people of the United States. Such information should be based on sound scientific facts and "scare" type rumors and reports must be virously refuted.

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REPORT OF COMMITTEE

RADIOACTIVE FALLOUT AND ITS EFFECT ON ANIMALS AND FOOD PRODUCTS OF ANIMAL ORIGIN

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Burro


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REPORT OF THE COMMITTEE ON RABIES

A. L. Brueckner, Baltimore, Maryland, Chairman; E. S. Tierkel, Atlanta, Georgia, Vice-Chairman; R. L. Elsea, Harrisburg, Pennsylvania; J. G. Flint, St. Paul, Minnesota; D. Ibsen, Little Rock, Arkansas; G. S. Kaley, Albany, New York; E. E. Saulman, Washington, D. C.; J. V. Smith, Hartford, Connecticut; L. E. Starr, Atlanta, Georgia

The report contains a tabulation prepared by the Communicable Disease Center covering the years 1953 through 1961. A footnote explains the source of the information.

INCIDENCE OF RABIES IN THE UNITED STATES BY TYPE OF ANIMAL

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<td>371</td>
<td>794</td>
<td>1,281</td>
<td>631</td>
<td>41</td>
<td>126</td>
<td>10</td>
<td>5,846</td>
</tr>
<tr>
<td>1957</td>
<td>1,758</td>
<td>382</td>
<td>714</td>
<td>1,021</td>
<td>775</td>
<td>31</td>
<td>115</td>
<td>6</td>
<td>4,802</td>
</tr>
<tr>
<td>1958</td>
<td>1,643</td>
<td>353</td>
<td>737</td>
<td>845</td>
<td>1,005</td>
<td>68</td>
<td>157</td>
<td>6</td>
<td>4,814</td>
</tr>
<tr>
<td>1959</td>
<td>1,119</td>
<td>292</td>
<td>751</td>
<td>920</td>
<td>789</td>
<td>80</td>
<td>126</td>
<td>6</td>
<td>4,083</td>
</tr>
<tr>
<td>1960</td>
<td>697</td>
<td>277</td>
<td>645</td>
<td>915</td>
<td>725</td>
<td>88</td>
<td>108</td>
<td>2</td>
<td>3,457</td>
</tr>
<tr>
<td>1961</td>
<td>594</td>
<td>217</td>
<td>482</td>
<td>614</td>
<td>1,254</td>
<td>186</td>
<td>120</td>
<td>3</td>
<td>3,470</td>
</tr>
</tbody>
</table>

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

The continued decrease in the number of cases of rabies in dogs and cats reflects the value of control programs in the state which in most instances are satisfactorily conducted. However, unless these procedures are followed with vigor the disease may again become a problem in these species. There has also been a decrease in the cases in foxes but an increase in skunk rabies. The deaths in farm animals has also decreased.

The most alarming figures are those reporting cases in bats and this feature makes for caution in predicting the future of the disease in all species, including man. Although the human deaths from rabies has remained at a low level for the past two years extreme caution should be observed and livestock sanitary and public health officials should continue to impress upon the public the dangers of outbreaks from exposure to bats which may be harboring the virus.

RABIES INCIDENCE AND TRENDS - CALENDAR YEAR 1961

The total number of laboratory confirmed animal rabies cases reported for calendar year 1961 was 3,470, representing an increase of 13 cases over the total number of confirmed cases reported during the previous calendar year.
The numbers of confirmed cases reported for 1961 by type of animal included 594 dogs; 217 cats; 482 farm animals; 614 foxes; 1,254 skunks; 186 bats; and 120 cases in other types of animals. A decline occurred in dogs, with a drop of 103 cases between 1960 and 1961. The total of 594 canine cases is the all-time low reported for the entire country.

In 1961, there was a substantial decrease of rabies in foxes. One-third fewer cases were reported than in 1960. The southeastern states, which long have been areas of high fox rabies, are now virtually free of it.

Reported skunk rabies cases increased by 73 percent in 1961. This sharp rise is not due to a single regional epidemic but to 100 percent increases in the number of rabid skunks reported in each of three widely separated foci of skunk rabies in California, Texas and Iowa.

The number of rabid bats in 1961 was 186 as compared to 88 for the previous year. These cases came from 28 States. A number of these, such as Massachusetts, Montana, New Jersey, Utah, Oregon and Washington, seldom report rabies in terrestrial animals.

The New England States remained rabies free in 1961 with the exception of one reported bat case in Massachusetts. This is the first reported case of animal rabies in the State of Massachusetts since 1949. There were 23 states in which there was an increased incidence in total rabies cases over the previous year: Massachusetts, New Jersey, Indiana, Illinois, Michigan, Wisconsin, Minnesota, Iowa, Missouri, South Dakota, Nebraska, Kansas, Maryland, Florida, Kentucky, Louisiana, Oklahoma, Texas, Arizona, Utah, Washington, Oregon, and California. Of these, there were four states in which the increase was by more than 50 cases, Illinois, Iowa, Texas, and California. The principal problem in each of the four states has been one of skunk rabies. Texas showed the highest incidence in the country with a total of 658 cases of animal rabies for 1961. Iowa showed the greatest increase with 160 more cases for 1961 than for the previous year.

In 17 states there was a decrease in the number of cases reported: New York, Pennsylvania, Ohio, North Dakota, Virginia, West Virginia, North Carolina, South Carolina, Georgia, Tennessee, Alabama, Mississippi, Arkansas, Montana, Colorado, New Mexico and Alaska. In New York and Arkansas the decrease was by more than 50 cases. The most significant decrease in these two states was in fox rabies.

RABIES TRENDS BY UNITED STATES PUBLIC HEALTH SERVICE REPORTING REGIONS - 1961

New England
(Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, and Connecticut)

The only reported case of rabies during 1961 was in Massachusetts in a bat.

Middle Atlantic
(New York, New Jersey, and Pennsylvania)

New York’s incidence dropped from 455 cases in 1960 to 92 cases during 1961, a decrease of 363 cases. This was the largest decrease in the
country. New Jersey showed a slight increase, reporting seven cases during 1961 as compared to one case the previous year. On the other hand, Pennsylvania reported a decline from 18 cases to 14 cases. Thus, this reporting region had a decrease of 361 cases.

**East North Central**
(Ohio, Indiana, Illinois, Michigan, and Wisconsin)

Each state in this reporting region showed an increase with the exception of Ohio, which reported 38 fewer cases for 1961 than the previous year. Total increase for these states was from 380 cases to 454 cases.

**West North Central**
(Minnesota, Iowa, Missouri, North Dakota, South Dakota, Nebraska, and Kansas)

This reporting region showed an increase of 228 cases over the previous year: 160 of these were reported from Iowa.

**South Atlantic**
(Delaware, Maryland, District of Columbia, Virginia, West Virginia, North Carolina, South Carolina, Georgia and Florida)

This reporting area showed a decline of 65 cases in 1961 with every state showing a decrease except Maryland and Florida.

**East South Central**
(Kentucky, Tennessee, Alabama, and Mississippi)

Slight decline from 405 to 379 for this reporting region, with Kentucky the only state showing an increase.

**West South Central**
(Arkansas, Louisiana, Oklahoma, and Texas)

This reporting region showed an increase of 66 cases with every state except Arkansas showing increases. Arkansas reported 92 cases as compared to 154 cases during 1960. Texas showed the highest incidence in the country again this year with 658 cases, an increase of 97 cases over the total for 1960.

**Mountain**
(Montana, Idaho, Wyoming, Colorado, New Mexico, Arizona, Utah, and Nevada)

A slight decline from 82 cases in 1960 to 68 cases during 1961.

**Pacific**
(Washington, Oregon, California, Alaska, and Hawaii)

This reporting region showed an increase of 110 cases from 1960 to 1961. The bulk of the cases was reported from California. Hawaii remained rabies-free.
RABIES

HUMAN RABIES DEATHS - 1961

Three human rabies deaths were reported in the United States for calendar year 1961. The first case occurred in a 53-year-old woman who died of rabies in Harlan County, Kentucky, on January 6, 1961, 59 days after being bitten on the leg by a gray fox. She was bitten when she kicked at the animal to scare it away from her puppy. The fox was killed by the woman's husband. The next day she saw a physician and immediately began a series of 14 doses of duck-embryo rabies vaccine.

A 76-year-old man died on January 20, 1961, 44 days following the bite of a rabid dog near the Imperial Dam, Imperial County, California. The dog jumped from the bank above and attacked him on December 7. The five-inch bite wound on the right wrist was washed following the attack and a series of 14 doses duck-embryo rabies vaccine was begun six days after the biting episode. Onset of illness was 31 days after the bite and death occurred on the 13th day after onset of illness.

The third human rabies death was also reported from Kentucky due to a bite of a rabid fox. On May 15, a 74-year-old resident of Powell County investigated a commotion in his chicken house and found a fox under the shed. On attempting to chase the fox away, he was bitten on his left thumb. The fox was killed and discarded. The man refused rabies vaccination initially, but after two calves had died of apparent rabies, within the following two weeks, he consented to vaccination. Beginning on June 7, two and one-half weeks after the exposure, he received 14 doses of duck-embryo vaccine. The last dose was administered a day before onset of symptoms. The patient died on June 27.

U. S. HUMAN RABIES DEATHS - 1961

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date Died</th>
<th>Age</th>
<th>Sex</th>
<th>Nature of Exposure</th>
<th>Incubation Period</th>
<th>Length of Illness</th>
<th>Treatment</th>
<th>Biting Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harlan County, Ky.</td>
<td>1/6/61</td>
<td>53</td>
<td>F</td>
<td>Bitten on leg</td>
<td>52 days</td>
<td>7 days</td>
<td>14 doses vaccine - next morning</td>
<td>fox</td>
</tr>
<tr>
<td>Imperial County, Calif.</td>
<td>1/20/61</td>
<td>76</td>
<td>M</td>
<td>Five-inch wound on right wrist</td>
<td>31 days</td>
<td>13 days</td>
<td>14 doses vaccine - 7 days later</td>
<td>dog</td>
</tr>
<tr>
<td>Powell County, Ky.</td>
<td>6/27/61</td>
<td>74</td>
<td>M</td>
<td>Bitten on left thumb</td>
<td>39 days</td>
<td>5 days</td>
<td>14 doses vaccine - 21/2 weeks later</td>
<td>fox</td>
</tr>
</tbody>
</table>

HUMAN RABIES DEATHS - 1962 TO DATE

At this writing (October 29, 1962) there have been only two human rabies deaths reported for calendar year 1962. In the first case, a
three-year-old white child, from Cameron County, Texas had onset of illness on July 16, 1962. The child was one of a number of children who had played with a puppy which became ill and died several weeks before onset of the child's illness. About that time a stray dog was seen in the neighborhood which subsequently was proven rabid by laboratory confirmation. There was no history of an animal bite. The child died on July 24. The diagnosis in this case was not made until post mortem and if there had been no autopsy, the case would not have been diagnosed.

The second case was in an 11-year-old boy who died on October 8 in Grace, Idaho, some 35 miles from the Wyoming border. Confirmatory diagnosis by the fluorescent antibody test was carried out by the Communicable Disease Center laboratories. While sleeping in his back yard early in September the boy was bitten by a creature which left three tooth marks on the upper left cheek and one mark on the lower left cheek. The biting awakened him, but he was unable to see what had bitten him. He received no treatment, was admitted to the hospital on October 5 and died on October 8.

THE RABIES PICTURE - OVER THE YEARS

Although the number of rabies cases in dogs declined, the number of wildlife cases increased during 1961, thus bringing the total slightly higher for 1961 than it was in 1960. This is illustrated in the following table:

<table>
<thead>
<tr>
<th>Year</th>
<th>Wildlife</th>
<th>Cats</th>
<th>Livestock</th>
<th>Man</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956</td>
<td>2,592</td>
<td>2,079</td>
<td>371</td>
<td>10</td>
<td>5,846</td>
</tr>
<tr>
<td>1960</td>
<td>697</td>
<td>1,836</td>
<td>277</td>
<td>2</td>
<td>3,457</td>
</tr>
<tr>
<td>1961</td>
<td>594</td>
<td>2,174</td>
<td>217</td>
<td>3</td>
<td>3,470</td>
</tr>
</tbody>
</table>

NATIONAL RABIES PICTURE - FIRST NINE MONTHS - 1962

The total number of animal rabies cases during the first nine months of 1962 is 2,909. These are provisional figures and are based on cumulative totals reported on a weekly basis to the Communicable Disease Center, United States Public Health Service. This represents an increase of 305 cases as compared with the same period in 1961. In this period a rise in incidence was noted in Ohio, Indiana, Minnesota, and California. Notable declines in incidence were reported from Missouri, Virginia, Arkansas and Louisiana.

ANIMAL RABIES OUTBREAK - UNITED STATES-CANADIAN BORDER

Of newest concern this year is an outbreak of rabies in animals in Canada along the United States Border. Dr. Dean H. Fisher, Commissioner, Maine Department of Health and Welfare, in conversation with the Quebec Province Veterinarian regarding a confirmed case of rabies in a
Rabies in Animals
First Nine Months - 1961-1962
(CDC—United States Public Health Service)

<table>
<thead>
<tr>
<th>State</th>
<th>1961 (First Nine Months)</th>
<th>1962 (First Nine Months)</th>
<th>State</th>
<th>1961 (First Nine Months)</th>
<th>1962 (First Nine Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New England</td>
<td></td>
<td></td>
<td>South Atlantic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maine</td>
<td>0</td>
<td>0</td>
<td>West Virginia</td>
<td>96</td>
<td>105</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>North Carolina</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Vermont</td>
<td>0</td>
<td>0</td>
<td>South Carolina</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>0</td>
<td>1</td>
<td>Georgia</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Rhode Island</td>
<td>0</td>
<td>0</td>
<td>Florida</td>
<td>56</td>
<td>54</td>
</tr>
<tr>
<td>Connecticut</td>
<td>0</td>
<td>0</td>
<td>East South Central</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle Atlantic</td>
<td></td>
<td></td>
<td>Kentucky</td>
<td>84</td>
<td>106</td>
</tr>
<tr>
<td>New York</td>
<td>64</td>
<td>71</td>
<td>Tennessee</td>
<td>156</td>
<td>175</td>
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<tr>
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<td>Alabama</td>
<td>46</td>
<td>21</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>12</td>
<td>33</td>
<td>Mississippi</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>East North Central</td>
<td></td>
<td></td>
<td>West South Central</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>61</td>
<td>336</td>
<td>Arkansas</td>
<td>165</td>
<td>59</td>
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<tr>
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<td>76</td>
<td>179</td>
<td>Louisiana</td>
<td>58</td>
<td>18</td>
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<td>79</td>
<td>84</td>
<td>Oklahoma</td>
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<td>23</td>
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<tr>
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<td>35</td>
<td>Texas</td>
<td>460</td>
<td>435</td>
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<tr>
<td>Wisconsin</td>
<td>18</td>
<td>32</td>
<td>Mountain</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>West North Central</td>
<td></td>
<td></td>
<td>Montana</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Minnesota</td>
<td>104</td>
<td>174</td>
<td>Idaho</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Iowa</td>
<td>280</td>
<td>299</td>
<td>Wyoming</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Missouri</td>
<td>172</td>
<td>131</td>
<td>Colorado</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>North Dakota</td>
<td>25</td>
<td>47</td>
<td>New Mexico</td>
<td>23</td>
<td>12</td>
</tr>
<tr>
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<td>7</td>
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<tr>
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<td></td>
<td>Pacific</td>
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</tr>
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<td>0</td>
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<td>Oregon</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>District of Columbia</td>
<td></td>
<td></td>
<td>California</td>
<td>172</td>
<td>207</td>
</tr>
<tr>
<td>Virginia</td>
<td>167</td>
<td>116</td>
<td>TOTALS</td>
<td>2,604</td>
<td>2,909</td>
</tr>
</tbody>
</table>

Fox killed at the Lac Frontiere Custom House, learned that approximately 50 cases of animal rabies have been recorded since September 1961, within a 25-mile radius of the custom house. Of the 50 cases, there were 25 in foxes and 25 in horses, cows, pigs, dogs and cats. No human cases have been reported during this period.

The Maine territory immediately south of the custom house is a large spruce-fir forest worked by scattered crews of lumberjacks and visited by fishermen and hunters. Except for one north-south state highway in the area all roads are owned and operated by various lumber companies.

Officials of the Maine Department of Health plan to enlist the immediate cooperation of the national health authorities in Ottawa and the Maine Fish and Wildlife Service in a program aimed at reducing the fox population through poisoning and trapping on all roads in the subject area. In addition, no dogs or cats will be permitted in the lumber camps and winter
hibernation of skunks and raccoons will eliminate the hazard of human contact with these infected animals.

The New York State Health Department reports numerous cases of rabies to Vermont via foxes has been a distinct possibility. Clinton, Franklin and St. Lawrence Counties (New York) have reported 18 cases of rabies in foxes during 1962, 15 of these having been recorded since April 1962.

RABBITS PREVALENT IN QUEBEC ALONG THE VERMONT BORDER

According to reports from officials of the Division of Communicable Disease Control, Vermont Department of Health, several confirmed cases of rabies have occurred recently in Quebec along the Vermont Border.

The closest case occurred in a bovine in Dixville, Quebec, only five miles from Norton, Vermont. This case was confirmed in the laboratory on July 27, 1962. In Magog, Quebec, 17 miles from Derby Line, a positive diagnosis of rabies was made in a horse on August 7, 1962. Rabies was diagnosed in a dog in Sherbrooke, Quebec, 29 miles from Norton on April 5, 1962. The disease was diagnosed in two dogs and one cow during the latter part of April 1962 in Richmond, Quebec, about 50 miles north of Derby Line.

Canadian officials say that rabies has been a problem in northern Quebec during the last two years, but only in the past two years has it moved south.

Recently the Vermont Department of Health sent a letter to all physicians and health officers in the State pointing out that Vermont can no longer be considered a rabies-free area and that all animal bites should be evaluated with much care. Health officers were urged to promote vaccination of dogs against rabies in their communities and also promote a humane program for the elimination of stray dogs. Hospital pharmacies were urged to stock anti-rabies serum. The letter recommended that biting animals should not be killed but should be confined and observed for signs of rabies. If such animals should be killed, their heads should be refrigerated (not frozen) and sent to the State laboratory at once.

U.S.-MEXICO BORDER RABIES CONTROL PROGRAM GETS UNDERWAY

At the special conference of rabies held in Mexico City November 2-3, 1961, representatives of the United States Government, the Mexican Government, and the Pan American Sanitary Bureau outlined the following program for rabies control in the area of the United States-Mexico Border:

1. Rabies is a disease problem of major importance in the United States of America and in the United States of Mexico.

2. The health authorities of the two countries should therefore expend maximum effort and cooperation for the development of antirabies programs.
3. The various programs will be more effective if they are coordi-
nated from a single point and in such a way as to result in a single
program for the Border Area.

4. Coordination of the antirabies programs for the Border Area will
be provided by the Pan American Sanitary Bureau through its
United States-Mexico Border Field Office.

5. All the activities related to cooperation in, and coordination of the
rabies control program should be carried out, taking into consider-
atation the authority and responsibility of the various agencies con-
cerned at national, state and local levels.

It was deemed that the responsibilities of the various parties would
be:

1. State and Local Government Agencies

(a) To develop a system for the reporting of cases of rabies by
veterinarians, physicians, hospitals, laboratories and others
concerned, and to convey this information to the Field Office of
the Pan American Sanitary Bureau.

(b) To promulgate or revise legislation so that the programs of
rabies control may be more effective.

(c) To plan and conduct all antirabies measures necessary to re-
duce and eventually eliminate this public health problem.

(d) To provide the funds, personnel, supplies, equipment and labor-
atory services necessary for the implementation of the meas-
ures mentioned above, within their capabilities.

(e) To establish local rabies advisory councils integrated by tech-
nical and community representatives which will work together in
the rabies control programs and which will participate in the
respective regional rabies advisory councils.

(f) To cooperate and collaborate fully with the national public health
officers and with the Field Office of the Pan American Sanitary
Bureau in the coordinated rabies program for the Border Area.

(g) To carry out programs of public health education, emphasizing
the dangers represented by dogs and other animals in the trans-
mission of rabies.

2. National Government Agencies

(a) To provide training services in all aspects of rabies control in-
cluding the conduct of courses, seminars and demonstrations
programs.

(b) Provision of consultation and technical assistance, including as-
sistance for field studies, assignment of personnel, reference
laboratory services and aid for wildlife control.

(c) Quality control of biologics used in rabies programs.

(d) Conduct of, and assistance in, applied research and studies in
ecology, epidemiology and rabies control.

(e) To collaborate with, and to support as far as possible, the state
and local agencies and the Field Office of the Pan American San-
itary Bureau, to attain a single coordinated antirabies program
for the Border Area.
3. The United States–Mexico Border Field Office of the Pan American Sanitary Bureau

Consistent with the policies and responsibilities of the National health services and keeping these services advised at all times, the activities of the Field Office of the Pan American Sanitary Bureau will be as follows:

(a) To assist the national, state and local health services in the development of rabies reporting services, both routine and emergency. This information will be centralized and will be distributed to all interested agencies by the Field Office. The system adopted will include the trial operation of various methods until the most efficient is found.

(b) To collaborate with governmental agencies in the border area in the study of legislation, and to prepare such amendments as are deemed necessary to effectively support the antirabies program.

(c) To collaborate with official agencies in the study of needs in personnel for the carrying out of control programs and to make the necessary recommendations.

(d) To study the need for laboratory services in the area and to inform the appropriate officers when these laboratories should be extended in number or in services, for rabies diagnosis.

(e) To propose modification in the program in accordance with local requirements.

(f) To assist in the establishment of local and regional advisory councils and to participate in these councils when possible, with the purpose of providing the corresponding information.

(g) To coordinate the rabies research activities which are carried out in the area, and to stimulate such research when necessary.

(h) To prepare and distribute a monthly newsletter in both languages, in rabies control and related subjects carried out in the area.

In testimony of the importance of the foregoing conclusions and for the success of the rabies control activities in the Border Area, the document was signed by representatives of the Public Health Service, the Mexican Ministry of Health, the Pan American Sanitary Bureau, the United States Fish & Wildlife Service, and officials of local and State health departments on the border.
PNEUMONIA COMPLEX IN LAMBS
Gordon Shultz, D.V.M.*
Sacramento, California

Pneumonia complex in lambs can best be described as a multiple infection induced by exposure to a pneumonia virus and subsequent stress factors.

In California the disease appears most often in lambs that have been recently moved from native range to irrigated pasture or feed yards. Accordingly, the disease is more often noted in the large irrigated pasture and lamb feeding areas such as the Sacramento and San Joaquin Valleys. Occasionally, it has been observed in ewe lambs that have been retained for replacements in the breeding flock. Also, what clinically appears to be the same condition has been observed on native range in three month old lambs not yet weaned.

For many years pneumonia complex has been observed to be more prevalent in feeder lambs from the north coast areas of California and in western Oregon.¹

Investigations and Observations:

The primary cause of pneumonia complex is known to be a virus.²,³ Virus pneumonia in lambs may occur without being clinically evident. Slight to well marked lesions have been observed on autopsy of north coast lambs that were never moved from the ranches on which they were born. This indicates infection on the ranches before shipment is made to valley feeding areas.

Occasionally pneumonia complex may be observed on the home ranches, more commonly in the north coast area but also in the valley areas, when lambs are placed under stress through poor feed conditions, parasitism and climatic changes. Lesions found in these cases are identical with those found in valley areas.

Greatest losses are found, however, in lambs which have been affected with virus pneumonia and are subsequently moved to permanent pasture or feed lots in Sacramento and San Joaquin Valleys. Here again stress factors appear to be secondary. Gathering, weaning, shipping, shearing and climatic changes appear to be the causes that bring about lamb losses.

Lesions found on autopsy have varied. Pleurisy, adhesions, fibrin formation and numerous types of abscesses indicate that many opportunistic bacteria may be present. Autopsy specimens submitted to the California State Veterinary Pathology laboratories have revealed that pasteurella organisms were present in about 50 percent of the cases. The presence of this organism, which appears to be more virulent under these conditions, may cause death in apparently good strong lambs in a matter of hours.

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This is in contrast to the usual course of the disease, sometimes referred to as shipping fever in which symptoms are noted over a period of several days with noticeable weight loss, labored breathing and usually death. Sometimes lambs will survive the pneumonia complex attack only to die several weeks later from the effects of lung damage and multiple abscesses. Surviving lambs generally will be unthrifty and fail to make satisfactory weight gains.

Treatment:

When it became apparent that many different bacteria were involved, treatment had to be based on the use of a broad spectrum drug. Due to the labor required in handling large numbers of lambs, infrequent treatments were considered desirable. Further, frequent gathering and handling of the lambs would aggravate any weakened condition and be an additional stress factor.

Sulfa drugs have been tried in several forms and with varied degrees of success. Sulfamethazine in a 25 percent solution was used intraperitoneally at the rate of one grain per pound body weight per day. The dosage was given once daily for three days to all lambs in the affected flock. At the end of three days treatment, all visibly sick lambs were removed and held separately. This procedure has proven successful when used early in an outbreak.

A Shikles vaccinating outfit is used to administer the drug. The lamb is given the sulfa in the right flank while in a standing position. The lambs may be handled by crowding into a small pen, but the preferred technique is to use a long narrow crowding chute. Lambs should be identified as treated.

There are other methods used in administering sulfa drugs. A 12.5 percent solution of sulfamethazine administered orally proved economical and gave equally good results. Twelve and one-half percent solutions were not intended for intraperitoneal use, but they have been used with no apparent adverse effects. In feed yards where lambs are fed pellets or fine ground concentrate feeds, the powdered form of sulfa has been used in the feed with satisfactory results.

Sulfathiazole, a relatively low-cost sulfa, has been used in the powdered form mixed in feed. Sulfamethazine or sulfathiazole powder is mixed so that the dosage is from three-fourths to one grain per pound body weight daily. Seventy-five to eighty pound lambs will usually consume about four pounds of feed per day. Thus, if five pounds of sulfa drug is mixed in a ton of feed the lambs will usually consume a therapeutic dose. This is given for three days. Lambs not eating should be individually treated.

Sulfa drugs have also been used in the drinking water, but this method has some disadvantages. The daily consumption of water may vary greatly which, in turn, will result in varied daily intake of sulfa.

To date, antibiotics have not been tried either for prevention or treatment.

Vaccines have been considered. One was tried and reported ineffective in 1959.
Conclusion:

To insure against a pneumonia complex outbreak one can only advise good careful management practices plus a program of sulfa treatment if the feeder lambs are from a known dangerous area.

It should be kept in mind that pneumonia conditions have appeared in replacement ewe lambs kept on their native range without ever having been subjected to the stress of handling and shipping. Also the condition has never occurred in mature ewes on the same ranch or in adult ewes shipped into known danger areas. It is not known if there is an age factor involved or if all sheep have a mild latent form of the disease at an early age only to subsequently develop an immunity which carries throughout maturity. If an attack of the disease confers immunity it should be possible to produce an efficient vaccine.

REFERENCES

TRANSMISSION OF SCRAPIE TO GRADE LAMBS
BY SUBCUTANEOUS INOCULATION

Donald P. Gustafson and Melvin W. Stromberg*

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Since scrapie was first reported in the United States in 1947, it has been recognized in 26 states. Records12 of the United States Department of Agriculture indicate at least 113 flocks to have been infected. In these flocks there were at least 181 sheep showing signs of scrapie. Sixty-two percent of the infected flocks and 59 percent of the total number of infected animals were in Illinois, Indiana, Ohio, and California. About 95 percent of the cases have occurred in Suffolk sheep, the remainder in Cheviot and first cross animals.

Scrapie is an unusual disease. There are but few diseases of man or animals which resemble it in significant measure. The long incubation period characteristic of scrapie in sheep is approached by visna,8 a demyelinating slowly progressive encephalitis of sheep in Iceland. Pleocytosis is a common finding of visna not shared by scrapie or rida,8 another disease of sheep in Iceland which resembles scrapie to such an extent that it is considered by many to be identical. Kuru4 of man is a slowly progressive disease in which fine tremors of the head, trunk, and extremities and wasting are the signs which remind one of scrapie. Histopathologically Kuru is characterized principally by astrocytosis, microgliosis, and neuronal degeneration. The principal and distinguishing feature of scrapie which sets it apart from all other diseases whose agent of transmission is particulate and self replicating5,6 is that the agent of scrapie will withstand heating at 99.5 degrees Centigrade for as many as eight hours10 without destroying its ability to cause the disease.

At the time these studies were initiated in 1958, all cases of scrapie reported in the United States had occurred in purebred sheep. Interest was aroused in the susceptibility of grade lambs, in the heat stability of the agent in central nervous system (CNS) material, and in the transmissibility by subcutaneous exposure which would remove the necessity of interpreting changes in the CNS following intracerebral inoculation.

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Scrapie Agent. Approximately one-half of the cerebrum, cerebellum, and brain stem of a purebred Suffolk sheep affected with naturally developed scrapie killed when moribund was used as source material for the inoculum for the principals.

Preparation of the Inoculums. (A) Heat treated material. Representative portions of the available CNS material were placed in a partially closed vessel to prevent water loss during heating. The vessel was suspended in another containing boiling water and allowed to remain under these conditions for six hours. The material was removed, chopped finely, and suspended volumetrically in nine parts of Earle's balanced salt solution (BSS). It was centrifuged in 50 ml. Rockefeller tubes at approximately 2250 relative centrifugal force (r.c.f.) for 30 minutes. The supernatant fluid was harvested and saved to be used as the inoculum.

(B) Filtered Material. A representative portion of the available CNS material was chopped finely and suspended volumetrically in nine parts of Earle's BSS. It was centrifuged at approximately 2250 r.c.f. for 30 minutes. The supernatant fluid was harvested and passed through an 03 Selas filter under approximately 10 pounds positive pressure of five percent CO₂ in air. The bubbling pressure of the candle was 29 pounds of pressure. The filtered fluid was used as an inoculum.

(C) Crude Material. The inoculum was prepared as in (B) except that it was not filtered. The harvested supernatant fluid of the centrifuged 10 percent suspension was used as an inoculum.

(D) Control Inoculums. The brain of an apparently normal adult grade ewe was obtained to be used as source material. The inoculums were prepared at the same time as those in (A), (B), and (C) above from fresh material and in as nearly identical fashion as was possible. The inoculums were refrigerated at 6°C to 10°C Centigrade overnight and were administered approximately 20 hours following their preparation.

Experimental Subjects. Lambs approximately 4 months of age were purchased at a public sales place. They were a mixture of grade animals showing phenotypical resemblances to a wide variety of breeds. The lambs were held for approximately three weeks prior to inoculation. During this time they were examined individually for gross defects and blood samples taken from each. Serum samples were stored at -35°C. The lambs were shown to have intestinal nematodes and were treated with phenothiazine.

Inoculation of Lambs. All lambs were inoculated subcutaneously in the woolless area of the axillary space with approximately five ml. of the appropriate inoculum. An exception was the group exposed to heated scrapie material in which case the subjects were inoculated with approximately three ml. per inoculation. In addition to the inoculated animals, 11 lambs were left non-inoculated and served as contact controls. Two adult grade normal rams were included with the group at an appropriate time to serve the females at random. The groups of lambs and inoculums were as follows:
1. Scrapie heated material — 16 lambs
2. Scrapie filtrate — 20 lambs
3. Scrapie crude supernate — 24 lambs
4. Normal heated material — 10 lambs
5. Normal filtrate — 10 lambs
6. Normal crude supernate — 10 lambs

**Maintenance of the Flock.** The sheep were maintained together in pastures which were essentially isolated from other flocks in the community. During the three years of the experiment the flock was shifted from pasture to pasture of the farm during the grazing season. Shelter was provided in the winters near the principal buildings. Parasitological examinations were conducted periodically and the animals treated accordingly. Ewes heavy with lambs were brought to a sheltered lambing area. Lambs were docked and castrated prior to being returned to the flock with their dams.

**Cell Cultures.** Cultures of cells were prepared from the CNS of one of the sheep which became affected with scrapie 40 months following inoculation and from one apparently normal sheep. The cultures have been maintained over six months and have been subcultured at random intervals without difficulty.

Portions of the thalamus and midbrain of a sheep affected with scrapie were trypsinized and cultures were set in Leighton tubes and T-9 flasks. The material was minced and 0.25 percent trypsin at 37°C was added. The mixture was stirred for less than three minutes in a magnetic stirring device. The supernatant was decanted and refrigerated. This procedure was repeated three times. The four supernatant fluids were centrifuged at 10² C. for 20 minutes at 425 r.c.f. after which the supernatant fluids were discarded. The cellular portion was resuspended in approximately three volumes of Hanks' BSS and SPAM (streptomycin 100 mgm penicillin 100 units, Achromycin* .01 mgm, and Mycostatin** 50 units per ml. of Hank's BSS) and centrifuged; the supernatant fluid was discarded as before. Each of the four cellular harvests were suspended in complete medium. The medium was composed of 80 percent Eagle's Basal Medium, 10 percent human serum, and one percent SPAM. Because of the amount of cellular debris it was not possible to adjust the number of cells per unit volume of medium using ordinary hemacytometrical procedures. Dilution was achieved by estimation. Subsequent subcultures were prepared using 0.25 percent trypsin to replace the cells from the glass and adjusting the number of cells per ml. to approximately 400,000.

Similar cultures were prepared from an apparently normal sheep.

**Examination of Cell Cultures.** Cover slips on which the cells grew in the Leighton tubes were removed and paraffin mounts prepared for phase microscopy. The cultures on cover slips were subsequently fixed in alcohol and stained using the technique described by Jacobson.

**Examination of Cell Cultures for Pleuropneumonia-like Organisms (PPLO).** Freshly harvested fluids from cell cultures were cultured to

* Achromycin — tetracycline
**Mycostatin — nystatin
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determine whether or not PPLO were present. Fluids were cultured in the following media: Heart Infusion Broth with 0.5 percent Bacto Peptone was adjusted to pH 7.82. Semisolid media was prepared by adding 1.5 percent agar. The media was autoclaved at 121° C. for 15 minutes and 30 percent Human Ascitic Fluid added. Tubes and plates were inoculated with 0.5 ml. of cell culture fluid.

Index of Multinucleation of Cell Cultures. Permanent preparations of stained cell cultures on cover slips were examined for cells which contained more than one nucleus. One thousand cells per preparation were counted and the number of polynucleated cells was recorded. The number of multinucleated cells per 100 cells counted provided an index of multinucleation for the cell cultures.

Examination of Scrapie Affected Sheep for Pathological Changes.

(A) Observations of Signs and Symptoms. Daily observations of pulse, temperature, and respiration were made on those sheep which showed symptoms associated with scrapie. Cinematographic records were made of each at various intervals.

(B) Gross Post-Mortem Examination. The disease was permitted to nearly run its course in two affected subjects before they were killed. Two died naturally and were examined within two hours of death. A general post-mortem examination was conducted on all four animals affected with scrapie and on others that died except when the condition causing death was readily recognized through signs and symptoms prior to death or when the corpse was in such condition as to make the examination impractical.

(C) Histopathological Examination. Brains from the four experimental sheep showing clinical signs of scrapie were removed at necropsy and with the exception of the left half of the brainstem (retained for tissue culture purposes) were fixed by immersion in 10 percent neutral formalin. The entire spinal cord was collected from one of these animals.

After proper fixation, each of three brains was sliced transversely into approximately 14 equal parts and the fourth brain in five equal parts. Sections 15 μ in thickness were cut from each level of each brain and stained with hematoxylin and eosin. Weil's stain for myelin sheaths and thionin for Nissl bodies were used on sections from the same levels in three of the above animals. Each segment of the spinal cord was sampled and stained in the same manner.

By means of the above procedure, parts of the cerebral cortex basal nuclei, thalamus, hypothalamus, epithalamus, midbrain, pons, medulla oblongata, and spinal cord were carefully examined for pathological changes. Brain sections from normal sheep were used for comparison, Whenever possible, the same structures were examined as listed by Hadlow2 in his work on experimental scrapie in the goat. This involved over 60 specific nuclei and fiber tracts most of which are brainstem structures.

RESULTS

In the 37 months prior to the recognition of the first sheep affected with scrapie, 20 had died of various causes. Forty-four wethers were sold for slaughter at 33 months. At the same time the first animal was noticed
showing signs of scrapie, there were seven in Group one, six in Group two, 10 in Group three, four in Group four, five in Group five, one in Group six, and four in Group seven. A total of four sheep, all in Group three, developed recognizable signs of scrapie.

(A) Syndrome Observations. Two of the four affected with scrapie showed symptoms commonly associated with scrapie as it has been witnessed in the United States of America. These shared the common observations of having separable, dry, brittle wool; gradual debilitation in the presence of a continuing appetite; altered gait; staring eyes; viper-like flicking of the tongue with the head slightly elevated when rubbed over the tailhead; and of periodic licking, biting, and rubbing of various parts of the limbs. One was much more nervous and easily excited than the others. Although its wool was dry and brittle, there were no large areas denuded by rubbing and biting. One was quite quiet and not readily moved by rubbing over the tailhead into a spasm of licking and biting. Patient quiet observation revealed the animal to rub and to lick at its limbs periodically. This individual seemed to fit the activity of the "sleepy type" described by workers in Great Britain. The pulse, temperature, and respiration of each of the four affected animals remained essentially normal through the course of the syndrome.

(B) Pathology. On post-mortem examination all showed evidence of parasites in the gastro-intestinal tract. Blood-vascular, respiratory, and urinary systems showed no gross changes which seemed related to scrapie. Careful examination of the musculature of the pelvic limbs for evidence of myopathy was negative. However, bundles of the sacral portion of the m. multifidus dorsi of each affected animal were progressively pale pink to white, indicative of degeneration.

The nature of the histopathological changes was found to be generally similar to those described by Hadlow\(^2\) for the goat. The most widespread change was shrinkage, vacuolization, and apparent degeneration of some neurons. Areas of "spongy degeneration" were fairly common in the gray matter at various levels of the brainstem and were sometimes noted in the cerebral cortex. These areas often contained large pale astrocyte nuclei with a variety of forms such as oval, elongate, flask-shape, U-shape, and some with extremely irregular or lobulated nuclear outline. These cells could be found scattered randomly in the gray substance or else as clumps of several nuclei. Occasionally these cells were found near degenerating neurons.

A second type of darker staining elongate or otherwise pleomorphic nuclei were prominent in some areas. These were interpreted to be microglia cell nuclei and were not as plentiful as the large pale nuclei. Some of this latter type could also be identified in certain fiber tracts. Neither type of cell is felt to be pathognomonic for scrapie since small numbers could be identified in brains from clinically normal sheep.

Apparent neuronal degeneration was prominent in some areas of two scrapie brains but much less so in the other two. In one brain neuronophagia was clearly demonstrable in the hippocampus, the stellate cell layer of the dentate gyrus, the amygdaloid nuclei, and several thalamic nuclei. A large share of the cerebral cortical neurons of the frontal area

in this animal had been replaced by glial elements. Large portions of the
cerebellar vermis were almost devoid of Purkinje cells.

Brains of the other three scrapie animals showed slight or no cerebral
cortical damage so the pathological changes mentioned above could well be
due to a neurological problem other than scrapie.

(C) Tissue Culture. Cell cultures derived from the CNS of one sheep
affected with scrapie were observed under phase microscopy to have many
polynucleated cells. This was confirmed in stained preparations when it
was found that the index of multinucleation of such cultures was greater
than that of comparable cultures from an apparently normal sheep by an
average factor of six. However, when cultures from the normal sheep
were exposed to fluids from cultures from the scrapie affected animal,
they developed an index of multinucleation larger than that from those not
so exposed by an average factor of six.

Cells of the cultures from both the normal and scrapie affected sheep
were morphologically comparable in general. There were apparently
three types of cells in both and probably all three types were derived from
glial elements. One type was very large. Its nucleus commonly was five
to 20 times as large as that of the small type which had little cytoplasm.
The large cell had a remarkable amount of cytoplasm. These large cells
did not tend to form continuous sheets but were found in small groups or
singly. The medium sized cells seemed to pile up, forming pearl-like ag-
gregations, and also to be the most prolific. These tended to form con-
tinuous colonies of cells about the so-called pearls. The medium sized
cells were phagocytic as indicated by cellular fragments contained in the
cytoplasm of many.

The cultures from the scrapie affected subject had a wider range of
nuclear and cytoplasmic size than those from the normal animal. Cultures
from the normal sheep exposed to fluids from the cultures from the scrapie
affected animal resembled those from the scrapie source after four weeks
of semi-weekly exposure. Pleuropneumonia-like organisms were not found
to be present in any of the cultures examined for multinucleation.

DISCUSSION

The report by Gordon3 indicated that experience had shown the short-
est incubation period in lambs inoculated subcutaneously with the super-
natant fluid of a 10 percent saline suspension of CNS material from a sheep
affected with scrapie to be about 16 months. When 33 months of the ex-
periment had passed a large segment of the flock on experiment was sold
and the prospects for success were considered to be rather dim. This
disparity in results may have stemmed from one of several sources or a
combination of factors. The literature contains many comparisons of the
susceptibility of the various breeds but relatively little of the disease in
grade animals. Consequently extensive information of susceptibility for
grade animals is not available and it is difficult to evaluate the suscepti-
bilities of lambs with unknown genetic values. The optimum point in the
progress of the disease to harvest materials for transmission has not been
carefully studied. Therefore the infectious titer of a given source material
for the preparation of an inoculum is open to question. Dilution of the inoculum has been shown to lengthen the incubation period and to have a lesser but constant effect on decreasing incidence in an exposed group.\textsuperscript{3} It is possible that techniques in preparation and application of inoculums are important factors in success of transmission. It is interesting to note that of the 10 sheep remaining in the group inoculated with supernatant fluid of a 10 percent suspension of minced CNS of scrapie affected sheep that four became visibly affected with scrapie at 38 to 40 months. This number expressed in percent compares favorably with the transmission rate reported by others.\textsuperscript{3}

In the period of the experiment, some 48 months, none of those inoculated with heated material or with 03 Selas filtrate became affected visibly. The possibility of low titer of scrapie agent in the inoculum and unknown susceptibility of the experimental subjects seem to provide a reasonable explanation for the results. In addition, the method of heating is difficult to evaluate for in this case the brain material was heated in a double boiler arrangement and prepared in a 10 percent dilution in BSS subsequent to heating, whereas others have heated the material after mincing and suspension. It is possible to have altered the dilution factor to the extent that the second method may have lost water from the suspension fluid during the boiling period. The method employed lost nothing in dilution in BSS but may have lost critical tissue hydration instead. Although the heat may be present in the tissue for the specified period, the conditions of application may vary and be of significance in survival of sufficient agent to cause overt signs of the disease in an animal whose susceptibility value is essentially unknown. The absence of positive results found using 03 Selas filtrate gives further substance to the considerations of titer of inoculum and subject susceptibility. The average pore diameter (a.p.d.) of an 03 Selas filter is 0.65\(\mu\) which is comparable to the 0.69\(\mu\) a.p.d. reported for the gradacol membranes used in similar studies by workers in Great Britain.\textsuperscript{3} Experience with similar material containing virus particles in filtration through such filters has shown readily demonstrable reduction in titer following the excursion.

The implications of the differences between similar cell cultures derived from a scrapie affected sheep and an apparently normal sheep are interesting. However, the presence of the agent of scrapie in those cultures derived from a scrapie affected sheep has not been established.

The disease has had considerable attention from the standpoint of pathology and epizootiology. Beginnings of studies in characterization of the agent have been made and should be expanded. It would seem that the neurologist could contribute much in studies of the prodromal period as well as the syndrome through mapping efforts, electroencephalography, and studies of interference with neurological pathways with reference to the seat of the lesions which cause manifestations of the disease.

**SUMMARY**

One hundred and one grade lambs were included in an effort to transmit scrapie by subcutaneous exposure in three groups inoculated with
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(a) heat treated material from the central nervous system of a scrapie affected sheep, (b) 03 Selas filtrate of CNS material, and (c) supernatant fluid of CNS material. Suitable controls were included in the group. The flock was maintained as a unit. The first case of scrapie was observed at 37 months post-inoculation and a total of four had occurred at 40 months without further cases by the end of the experiment at 48 months. All were in group (c) above.

Histopathological studies performed on brains of four affected sheep revealed variable evidence of neuronal degeneration and hypertrophy of astrocytes at several brain stem levels. Changes were most pronounced in nuclei of the thalamus. In general, the evidence of central nervous system damage was not commensurate with the severity of symptoms seen.

Cell cultures from one of the affected animals were prepared and showed a markedly higher index of multinucleation than did comparable cultures from an apparently normal sheep. Similar results occurred when cultures from an apparently normal sheep were exposed to fluids from cultures of the scrapie affected sheep. However, this finding yet requires substantiation of its significance.

REFERENCES

REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SHEEP AND GOATS


Your Committee has carefully reviewed reports of the progress of scrapie eradication in this country. The Committee has also carefully reviewed the following:

3. Report of the lecture by Dr. Allen G. Dickinson, Moredun Institute, Edinburg, Scotland, presented at a seminar held at the University of Wisconsin, April 13, 1962.
4. H. R. 11993 introduced June 5, 1962, to provide full indemnity for sheep slaughtered for the eradication of scrapie—not only for sheep to be slaughtered, but for those previously slaughtered. Note: The first scrapie infected flock was disposed of in California ten years ago.

The Committee believes that there is strong evidence that scrapie (1) was transmitted to sheep by contact, and (2) was transmitted to goats by contact with infected sheep, and that there is experimental and epidemiological evidence to indicate that scrapie is an infectious disease.

The Committee has also considered that the scrapie study group designated by the United States Department of Agriculture, which convened in Washington, D.C., March 23, 1962, was of the opinion that this country should continue the national scrapie eradication program as it is now being conducted until additional information is available.

Your Committee has also canvassed all of the State Regulatory Officials in the United States to determine whether they felt the present program was successful, whether indemnities were adequate, and whether any change should be made in the program. With very few exceptions the response was that the program was successful, indemnities were adequate, and that no change should be made in the present program. There was some indication that indemnities in some states are not adequate and are very likely detrimental to the program.
In view of the above findings and the apparent success of the eradication program in this country, your Committee recommends that no changes be made in the national scrapie eradication program. We further recommend that the problem be given continuous study during the ensuing year and be re-evaluated just prior to the next meeting of the Association.

Those states not providing indemnity for scrapie eradication or that are only providing inadequate indemnity are urged to take the necessary steps to provide for adequate indemnity for scrapie eradication. The Committee again requests that the responsible officials in the Agricultural Research Service of the United States Department of Agriculture continually evaluate both research findings and progress of the program in this country, Canada, Britain, and elsewhere and make recommendations as they deem appropriate in light of additional knowledge; further that they keep the Committee informed of new pertinent information as it becomes available.

**SCABIES ERADICATION** - Scabies in cattle and sheep not only continues to exist in the United States, but is on the increase. Technical knowledge necessary for its eradication has been available for many years. It seems inconceivable that a modern nation which prides itself in a high order of technical and sanitary competency in the control and eradication of animal diseases would tolerate this costly disease which can be eradicated.

We therefore recommend that the Secretary of Agriculture request, and the Congress appropriate, the sum of $1,450,000 for scabies eradication in cattle and sheep.

**RESPIRATORY DISEASES OF SHEEP** - Over the past several years, respiratory diseases of sheep, and particularly pneumonia in lambs being fattened for market, have been recognized as a major source of economic loss to the sheep industry. Large lamb producing areas, such as California, western Oregon, and some midwest states have for years been plagued with respiratory diseases. Considerable research regarding the etiology of some specific pneumonias has been accomplished; however, there remain many large gaps in our knowledge of transmission and control.

Because of its economic impact and because the respiratory disease complex of sheep will require markedly accelerated research before adequate control can be accomplished, this Committee recommends that Federal monies be made available for appropriate regional research projects on respiratory diseases of sheep, also that the states engage in active research on these diseases.

The remainder of the Committee's report consists of situation reports from various authorities on transmissible diseases of sheep that are of special concern.

**VACCINATION FOR JOHNE'S DISEASE**
*(A Situation Report)*

The Agricultural Research Service, United States Department of Agriculture, has an experiment under way to determine the efficacy of a vaccine against Johne's disease in sheep. When this experiment is completed, we will have information on 50 vaccinates and 50 controls which were all exposed to Johne's disease on a ranch in Montana and kept under observation for 5 or 6 years.
The vaccine is similar to the one used by Dr. D. Sigurdsson Iceland, except that it was modified to reduce the size of the local lesion which develops at the inoculation site, and it was administered to the lambs when they were only a few weeks of age. Part of the lambs received a second dose of vaccine when they were about six months of age.

This experiment was initiated in 1957, and it is planned to complete it next year. When it is completed, a report of the results will be made. (Report submitted by Aubrey B. Larsen, D.V.M., National Animal Disease Laboratory, Agricultural Research Service, United States Department of Agriculture, Ames Iowa.)

VIBRIONIC ABORTION
(A Situation Report)

Research on ovine vibrionic abortion in the last two years has produced the following information.

Vaccination studies have shown a difference in sera types of the various strains of vibrio fetus found in the field. Hence, vaccines made from one sera type are not giving adequate protection from a different sera type. Various workers are still gathering information which would indicate that the use of a vaccine in the face of an outbreak will stop subsequent abortions in approximately ten days. As yet this is an assumption only, as no controlled experimentation has been accomplished.

Reservoir of infection studies continue to point an accusing finger at the gall bladder as an organ where the vibrio fetus may retain its pathogenicity for long periods of time. Recently bile isolates have been shown to be vibrio fetus and also have demonstrated an ability to produce abortion in pregnant ewes. Additionally in unrelated work a vibrio fetus organism was isolated from the gall bladder of a ram. To date no significant pathogenicity studies of this isolate for pregnant ewes has been accomplished.

Possible reservoirs of infection other than sheep have always posed a problem in research on this disease. Within the next two years an increasing emphasis will be placed on this aspect by many stations engaged in research on this disease.

There is a continuous effort to identify and isolate nutrients or micro-nutrients which will enhance the growth of vibrio fetus. In addition, media which will contribute to the stability of this organism grown in vitro are constantly being sought.

Results obtained from physiological studies indicate that a given vibrio fetus may have progeny with different physiological properties when subsequently passed in ewes. Physiologically different strains were isolated from aborted lambs in the same herd.

Efforts to identify vibrio fetuses in infected tissues with fluorescent antibody techniques have so far been unsuccessful. However, efforts will continue to utilize this procedure.

As a result of the regional attack on the problem of vibriosis in sheep the following items of knowledge of the disease appear to have been established.
1. A multivalent vaccine is necessary to protect against outbreaks of vibriosis.
2. Vibrio-fetus-like organism cultured from naturally infected gall bladders produce vibriosis. Therefore, this organ may serve as a reservoir of infection.
3. Preliminary studies suggest that the fluorescent antibody technique may be useful for serotyping *vibrio fetus* isolates and diagnosing vibriosis.

(Report submitted by Blaine McGowan, Jr., D.V.M., Associate Professor of Veterinary Medicine, School of Veterinary Medicine, University of California, Davis, California.)

**ARTIFICIAL INSEMINATION, EWES, 1961**

*(A Situation Report)*

*Kern County Project* - One thousand ewes were inseminated between July 10, 1961 and August 15, 1961 by a practicing veterinarian with semen both fresh and frozen, flown from Utah.

Conception rate was probably below 50 percent.

*Senoma County Project* - One thousand ewes were inseminated between June 15 and July 25, 1961. Due to delay in the appearance of estrus in the ewes, a progesterone product was fed for 18 days to hold up ovulation. When the product was discontinued, the ewes developed estrus in large numbers in three to five days. Subsequent introduction of "Marker Rams" indicated that a conception rate of less than 50 percent had been obtained with artificial insemination.

There are no plans currently for the use of artificial insemination in ewes in 1962. (Report submitted by Dr. Carl Bills, Staff Veterinarian, Bureau of Animal Health, Division of Animal Industry, California Department of Agriculture, Sacramento.)

**BLUETONGUE**

*(A Situation Report)*

The incidence of Bluetongue in Sheep in the United States during 1961 was reported from six states, namely: California (2), Colorado (2), Idaho (3), Oklahoma (1), Utah (3) and Washington (2). Bluetongue virus was recovered from the blood in 10 of the 13 samples submitted for diagnosis. The Oklahoma and one Washington sample was negative while one of the Idaho samples is as yet undiagnosed.

Again, it is logical to assume on the basis of the history and the number of states reporting the disease that many unreported cases occur.

The virus was again isolated from a bovine. In this case it was obtained from a one year old male that was emaciated and had an ulcerative mouth condition. The animal was part of a herd of 20 cattle and located in Utah where there has been no reported cases of Bluetongue. (Report submitted by John G. Bowne, D.V.M., Acting Director, Animal Disease Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Denver, Colorado.)
In the 1961 report of the Committee on Transmissible Diseases of Sheep and Goats there was a report of a national foot-rot conference held in Lexington, Kentucky, in May, 1961. Following that meeting a committee of 20 men was set up to stimulate action toward controlling this serious hazard to the sheep industry. That committee included veterinarians, sheep producers, market operators, extension specialists and representatives of wool growers associations, from various sections of the country.

This Committee is not yet in a position to report much general progress. However, in several states activity on the foot-rot project is in progress. The most outstanding action which has been brought to our attention is the program of education and demonstration which is under way in Ohio, under the leadership of Sheep Health Committee of the Ohio Sheep Improvement Association. The Ohio "Task Force" on sheep foot health includes representatives of the State Extension Service, the state and federal regulatory organizations, the State Experiment Station, three producers associations, the Livestock Marketing Association and the Sheep Shearers Association.

Research work on the further clarification of the bacteriology and diagnosis of the disease is in progress in Australia and to a limited extent in this country. (Submitted by Hadleigh Marsh, D.V.M., Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, Montana.)

REPORT OF PROGRESS AND RESEARCH ON SCRAPIE

During fiscal year 1962 there were 13 scrapie-infected flocks reported in five States. The infected flocks, source flocks, exposed sheep, and their immediate progeny slaughtered as a result of these outbreaks involved the payment of Federal indemnity on 315 claims for 6,676 sheep slaughtered from 15 States. Indemnities totaled $341,195 Federal and $74,085 State indemnities. There are presently some 1,236 flocks of approximately 565,000 sheep in 37 States under surveillance for scrapie. This is a decrease from last year when 1,334 flocks were under surveillance in 41 States.

Idaho. Idaho's third, fourth, and fifth outbreaks involved Suffolk sheep and were reported in fiscal year 1962. Two of the infected flocks were in Bear Lake County. Infected flock No. 3 included 164 sheep of which nine were showing signs of scrapie. The outbreak was reported by the owner and apparently goes back to imported sheep purchased several years earlier. The second infected flock (flock No. 4), of 14 sheep, was found when a regulatory inspector observed that exposed ewe No. 370 from the infected flock mentioned above was showing symptoms of the disease. The most recent outbreak (infected flock No. 5) was reported in Bonneville County by a veterinary practitioner. Some ten cases appeared in a flock of 1,733. The owner had seen the scrapie film and recognized the disease. It was not possible to pinpoint the source of the outbreak. Officials concerned
endeavored to slaughter all exposed sheep moved from the infected flocks and their immediate progeny.

**Illinois.** Illinois reported six infected flocks during the year bringing their total to 24—more than any other State. Infected flock No. 19 was reported by the owner. A previous flock of the same owner had been found to have scrapie in 1956 and was slaughtered in August of that year. Infected Suffolk ewe No. 162, found during fiscal year 1962, was the immediate progeny of a ewe purchased from an infected flock in Daviess County, Missouri, but had not been previously identified as such. The Illinois flock of 40 sheep was slaughtered in September 1961. Infected ewe No. 162 was by the same ram as the infected ram in flock No. 20. Infected Suffolk ram No. 28 in flock No. 20 was reported by a veterinary practitioner. The flock of 30 was slaughtered in September 1961. The flock from which the infected ewe in flock No. 19 and the infected ram in flock No. 20 were bred was considered a source flock and also slaughtered. The owner of a Hancock County flock (flock No. 21) notified State officials of the suspicious signs in his Suffolk ram No. 414. Following laboratory confirmation of scrapie, the flock of six sheep was slaughtered in September 1961. The infected ram's dam was also the dam of an infected ewe found previously to have scrapie in another Hancock County flock which had been slaughtered in January 1960. Regulatory officials had endeavored to slaughter all exposed sheep sold from the infected flock but were not aware of the existence of infected ram No. 414. Suffolk ram No. 59-216 was found to have scrapie in infected flock No. 22 on a routine scrapie surveillance inspection. The flock of 82 sheep was slaughtered in November and December 1961. Infected ram No. 59-216 had been purchased from a Livingston County flock found infected in January 1961 but had left the latter flock prior to the exposure date and thus had not been considered subject to slaughter. Infected flock No. 23 in Vermillion County is the sixth infected Cheviot flock reported in this country. Infected Cheviot ram No. 1116 in flock No. 23, as well as two infected Cheviot sheep found previously—infected ewe No. 1077 in flock No. 17 in Illinois, and infected ram No. 1098 in flock No. 4 in Oregon—had been bred in and purchased from a St. Clair County, Illinois flock. Three earlier outbreaks in Cheviots had occurred in Ohio. The St. Clair County flock was considered a source flock and also slaughtered. Suffolk ewe No. 4 was found to be showing signs of scrapie in infected flock No. 24 by inspectors conducting farm-to-farm sheep scabies inspections. The 38-sheep flock was slaughtered in February 1962. The dam of infected ewe No. 4 was purchased from a Sangamon County source flock in August 1953. This source flock had been slaughtered in June 1957 and ewe No. 4 should have been slaughtered as the immediate progeny of an exposed sheep; however, her existence was not known by regulatory officials. Regulatory officials in Illinois and other States concerned endeavored to slaughter all infected and source flocks and exposed sheep moved from them and their immediate progeny.

**Texas.** The second outbreak of scrapie reported in Texas was brought to light in a flock of 50 sheep in Kerr County by a veterinary practitioner. Infected Suffolk ewe No. 606 was 54 months of age. She had been bred in Virginia, born in January 1958, sold in July 1958 into a Grayson County,
Texas flock, sold in June 1959 into a Kerr County, Texas flock and sold in September 1961 into the flock where she was showing symptoms of scrapie eight months later. Both Kerr County flocks were slaughtered as were exposed sheep sold and their immediate progeny.

**Virginia.** Virginia experienced her third and fourth outbreaks of scrapie during the fiscal year. The third outbreak was disclosed when a Wythe County flock owner took an infected Suffolk ram to a State Regional Diagnostic Laboratory. The infected flock of 26 sheep was slaughtered in September 1961. There had been no movements of exposed sheep from the flock. The origin of the outbreak may have been the same Augusta County flock mentioned below as having been slaughtered in July 1961. The fourth outbreak was discovered when the owner of an Augusta County flock suspected two of his Suffolk rams had the disease. Regulatory and laboratory investigations confirmed his suspicion and the flock of 314 sheep was slaughtered in November 1961. The source of the outbreak is believed to be an infected Augusta County flock slaughtered in July 1961.

**Wisconsin.** Wisconsin's third outbreak was disclosed when the owner of a Rock County flock contacted a veterinary practitioner who advised him to take his Suffolk ewe No. CR80 to a State Diagnostic Laboratory. The infected flock of 26 sheep was slaughtered in December 1961. The source of the outbreak could not be definitely determined. There were no exposed sheep to be slaughtered.

As of September 1962, 116 infected sheep flocks had been reported in 92 counties in the following 26 States: Alabama (two), California (eight), Connecticut (two), Georgia (one), Illinois (26), Indiana (23), Iowa (one), Kentucky (one), Maryland (two), Michigan (two), Mississippi (one), Missouri (three), New York (three), North Carolina (two), Ohio (12), Oregon (four), Tennessee (two), Texas (two), Utah (one), Virginia (four), West Virginia (one), Wisconsin (three), Wyoming (two), Pennsylvania (two), South Dakota (one), and Idaho (five). The first report of scrapie was from Michigan in 1947. No additional outbreaks were reported until fiscal year 1953 when 10 were disclosed. There were three in 1954, 11 in 1955, 23 in 1956, 12 in 1957, seven in 1958, 11 in 1959, 13 in 1960, nine in 1961, 13 in 1962, and three to date in 1963.

**Scrapie Research Developments (A Progress Report)**

Last year your Committee's report included a summary of the considerable work and progress being made in the field of scrapie research. Attention was invited to the following aspects of research findings reported: P. L. 480 Grants, by the United States Department of Agriculture for Expanding Scrapie Research in England and Scotland; Transmission to Mice; Work on Diagnostic Tests; Work with Goats at Compton; Scrapie Agent Possibly Becoming Altered or Adapted by Passage Through Goats; Transmission by Mouth Demonstrated at Compton; Age of Scrapie Manifestations; Additional Histopathological Studies; Heat Resistance of Scrapie; and Additional Attempts to Characterize the Scrapie Agent. Since this report, active research has continued and work is being done at several locations including some in this country. The following is a brief summary of recent research activities:
Further Research Information that Scrapie can Spread by Contact

Dr. John T. Stamp, Director, Animal Disease Research Association, Moredun Institute, Edinburgh, Scotland, recently furnished us with the following information: Further results are not available in regard to contact exposure experiments at the Moredun Institute. Six goats, one-day old, were placed in contact with scrapie sheep. The exposure was continued by putting additional scrapie sheep in beside the goats following death of the scrapie sheep and as the goats grew older. One of the goats died when it was about 2 1/2 years old having shown no signs of scrapie. Of the remaining five, all developed the disease. The goats began showing signs of scrapie at about 40 months of age. Dr. Stamp felt this indicates that contact infection does occur in scrapie and adds considerable weight to the results of other experiments at Moredun. At the Institute they now have little doubt that contact infection does occur. These results add further information to other work in progress at Moredun in which "clean" sheep purchased from each of five farms developed scrapie (either in the contact experiments or in the clean flock at the Institute) while the sheep remaining on the five farms have not.

The isolation of the "clean" flock was not complete at the Institute. However, in this flock, there has been a very definite age shift in the onset of scrapie which is occurring in older sheep than one would expect. This might be explained due to delayed contact—contact occurring for the first time when the "clean" sheep were brought to the Institute.

Two uninoculated control goats, housed together with inoculated animals, developed scrapie.

Dr. P. J. G. Plummer, Director of the Canada Department of Agriculture, Production and Marketing Branch, Health of Animals Division, Animals Pathology Laboratories, notes that four of 44 sheep inoculated with normal brain material developed symptoms closely resembling those of scrapie. All animals including these 44 were housed in the same building. He suggests that one considers the possibility that the disease spread from the scrapie infected animals to the control animals by contact or that the brains from apparently normal animals carried the agent in a masked form. Further study in this area will be required.

Failure to demonstrate contact spread of scrapie in earlier experiments may have been because the animals used were not susceptible or because the test animals were disposed of too soon for symptoms of scrapie to appear.

Uninoculated Offspring of Inoculated Animals Develop Scrapie

During the course of experiments conducted during the period 1956-58 Gordon observed scrapie in nine uninoculated lambs, the immediate progeny of inoculated ewes and rams. Clinical signs were seen in the "lambs" when they were seven to 18 months old. Seven of the nine lambs were out of dams that had developed scrapie—the dams of the other two lambs had not. In 1962 Stamp noted similarly that uninoculated mice whose mothers had been inoculated developed the disease. In both instances the uninoculated sheep and mice had been maintained in an infected environment.
Research Scientists and Regulatory Officials Attend Lectures Given by Scrapie Research Workers

In March 1962 the Animal Disease Eradication Division sponsored a Washington meeting of research scientists and regulatory officials to hear Dr. H. B. Parry discuss scrapie. Dr. Parry is a veterinarian affiliated with the Nuffield Institute for Medical Research, Oxford University; Oxford, England. Dr. Parry's trip to the United States was arranged by the National Foundation for Neuromuscular Diseases. His work is, in part, supported by a grant from that foundation. Individuals hearing Dr. Parry and participating in the discussions represented many special fields of virology and genetics including animal, plant, and poultry geneticists and virologists of the Department's Animal Husbandry and Crops Research Divisions; virologists, pathologists, and research workers of USDA's Animal Disease and Parasite Research Division, the Armed Forces Institute of Pathology, Walter Reed Army Institute of Research, National Institute of Health, and of other agencies. Participants also included the Scrapie Study Group, assigned in 1958 to review the problem of scrapie in its entirety.

Dr. Parry presented his views which are as follows: Scrapie is a wasting disease of sheep, with rubbing and ataxia fatal in six weeks to one year; it has been known since 1720. Data are presented on 1200 affected sheep among a recorded population of 20,000. The disease may appear at any time after 1 1/4 years of age up to 11 years, with a mean of 3 1/4 years. It occurs with variable incidence in many improved breeds of sheep. Males and females are affected equally. Seventy percent of a female birth-group may be affected but the attack-rate is more usually five to 10 percent. Ninety percent of all animals that will be affected manifest the disease by 4 1/2 years old. This age has therefore been taken as a level for assessing the performance of all animals. The disease is due to a single autosomal recessive gene ss. The genetic disease is artificially infectious by inoculation, but never naturally. The scrapie gene is closely linked to certain preferred breed characteristics selected to show animals, so that ss and Ss sheep are at a selective advantage. Scrapie is thus an example of balanced polymorphism, unconsciously favoured by current methods of selection, which allow the frequency of the gene to reach very high levels. Scrapie arises by a dual mechanism of gene and provirus. The scrapie provirus is gene-determined but it lacks certain properties of a natural virus. Scrapie falls into a special category of genetic disorder, in which the physiological action of the gene is mediated by a specific particle, with independent powers of self-replication and pathogenicity.

Dr. Parry indicated that losses are such that scrapie is a problem that one must do something about and that the disease can't be allowed to go unchecked. The flock incidence, i.e., the number affected per 100 breeding females per annum, varies widely from flock to flock, even of the same breed, and from year to year. Many flocks may be considered as free, i.e., no manifest cases for at least five years; in many the incidence is low (one to three percent) or sporadic (one percent), in some it is medium (four to 10 percent) and in a few flocks it is high (10 percent), and may exceed 20 percent. In a particular flock 75 percent of a lamb crop
eventually died of scrapie. It can be, and in some areas has been, a decimating disease forcing disposal of the remaining sheep in the flock.

Dr. Allen G. Dickinson, who works with both Moredun Institute, Edinburgh, Scotland, and the Agricultural Research Council Field Station, Compton, England, addressed a seminar at the University of Wisconsin and in Washington, D.C., in April 1962, on scrapie in sheep. Dr. Dickinson believes the causative agent is a transmissible, filterable virus. Twenty-two successive passages have successfully been made through sheep with the agent retaining its ability to reproduce the disease. The disease has been reproduced from almost all body tissues, except serum and skin, when injected into susceptible animals. Many routes of injection can be used to reproduce the condition. However, intracranially has been adopted as the routine method due to a shortened incubation period. The causative agent is not killed by chemical means or boiling, but can be killed by autoclaving. It will pass through a 650-210 μ filter but will be filtered out by 120-27 μ filters. Sheep originating in previously free flocks when exposed to infected animals will become infected at about 4 1/2 years on up. Sheep which are injected with the infective agent will show clinical scrapie starting at four months following injection to about eight months, reaching a peak at about six months. Lambs born to ewes which were injected with the infective agent within 30 days of conception will exhibit scrapie in nine to 12 months after birth. Natural scrapie has never shown up prior to 18 months and usually not before 2 1/2 years of age. Dr. Dickinson points out that these experiments indicate maternal transmission must be seriously considered.

To summarize, Dr. Dickinson believes that scrapie is a transmissible viral disease of sheep. The causative agent is present in almost all body tissue of an infected animal. It can be transmitted by contact as well as through infected parents. Clinical signs can be confirmed by histopathological examination of brain tissue for vacuolated neurons. Dr. Dickinson also stated that the USDA program for eradication of scrapie through the slaughter of sheep was warranted, and if it had not been followed, the disease would be much more widespread in this country today.

Transmission to Mice Represents Important Breakthrough in Scrapie Research

In June 1962 in Washington, D.C., Dr. Richard L. Chandler addressed a group of Animal Disease Eradication Division Veterinarians in Charge, Teachers of Infectious Diseases from various veterinary colleges, Washington, D.C. Animal Disease Eradication Division and Animal Inspection and Quarantine Division personnel, and others on the subject of scrapie. Dr. Chandler, Principal Scientific Officer, Agricultural Research Council, has been working on various livestock diseases with Dr. W.S. Gordon et al. at the Agricultural Research Council Field Station at Compton, England over the past four years. For about one year he has devoted his research efforts toward propagation of the scrapie agent in laboratory mice. He has also had research experience in Africa and New Zealand. He had been in this country about three months conferring with various research workers including those engaged in transmission of scrapie to mice and indicated he was encouraged by the work he had seen. Dr. Chandler said he planned
to greatly enlarge the project upon his return to England. Dr. Chandler has successfully produced scrapie in laboratory mice and has reported the earlier aspects of this work in *The Lancet* (June 24, 1961, and January 13, 1962). He indicated he had infected mice by inoculating them with brain, spleen, kidney, and liver material from infected mice by various routes of inoculation and by oral administration. He showed several two by two inch slides depicting scrapie lesions in histological sections of brain from infected mice and stated that these lesions were identical with changes noted in similar material from sheep and goats. In addition to the cases in Swiss mice which occurred 7 1/2 to 10 months after inoculation; cases occurred later in the C.B.A. mice nine to 15 months after inoculation; and in the C.57 mice, 12 to 15 months after inoculation. The differences in susceptibility of the three breeds of mice were therefore not absolute, but related to varying incubation periods.

An experiment, repeating the original work, has been made using Swiss mice, inoculated intracerebrally with both "drowsy" and "scratching" types of scrapie brain material obtained from other goats and controlled with normal goat brain material. The encephalopathy syndrome again occurred, commencing six months after inoculation, in the mice inoculated with the "drowsy" type of scrapie. In a similar experiment, but using the intraperitoneal route of inoculation, the encephalopathy syndrome occurred, commencing five months after inoculation, in mice inoculated with the "drowsy" type of scrapie.

Other general points of interest Dr. Chandler mentioned included: All scientific evidence to date indicates the causative agent to be a virus; variations in the genetic susceptibility of the host apparently occur; transmission of scrapie by direct contact and from exposure to infected premises is a reality; indirect transmission is probably by ingestion of the virus; the transmissible agent multiplies during serial passages; it is most unlikely that scrapie is purely a genetic disease; scrapie can be produced in animals such as mice completely unrelated genetically to sheep and goats; the ewe is probably more important than the ram in transmission.

Transmission of the condition from mouse to mouse, by intracerebral inoculation, has been achieved. Two consecutive passages had been made in Swiss mice, the incubation period for the majority of the mice being four months, but in some cases three months. The histopathology of the brain was similar in character to that previously described but the vacuolation was of even greater magnitude and distribution; the spinal cords showed similar changes. Thus, there was evidence of adaptation of the agent in mice. The evidence indicated that, in mouse-to-mouse passage, the infection rate was 100 percent. The mouse encephalopathy syndrome has occurred in mice inoculated with heated scrapie goat brain material or with heated mouse encephalopathy brain material which had been immersed in sealed ampoules in boiling water for 30 minutes.

*Other Scrapie Researchers Confirming Dr. Chandler's Work*

Informal reports from Dr. J. T. Stamp, Director, Moredun Institute, Edinburgh, Scotland; Dr. W. S. Gordon, Director, Agricultural Research Council Field Station, Compton, Berkshire, England; Dr. A. J. Morris,
Chief, Section on Respiratory Viruses, Division of Biologics Standards, Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, Maryland; and Dr. W. J. Hadlow, Veterinary Pathologist, Department of Health, Education, and Welfare, Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana; relate additional interesting and useful preliminary information. These individuals plan to publish results of their mouse work.

In summary, following Dr. Chandler's findings, these workers successfully inoculated mice with scrapie-infected goat and sheep tissues. Several isolates are being passaged in various strains of mice. Sub-passages have been effected. Sheep and goats have been inoculated with the first passage mouse brain. To date only the goats have developed the disease. At one location in the United States the incubation period was approximately three months with two strains of mice and approximately eight months with three other strains. Some incubation periods have been longer.

Plans are underway for experiments in which female mice and male mice are inoculated and then bred. An elaborate procedure will be followed in which Caesarian sections will be performed on certain females for the purpose of placing some progeny of inoculated females in an uninfected environment to suckle normal females, and to allow progeny of normal females to suckle inoculated mothers. Further procedures will also be undertaken to learn if the progeny may acquire the disease either before or after birth. Additional phases of study include the possible role of the inoculated male.

Further recent work with mice includes that of Dr. D. P. Gustafson, Professor, Purdue University, School of Veterinary Science and Medicine, Department of Veterinary Microbiology, Pathology, and Public Health, Lafayette, Indiana. Dr. Gustafson inoculated four strains of mice with scrapie-sheep brain. The sheep was one of four that developed the disease at Purdue following subcutaneous inoculation from previous field cases of scrapie in Indiana sheep.

Other workers who plan to study mouse-adapted scrapie virus includes Dr. Hilary Koprowski, Director, The Wistar Institute, Philadelphia, Pennsylvania.

Earlier attempts had been made at Moredun Institute in Scotland to use small animals for experimental study. In 1953, groups of sheep were inoculated with brain material from mice, guinea pigs, or rabbits which had been inoculated with scrapie infective material 4 1/2 to 10 1/2 months previously. The laboratory animals had shown no symptoms of disease; however, one sheep which received mouse brain as inoculum developed scrapie after an incubation period of 17 months and three sheep which received guinea-pig brain developed scrapie after incubation periods of 6 1/4, 7 1/4, and 11 1/2 months respectively.

Research Involving Use of Different Tissues and Routes of Inoculation

Research workers in Canada, England, France, Iceland, Scotland and the United States have shown that scrapie can be produced in animals by inoculation with brain tissues (and by inoculation of frontal cortex or brain
stem), cerebrospinal fluid, pituitary body, lumbar spinal cord, sciatic nerve, muscle, adrenal gland, spleen, salivary gland, pancreas, and liver from affected animals. They have also reported transmission by the following routes: intracerebral, epidural, intravenous, intraocular, intradermal, subcutaneous, by applying the inoculum to the scarified skin and most recently, by feeding the animal brain tissue from affected animals.

Significance of Recent Research Findings

The results coming from the mouse work and additional information from experiments involving sheep and goats is in accord with the view that the scrapie agent is a virus and perhaps less mysterious than previously thought. The evidence is also becoming more conclusive that scrapie can be contagious. Breeding experiments to date do not support the hypothesis that scrapie is due to a simple autosomal recessive gene. In these experiments, maternal transmission seems to play a major part although there is now some experimental evidence that the ram can also transmit the disease on occasion. It is more likely that the heritable factor is one of resistance and susceptibility similar to the phenomenon observed in the resistance of nearly all species to most infectious diseases.

(Report submitted by J. L. Hourrigan, D.V.M., Chief Staff Officer, Special Diseases Eradication Section, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Washington, D.C.)

OVINE MASTITIS AND OVINE ABORTION

MASTITIS OF EWES

Mastitis or blue-bag, is an abnormal condition of the mammary tissue, usually infectious in nature and ordinarily affecting but one side of the udder. It is distinct from the usual forms of udder inflammations which occur during and immediately after lambing. Losses from mastitis in the western range states are of economic importance affecting approximately four percent of the ewes. The lamb loss resulting from stunting and buming is also heavy.

Occasionally a non-infectious mastitis develops that is purely physiological in nature, with no demonstrable bacteria being involved, and requiring no treatment. But in general there are two types of infectious mastitis. One of them develops before the ewes are moved from their lambing grounds. The other, more common type caused by Pasteurella mastitidis is found when the sheep are already on dry fresh grazing grounds and lambs are several months old.

The bulk of the early occurring mastitis cases is caused by infection with a variety of filth bacteria such as those found in unsanitary surroundings.

CAUSE

Investigation by the Montana Veterinary Research Laboratory showed that a very large percentage of the later occurring types of mastitis developed as a result of infection with a specific organism. It is difficult to understand why this disease should appear in ewes on presumably clean range after the lambs are three or four months old. This particular mastitis occurs quite generally in the western states.
Transmission of the infection would seem to take place by contact of the end of the teat with infection of the bedground. However, we know that some normal udders carry the organism from year to year. However, the abnormal or injured udder would seem more prone to infection. Heavy lambs are rough with the ewes when nursing, sometimes raising them off the ground. Bruising of the gland may easily take place, allowing invasion of the pasteurella organism. Nodules or cysts of any size, consistency, or location in the udder are potentially dangerous, for some of them harbor the organism, eventually breaking down to produce mastitis. Even the normal appearing and feeling udder may harbor the organism for an entire season without producing the disease, but may act as a source of infection for other ewes.

SYMPTOMS

The first noticeable symptoms are those of loss of appetite and dejection, the ewe standing off by herself, dropping behind while being moved or trailed, and not allowing her lamb to nurse. The important early symptom is lameness in one hind leg. She swings the hind leg of the affected side outward to avoid contact with the painful side of the udder. During the first day, the udder is painful and hot without too much enlargement, no discoloration, and the milk is normal.

After the first day the gland enlarges, begins to harden and the secretion changes through the successive stages of whey separation, casein coagulation and finally a caking of the udder in which there is no secretion. This process usually takes place in four to six days. The udder may or may not become bluish, which is responsible for the term "blue-bag." This is a misleading term for this discoloration occurs in a minority of cases. The infection is practically always on one side. The unaffected side and milk are normal unless the animal remains extremely ill for some time, then milk secretion ceases. The process terminates in a hard, enlarged udder or an abscessed one. In either case the ewe's economic reproductive life is ended. She should be marked for culling.

TREATMENT

Treatment has proven of value when given at the first sign of the disease. Several antibiotics have proved effective but sulfamethazine is the best treatment, being both economical and efficient. It is administered in a 15 gram bolus by the mouth with a repeat in 18 hours. If treatment is started early, experience has proven that at least 50 percent of affected udders can be restored to normal milk production. Without treatment, no udders are saved and 20-25 percent of affected ewes die. Regardless of recovery, the ewe should be culled in the fall. None of the vaccines now available are of any value whatsoever in prevention of mastitis.

CONTROL

Control of the disease is furthered by isolating the affected ewes to avoid spreading the infection from the discharging teats and udders. Avoid
milking the affected udders on ground or bedding that is accessible to other lactating ewes. Wash the hands after handling these mastitis cases.

Vigorous culling of all ewes with visibly abnormal udders including the nodular or cystic types should be followed. This repeated annual culling will reduce the cases but will never entirely eliminate the disease.

ABORTION OF SHEEP

NON-INFECTIONOUS ABORTION

Although the heaviest abortion losses in sheep are due to infection with microorganisms, losses may be due to other causes such as physical injury to the ewe or the ingestion of injurious substances in the feed.

*Sweetclover* poisoning, if severe, may result in the death of ewes due to disturbance of the blood-clotting mechanism. If the degree of poisoning is less severe the ewes may not show visible symptoms, but full-term lambs may be born dead which are "bled out." This is not a true abortion in which the lambs are expelled prior to full-term. Dicumarol, which prevents the blood from clotting, is most common in sweetclover that is slightly spoiled or moldy. We have no evidence to indicate that other types of moldy feed cause abortions.

*Nitrate poisoning* has caused abortions in cattle and there is a possibility that it can cause the same problem in sheep. This condition is commonly known as "oat hay poisoning" and may result from the ingestion of oat hay, Russian-thistle, or nitrate fertilizer. The nitrate itself is not poisonous but it may be converted to the poisonous nitrite form in hay that has been subjected to considerable wetting. There is also a possibility that animals may be able to convert the nitrate to the poisonous form in the digestive tract.

INFECTIOUS ABORTION

*Listeriosis*. This disease is commonly known as "circling disease" because it usually infects the brain and infected animals may walk in circles or push against fences or each other. This form of the disease is usually fatal and has occurred in both sheep and cattle in Montana. The organism that causes this condition has also caused abortions in cattle in the state, apparently without infecting the brain. So far we have not seen abortions in sheep due to this organism but we suspect that they take place.

*Paratyphoid abortion*. This type of abortion is caused by bacteria which belong to the group known as Salmonella. This is not a common cause of abortion but the condition has been diagnosed in the state a number of times. The method in which the disease is spread is not definitely known, but contamination of feed and water is a good possibility.

*Brucellosis*. Although brucellosis of goats is known to exist in Texas, New Mexico, Arizona and Colorado, the disease has only been reported once in sheep in the United States.

*Tularemia*. This disease, as a cause of death in adult sheep, has been recognized for many years in Idaho, Montana and Alberta. In a few
instances it has caused abortion of sheep in Montana with little death loss in the ewes, probably due to a difference in immunity between the ewe and the lamb developing in her body.

**Vibriosis.** The most important cause of infectious abortion of sheep in the United States is due to infection with *Vibrio fetus*. Abortions usually start some time during the last six weeks of pregnancy and may continue at a reduced rate after lambing has started. Weak or dead full-term lambs that are infected with the organism may be born after the ewes start lambing. The percentage of aborting ewes may range from five to 70 percent with an incidence of 10 to 20 percent being common. Death losses in ewes are usually very small.

Diagnosis of the disease can be made only by the examination of an aborted fetus or the fetal membranes in a laboratory. Any flock which has a higher than average number of stillborns during the last six weeks of pregnancy should be checked for vibriosis by examining the fetuses.

Once a diagnosis of vibriosis has been made in a flock, the only measures that can be applied to help decrease additional losses are those of management and sanitation. Ewes which have aborted should be placed in a hospital flock so that they will not contaminate the feed and water of ewes which have not aborted. Aborted ewes in the hospital flock which are seriously ill may be treated by a veterinarian. Aborted fetuses and membranes should be picked up and burned. If possible the ewes yet to lamb should be held in an area where crowding is not excessive and which has a clean water supply. Areas of poor drainage where run-off or snow water collect are to be avoided as ewes frequently drink from such readily contaminated pools.

After lambing is completed the aborters may be returned to the main flock. As abortion losses seldom occur the year following an outbreak and as the act of abortion apparently confers some immunity, there is no apparent value in disposing of aborters.

Vaccination with a commercially available bacterin confers adequate immunity for at least one year if given prior to or immediately following breeding; provided, serostrain 1 furnishes the exposure infection. There are two serostrains that seem to be responsible for most of the field outbreaks of vibriosis. The vaccine at present is prepared from only the one serotype organism, i.e., serotype 1. As yet the bivalent vaccine has not been prepared and proven.

**Virus Abortion**

Virus abortion of ewes was first recognized in America in 1958. It soon became apparent that the disease was present in many flocks in a number of states where it had doubtless existed for some years unrecognized.

It is not yet apparent what loss is sustained by sheepmen in this country as a result of virus abortion. It appears that the disease is widespread, but in most infected flocks, it has been present for a number of years, and the abortion rate is very low, about five percent or less. In some infected flocks, however, in which the disease was recently introduced, the abortion
rate is 30 percent or more. Heavy losses have been sustained by owners of large flocks during the first few years following introduction of the disease.

Although many infected ewes deliver normal lambs at term, others expel diseased lambs. These are usually aborted one to three weeks before term, but may be expelled up to eight weeks before or at term. There is a great variation in the appearance of these lambs. Of those which are aborted a week or more before term, a few are merely non-viable, i.e., though born alive, they very soon die. The majority, however, are born dead—some quite fresh with no obvious abnormality, other fresh but "pot-bellied" because of an accumulation of blood-stained fluid in the abdominal cavity. Some have obviously been dead in the uterus for days or weeks as judged by the cloudiness of the cornea of the eye, the ease with which the wool can be pulled out, and the general softness of the tissues. Others are in a remarkable state of degeneration which is unmistakable when once seen. The fetus is about one-half to two-thirds of the size expected in the last month of gestation. The orbits of the eyes are empty and the rims prominent. The skin, tightly drawn over the body, is almost black and is covered, not with the white curling wool of the late fetus, but with a scanty coat of dark brown hair. There is no appreciable odor. Presumably some degree of mummification has occurred. Dead or weak lambs born at or just before full term are also common in infected flocks. The dead lambs are often fresh, but may show degeneration to a degree varying from corneal opacity at birth to such a generalized softening of the tissues that the limbs and head are easily pulled away from the body when traction is applied at any assisted lambing. The weak lambs born at or just before term are often "pot-bellied." They make little or no attempt to feed from their dams. Even when brought into a warm atmosphere and hand fed, they scarcely survive for more than a day or two. The birth of one normal lamb at full-term immediately preceded or followed by a recently dead or degenerating twin is one of the clinical features of the disease.

The infected placenta may appear normal, or the cotyledons may be more or less necrotic and clay-colored and the membrane, of a watery, jelly-like consistency. Retention of the placenta is a fairly common occurrence.

While there is no apparent affect on many of the infected ewes, the disease has a more or less unfavorable affect on the health of the ewes which abort. They may be in poorer condition than their flockmates a few days or even a week or two before abortion occurs. They often have dirty tails due to a considerable vaginal discharge for a day or two before aborting. After abortion, there is a varying amount of vaginal discharge, depending mainly on the degree of retention of the placenta. When this is expelled within a few hours, the amount of discharge is no greater than after a normal lambing. When the placenta is retained for two to three days as is very common, the subsequent discharge may continue for several days. Such ewes are more seriously affected than others. Also when the disease causes death of lambs at full-term, mortality among the ewes may be high. This is probably due to infection following the assisted delivery of the dead lamb. Mortality among aborting ewes rarely exceed 10
to 15 percent, i.e., less than one percent of the total number of ewes in the flock. In many flocks no deaths occur although there is an average number of abortions. Some ewes may die a few days after aborting, whereas others may "pine" for several weeks before death occurs. After aborting once, ewes generally do not abort a second time. Their fertility is not impaired by the disease.

Very large quantities of the virus are present in the diseased placenta and in the uterine discharge occurring for a few days after, and occasionally before, abortion. As the virus cannot survive for more than a few days outside the animal's body, it appears that the disease can be spread only during the period when ewes are aborting and lambing. Where it is the practice to keep the ewes under very close observation during the lambing season, the crowding together of the animals for this purpose is probably responsible for the perpetuation of the disease. Infected and uninfected animals alike are confined in succession in the same pens. If a ewe has aborted or had a dead lamb and is fit to take a foster lamb, she is placed in a pen in the same way as a healthy ewe. It is thus obvious that there is every opportunity for the disease to spread by indirect contact. In Scotland on the hill grazing, where lambing pens are never used, this type of abortion is unknown. Infection probably occurs by mouth. Although the infection does no apparent harm to the non-pregnant ewe, it remains in the body until the animal becomes pregnant again in the autumn of the same year.

Owing to the spread of infection in the lambing pens it might be expected that the disease would be more common in second lamb ewes than in first lamb ewes, and this is found to be the case. Although abortion does occur commonly in first lamb ewes, much of it is due to causes other than infection, giving a lower proportion of infection in this age group. Virus abortion in first lamb ewes is the result of infection acquired before or soon after birth, through a ewe lamb being born of and/or reared by an infected ewe, or at 12 months of age, due to the common practice of turning out the aborting ewes with the yearlings.

Infection is acquired in a previously uninfected flock either by the introduction of infected ewes or by their being introduced into an infected flock. The infection is spread to the uninfected animals at the first lambing season after purchase when the infected ones abort or lamb, but the originally uninfected ewes do not abort until the second lambing season and the percentage of abortions in this group is high.

There is no evidence to show that the disease can be transmitted by the ram.

The occurrence of inapparent infections makes it impracticable to eradicate the disease by changes in management alone, but the following measures may be recommended:

1. If an aborting ewe is not required to rear a foster lamb, keep her apart from other ewes, especially yearlings, for at least four weeks after abortion has occurred.

2. In a flock producing ewe lambs for future breeding, give an aborting ewe a male foster lamb rather than a female.
3. Set aside a corner of the lambing shed for ewes obviously infected, or use separate pens.

4. Burn, bury, or drop into a barrel of disinfectant all placentas, even from apparently normal ewes.

5. If it were possible to buy female replacements from a flock known to be free from abortion, this disease could be brought under control in a few seasons merely by lambing the uninfected yearlings away from the infected part of the flock until the infected ewes have been completely replaced. The division of the flock into infected and non-infected groups would be necessary only during and shortly before lambing time.

6. Losses among ewes may be reduced by cleanliness during assisted deliveries of dead lambs.

7. The disease is successfully controlled in Europe by vaccination. However, commercially prepared virus abortion vaccine is as yet unavailable in this country due to the lack of demand.

Submitted by

Dr. E. A. Tunnicliff, Head Department of Veterinary Science and Veterinary Research Laboratory, Montana State College
Many procedures and techniques have been employed in the United States to promote disease control and eradication. Slaughter or test and slaughter have been among the measures taken to eliminate disease from infected herds. Quarantine and embargo have been used to prevent the introduction of infected animals into areas and herds free of a specific infection. Nominal control of some diseases has been accomplished by use of immunizing agents. The use of this procedure will not always eradicate a disease as has been demonstrated in hog cholera.

Disease eradication is most successful when infected animals are eliminated and healthy animals are used to repopulate the farm. Re-entry of disease into the herd is prevented by stringent quarantine. This procedure has proven effective in stamping out the pockets of infection of foot and mouth disease in this country. It has also been useful in maintenance of herds free of tuberculosis and brucellosis.

The application of some of these disease control techniques is the basis for the Specific Pathogen-Free (SPF) swine program. Elimination of infection in a new population by prevention of contact is accomplished through birth by hysterectomy followed by careful isolation and quarantine. Detailed description of these techniques can be found in veterinary literature. Success in maintaining herds free of atrophic rhinitis (AR) and virus pneumonia of pigs (VPP) is dependent on the diligence of the herd owner and his veterinarian. The results of their efforts are visible in a herd of thrifty, rapidly growing pigs. Since no farmer can successfully maintain a conventional herd and an SPF herd on the same premises comparisons are difficult to make. Young, et al. in 1959 reported on the performance of swine before and after repopulation in a given herd. Average 56 day weaning weights were from five to 13 lbs. heavier in the SPF animals and carried on to the 154 day weights when the difference was between 16 and 30 lbs. per animal in favor of the SPF hogs. Average daily gain was also greater in the SPF animals. A later report was published by Caldwell, et al. in 1961 covering results obtained under actual farm conditions from SPF swine. A summary of results on SPF farms covering four years of operation of the Nebraska SPF Swine Certification Program including 1951 litters has been compiled by the present program coordinator. The 1960 Wisconsin Swine Selection Cooperative Herd Analysis data and that of the 25 Iowa Master Swine Producers 1960 are used for comparison. This information is used for comparison to point out the advantages of stock free of chronic infectious disease.
The success of swine producers under the SPF program, in keeping their herds free of AR and VPP is pointed out by the fact that 92 percent of the farm herds qualified for certification under the program health requirements. This is in contrast to only 81 percent meeting performance requirements. The health requirements include a check on a given number of market hogs at the slaughter plant for AR and VPP. They must be free of brucellosis and leptospirosis as determined by blood tests and also free of external parasites. Freedom from external parasites has been an unexpected benefit of the SPF program. The finding of external parasites in an SPF herd is an indication of a break in the isolation program and contact with non-SPF animals has occurred. Other health requirements are hog cholera vaccination, control of erysipelas by vaccination if erysipelas is a problem and a program to minimize internal parasites.

Performance requirements for certification include:

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<th>Min. W. - 140 days</th>
<th>Max. B.F. 200 lbs</th>
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<tbody>
<tr>
<td>Average Herd requirement</td>
<td>155 lbs</td>
<td></td>
</tr>
<tr>
<td>Individual gilt</td>
<td>150 lbs</td>
<td>1.5 inches</td>
</tr>
<tr>
<td>Individual boar</td>
<td>170 lbs</td>
<td>1.3 inches</td>
</tr>
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</table>

Failure to meet health requirements drops the herd from the program. Failure to meet performance requirements does not, but no animal failing to meet individual requirements may be retained as breeding stock.

Herds under the SPF program contribute to the health of the swine population in another way. These herds act as sources for clean boars for use in conventional herds. Unpublished reports that SPF boars experienced difficulty when introduced into non-SPF herds stimulated an investigation of the problem. A survey covered 370 boars sold to 316 buyers in a nine-state area. It included boars going into both SPF and non-SPF herds. Data disclosed that SPF boars experienced more problems (20 percent) when going into SPF herds as compared to 17 percent in non-SPF herds. However, the problems in the SPF herds were minor and primarily due to immaturity. The boars entering non-SPF herds had fewer problems but those encountered were more severe. Disease was involved in 67 percent of the total problems these SPF boars encountered. Some of these could have been eliminated by proper prophylaxis prior to transfer or early treatment of disease. From this survey Underdahl et al. made the following recommendations which should be followed whenever boars are purchased whether SPF or not. They are:

1. Purchase mature boars over seven to eight months old (Preferably one year).
2. Anticipate needs and purchase boars at least three weeks before breeding season starts.
3. If erysipelas is a problem in the herd, have boar vaccinated three weeks before transfer to your farm.
4. Do not isolate completely but permit fence contact with breeding herd.
5. Boars going into non-SPF herds should receive 40-50 ml commercial anti-hog cholera serum and antibiotics prior to transfer.
Follow this with second administration of antibiotics four or five days later.

6. Observe boars closely and treat immediately if clinical symptoms appear.

By following these suggestions any breeder introducing a new boar or for that matter any new animal into his herd will have a better chance of maintaining the health of the new animal and his previous herd.

It has been suggested by Self that boars should not be used extensively until after they are at least a year of age. This permits the boar an opportunity to mature and as a result will function more satisfactorily as a herd sire. It is to be expected that the more mature individual would not experience as severe stress of a young animal would when moved to a new herd. The younger boar when put into service at nine or 10 months of age has not reached his full development. Heavy use will further lower resistance which has already been reduced by his movement to new surroundings and new management.

Hogs produced under the SPF program develop rapidly and producers are tempted to breed gilts at an early age. Some have tried farrowing gilts at eight months or earlier. The litter size and number of pigs weaned were disappointing. It is essential that both gilts and boars be mature before being used in the breeding herd.

The SPF program was originally planned to eliminate atrophic rhinitis, virus pneumonia, swine dysentery and brucellosis. However, decreased incidence of other enteric infections including TGE has also occurred. Leptospirosis has occurred in only one herd in the Nebraska program and was eliminated by a vaccination program for one generation.

Disease control principles based on clean stock, clean environment and quarantine have been employed in the SPF program and have proven effective in preventing the re-establishment of VPP and AR in herds of program members. It is impossible to determine what other disease outbreaks in these herds may have been prevented by participation in the program. Greater pride in faster growing, more efficient hogs is displayed by participating stockmen. These men have demonstrated to themselves and their neighbors that livestock diseases can be eliminated and denied re-entry by the use of repopulation, through sanitation and isolation of animals from outside contacts. SPF owners are extremely conscious of these principles and have come to expect their veterinarian to practice asepsis when doing their veterinary work. Such a group of stockmen are disease minded and will help to constitute a nucleus for programs to eradicate other diseases such as hog cholera.

REFERENCES


Transmissible gastroenteritis (TGE) was first identified as a specific entity in the swine enteritis complex in 1945 by Doyle and Hutchings.\(^1\) Doyle\(^2\) indicated that he had observed occasional outbreaks of disease with clinical features of TGE during the 10 or 12 years preceding that time, but it had showed no tendency to become widespread. In a review, H. C. Smith\(^3\) recalled that he had investigated a similar disease involving 23 herds of swine in central Minnesota in 1937. Soon after the report by Doyle and Hutchings, the disease was recognized in most of the important swine raising areas of the United States.\(^4,5,6\)

A disease similar to TGE was observed in Japan in 1956 and on the basis of cross protection tests with TGE virus strain New York I, Sasahara and his colleagues\(^7\) concluded that the virus was closely related to if not identical with TGE in the United States. A highly infectious gastroenteritis of pigs was encountered in England in 1957 by Goodwin and Jennings.\(^8,9\) They showed that the clinical and pathological features of this disease were very similar to TGE in the United States and that the infective agent was neutralized by serum produced in the United States. Gastroenteritis of pigs which appears to be very much like or identical to TGE has also been reported from Taiwan in 1958,\(^10\) Russia in 1957,\(^11\) Germany in 1959,\(^12\) and Poland in 1959.\(^13\) By following the literature it appears that TGE started as a disease of swine in the Midwest some 20 years ago and has spread through much of the important swine producing areas of the world. Whether this is true or not may remain a matter for conjecture. Certainly diarrheal diseases of young pigs have always been a problem wherever swine were raised in concentration. It seems unlikely, however, that a disease with such dramatic consequences and easy transmissibility could have gone long unrecognized in any area with competent veterinary service.

Very little information is available on the epidemiology of TGE. McNutt\(^6\) indicated that in his observations TGE appeared in clean herds only following the introduction of new stock. On the basis of observation of large herds in which almost every crop of pigs was affected with diarrheal
disease over a period of several years, he postulated that occasional swine become carriers of the virus for extended periods of time. He suggested that immunity of varying strength and duration develops in sows and is transmitted passively to their pigs protecting them for six or eight weeks after which they may become infected and thus perpetuate the disease in a herd. Goodwin and Jennings also presented evidence for persistence of TGE infection in a chronic form within a herd. This was based on the fitful emergence of diarrheal disease affecting all or parts of some litters for five months after the last deliberate infection in an experimental herd. Reber demonstrated that TGE infection could be transmitted through air in confined spaces and others have observed that the virus would spread from pig to pig in rooms where there was no direct contact.

The duration of the carrier state is of obvious importance in the epidemiology of TGE but the only reported work on actually testing the duration of the carrier state was done by Lee, Moro, and Baker who found that in a group of 13 pigs the virus was shed for intervals of time extending from two to eight weeks after inoculation.

The appearance of outbreaks far from known cases of TGE has prompted speculation as to whether species other than swine could serve as temporary carriers or reservoirs of TGE virus. Most of the common laboratory animals have been exposed to this virus but none have shown illness.

The purpose of this paper is to report some of our observations on natural outbreaks in Indiana, and preliminary experimental work on the inoculation and recovery of TGE virus in species other than swine.

OBSERVATIONS OF NATURAL OUTBREAKS

Over a period of 10 years we have had an opportunity to observe a fairly large number of outbreaks of TGE as they were reported to the Purdue Animal Disease Diagnostic Laboratory or referred to us directly. The diagnosis, in the majority of these cases, was made on post mortem appearance of affected pigs and a history of rapidly spreading diarrheal disease affecting all or most of the swine in a herd with high mortality only in baby pigs. In selected cases, the diagnosis was confirmed or negated on the basis of inoculation of test pigs with extracts of intestinal tracts or feces of infected pigs. Attempts were made to determine the means by which the virus entered the herd by questioning herdsmen as to movements of swine, equipment, personnel, and any other factors that seemed pertinent. Seventy-one of these herds were followed by means of letters, telephone, or repeated visits for periods of up to seven years to gain information on the continuing status of the herds with respect to TGE.

According to the records of the Animal Disease Diagnostic Laboratory at Purdue University, over 90 percent of the outbreaks occur during the period from late December through early April with the highest incidence in March. It is probably true that the actual percentage of occurrence is even higher during this period because outbreaks at other times of the year are more likely to be referred to the laboratory by virtue of their being unseasonal.
In most outbreaks we were unable to arrive at a satisfactory explanation as to how the virus got to the premises. In those instances where circumstances did point to certain factors, they were the introduction of actively infected or recently recovered pigs and transport of virus by persons, equipment, or feed. Many herdsmen were inclined to blame feed as the source of their trouble. Because it is a very common procedure to receive new feed on farms at intervals of a week or so, it usually was not possible to confirm or eliminate this on the basis of observations. In only one instance, we were able to find that more than one herd was infected after feeding from a single batch. In this case, three herds were affected within three days after they were fed home grown corn ground and mixed with supplement at a local elevator. On investigation at the elevator, it was found that the owners of these three herds had been there at the same time. It was a practice at this elevator for men who were waiting in line to gather in the mixing area. Most of them wore boots that were covered with mud from their home lots. When feed was spilled on the floor, it and whatever dirt came off their boots was swept into the hopper. Therefore, it seemed very possible that these three loads of feed had been contaminated with feces from this source.

There were five cases in which personnel had obviously carried virus in working between infected and clean herds and four others in which the disease followed quickly the use of live stock trucks to transport hogs from the herd.

Of 50 herds in which we obtained and recorded accurate information on the additions of swine to herds before outbreaks, 11 had added swine within one month, three between six weeks and two months, and 36 had not added swine for periods of four months or longer. Of the latter most of the additions were boars that sired the affected crop of pigs. Of the 11 herds to which swine had been added within a month, in two instances they were boars that had been through sales, were scouring when brought to the herd, and were placed in buildings with farrowing sows. In these herds, it seemed apparent that the virus was brought in by the boars. Feeder pigs had been added to three herds in this group at four days, six days, and two weeks respectively before the start of outbreaks. In none of these herds did the feeder pigs show diarrhea at the time of the outbreak. Since swine of comparable age that were native to farms on which outbreaks occurred almost always went through an episode of diarrhea, it appeared very possible that the feeder pigs were instrumental in bringing in the virus. Two of the herds in this group were composed entirely of sows that had been assembled 16 and 17 days before the start of outbreaks. It is possible that they were infected in trucks or in concentration points. Of the remaining herds, there was little evidence that swine that carried virus for long periods were instrumental in introducing the virus. The single instance in which this seemed to be a possible factor was in a herd in which two gilts had been added five months previously. All of the sows in this herd and their pigs sickened during the outbreak except for one of the added gilts and three of her five pigs.

Of 71 herds which were observed after outbreaks of TGE, nine had recurring episodes of "serious diarrheal disease." Four of these were
apparently not due to TGE virus; in two, the disease was treated successfully with antibiotics and sulfonamides. The other two were outbreaks of relatively mild diarrhea in which the possibility of TGE virus acting in the face of the herd immunity was considered. Attempts to demonstrate TGE virus from these herds by inoculation of test pigs was unsuccessful. Five of the 71 herds had frank outbreaks in following years. Three of these outbreaks occurred two years after the first without having TGE in intervening farrowings. The fourth outbreak occurred one year after the initial episode on the farm but the whole herd had been depopulated immediately after the first outbreak and the new stock had had one successful farrowing in the interim.

In the remaining herd, two outbreaks occurred in a six month period subsequent to the first one. This herd had been assembled with the intention of starting a "pig hatchery" by a man who worked regularly in two salesbarns. Four weeks after the original outbreak subsided, five pregnant sows were purchased and placed in the farrowing house which still held a few surviving pigs from the first episode. All of the pigs from these five sows were lost. The third outbreak occurred in the pigs of 16 sows that were said to have been in the herd during the first outbreak. These pigs were one to four weeks old at the time the disease broke out and only 15 of the younger pigs died. TGE virus was demonstrated in both the second and third outbreaks in this herd.

TGE VIRUS IN ANIMALS OTHER THAN SWINE

Materials and Methods

The general pattern of these experiments was to inoculate animals other than swine orally or by stomach tube and to test their feces or intestinal tracts for virus at various times thereafter by inoculation of pigs.

Virus

The virus was the seventh serial passage in pigs of TGE virus acquired from W. W. Bay in 1952. The material used to inoculate cats, mice, rabbits, and suckling pigs was from a pool (No. 544) made of intestinal tracts of four pigs killed two days after infection. The intestines and their fluid contents were ground with two volumes of 0.85 percent saline solution, centrifuged to remove coarse particles, and stored in screw capped vials at -18 C. A sample from this pool was shown to be infectious in pigs inoculated orally at a $10^{-5}$ dilution but not at $10^{-6}$. Dogs and foxes were fed crude ground intestines of pigs that had been exposed to the seventh passage.

Inoculation of Animals and Collection of Specimens

Mice

Twenty-nine Rockland Swiss mice four weeks of age were given one ml doses of pool 544 via stomach tube. Tests were made on the pooled intestinal tracts of two mice killed the day before and on the first, second, third, fourth, sixth, 10th, 14th, and 21st days after exposure.
Cats

Four six-week-old domestic cats were given two ml doses of pool 544 orally. Fecal samples were collected by giving a low enema with sterile 0.85 percent saline. Pooled samples of the day before and first, second, third, fourth, and seventh days after inoculation were tested.

Rabbits

Two wild cottontail rabbits about six weeks of age were given one ml doses of pool 544 via stomach tube. Stools were collected and pooled specimens from the day before and the first, second, third, fourth, and sixth day after inoculation were tested.

Dogs

Four nine-week-old mongrel pups were fed about ten grams of infective ground pig gut. Pooled fecal samples were tested from the day before, from the first seven, and from the 14th, 21st, and 50th days after exposure. Individual samples were tested from the first and seventh days after exposure. The dogs were inoculated again on the 50th day and samples were tested from eight of the first 11 days after the second exposure. A neutralization test was conducted using pooled serum collected before and 33 days after the first inoculation of these dogs. The serums were inactivated at 56 C for 30 minutes and mixed undiluted in two ml volumes with 10 fold dilutions of virus. Pre-inoculation serum was used against dilutions of $10^{-3}$, $10^{-4}$, and $10^{-5}$, and post inoculation serum against $10^{-2}$, $10^{-3}$, and $10^{-4}$ of pool 544 virus. The mixtures were incubated for 30 minutes before inoculation into pigs.

Foxes

Each of two wild red foxes about three months of age was fed about 15 grams of infective ground gut. Individual stool samples were tested before and at days one, four, eight, 15, 22, and 24 after this feeding.

Tests for Presence of TGE Virus

Samples were thawed and centrifuged at low speed to throw down coarse particulate matter. Penicillin was added to 10,000 units and streptomycin to 10 mg per ml of supernatant fluid and two ml doses were given orally to test pigs. The pigs were taken from sows in a herd established with pigs taken by casarean section or hysterectomy and housed individually in isolation units. There has never been any incidence of TGE in this herd.

A test was considered positive if a pig responded, usually within 18 to 30 hours, with vomition and diarrhea. Vomition usually preceded diarrhea and in most cases death ensued in from two to eight days. Pigs which remained healthy for four days were sometimes used to test a second specimen. If they remained healthy after the second exposure, they were finally given a dose of TGE virus of known infectivity. When pigs came down with TGE after these inoculations, the first two specimens were considered negative. Some pigs showed relatively mild diarrhea which might be misinterpreted as TGE. Such pigs responded typically to final inoculation with TGE virus. The occasional pigs that recovered from TGE did not sicken when exposed to a second dose of virus. From each species in which TGE
virus was recovered, a second passage was carried out in pigs as confirmatory evidence that the disease produced was TGE.

RESULTS

None of these animals other than swine showed clinical evidence of having been infected with TGE. The data on recovery of TGE virus from these animals is summarized in Table I. The fecal samples taken before inoculation were negative for TGE virus in all of these animals and the virus was not found in any of the samples from mice or rabbits after inoculation. One of two pigs used to test the pooled third day samples of cat feces in the first test developed TGE. The test was repeated, and again only the pig given third day feces developed TGE. Dilutions of this sample of $10^{-1}$ to $10^{-3}$ were negative. All of the pooled samples from dogs from the first seven days after exposure caused typical TGE in pigs while samples from the 14th, 21st, and 50th day were negative. Individual samples of all of the dogs on the first day, and three of the four, were positive on the seventh day. All samples taken after the second exposure of the same dogs were negative. In the neutralization test with dog serums, pre-inoculation serum had no effect on the infectivity of the virus as compared to previous titration. Post inoculation serum neutralized all dilutions including $10^{-2}$ (about 1000 infectious doses). More concentrated virus was not tested against this serum. Virus was found in the feces of both foxes on the first, eighth, and 15th day after being fed TGE infected gut. A fourth day sample of one of the foxes was infective at dilutions of one to four and $10^{-2}$ but not at $10^{-4}$ or $10^{-6}$.

DISCUSSION

It has been shown that at least two species other than swine are capable of being infected with and shedding TGE virus. These are dogs and red foxes. The finding of TGE virus for seven days after exposure and development of neutralizing antibody in dogs is strong evidence that they were

<table>
<thead>
<tr>
<th>Animal</th>
<th>No. Tested</th>
<th>Time after inoculation*</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Days</td>
</tr>
<tr>
<td>Mice</td>
<td>16</td>
<td>1 2 3 4 5 6 7 8 2 3 4 7</td>
</tr>
<tr>
<td>Rabbits</td>
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<td>-</td>
</tr>
<tr>
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<td>-</td>
</tr>
<tr>
<td>Dogs</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>Foxes</td>
<td>2</td>
<td>+</td>
</tr>
</tbody>
</table>

*Pre-inoculation sample from all species were negative

**Only pooled samples tested
actually infected as is the finding of TGE virus in stools of foxes for 15 days after exposure. The finding of a very low titer of virus in cat feces only on the third day after exposure could have been the result of survival through the alimentary tract. It is very likely that further studies may add more species to the list of those capable of being infected, and the fact that we were unable to find virus in the feces of rabbits or guts of mice does not preclude the possibility of their being infected under other conditions. The possibility that invertebrate hosts may play a part in the epidemiology of TGE is totally unexplored.

What, if any, importance the Canidae have in the spread of TGE between herds or in maintaining the virus between seasons remains to be shown. It appeared from this limited work that those animals which were infected shed virus for periods of only one or two weeks. Whether such animals may be infected naturally by contact with each other also needs to be shown. On many farms with outbreaks of TGE we have seen piles of dead pigs outside of farrowing houses or even scattered in fields with manure spreaders. We have seen dogs eating and carrying such pigs and may assume that foxes will do likewise. In the one instance in which we tried, we did recover TGE virus from the stool of a dog on a farm on which an infected pig had been eaten. Obviously, proper disposal of dead pigs is an important control measure in this disease.

There are many gaps in the information from which an accurate epidemiological picture of TGE could be drawn. The fact that the only practical indicator of the presence of TGE is a pig housed in isolation has made research on this disease expensive and progress slow. A number of groups including ourselves have attempted to adapt this virus to various tissue culture or other laboratory hosts. Most of these have not been published. The only report of successful adaptation has been the one by Lee, and, to our knowledge, this has not been repeated elsewhere. There is an urgent need for work in this area.

It seems reasonable on the basis of present knowledge to assume that the important natural route of transmission of TGE virus between herds is through the medium of ingestion of virus which is passed in feces of infected or convalescent animals. Although virus may be found in the other viscera and blood, the highest titers of virus in affected pigs have been found consistently in the intestinal tract with or without ingesta. There is undoubtedly a great amount of virus in stools of pigs in the early stages of disease. We have found titers of $10^4$ I.D. per gram in the first and second days and $10^5$ I.D. on the third day after infection. The most effective routes of inoculation are orally or intranasally. In the latter case a large part of the inoculum is swallowed. Young et al. found that a suspension of virus infective at a dilution of $10^{-6}$ by the intranasal route was infective only at $10^{-2}$ when injected intraperitoneally. Lee et al. were unable to produce typical disease by parenteral injection. We have produced typical TGE with a slight lengthening of incubation period by intramuscular inoculation of about $10^6$ oral infectious doses.

Our observations indicate that TGE is almost, but not quite, confined to the cold months of the year with the incidence increasing as long as cold weather continues and falling off sharply with the onset of warm weather.
More proof is needed, but it seems very plausible that this seasonality is due to characteristics of the virus and the fecal matter in which it is shed. We have kept TGE virus in the form of supernatant fluid of intestinal tract ground in 0.85 percent saline solution frozen at about -18 C. for four years without appreciable loss of titer.\textsuperscript{15} What evidence there is, on the other hand, indicates that the virus will not survive more than a few days at warm temperatures. Bay\textsuperscript{19} found that gut of infected pigs infected only two of four pigs three days, and none of four pigs 10 days after being left to dry and putrify at about 70 F. Young et al\textsuperscript{18} stated that the virus drops about one log in titer for each day's storage at 37 C.

Regardless of what animal deposits infected feces, there is some time interval before the accident of ingestion by a susceptible pig. This time interval may be extremely short where pigs are in direct contact and, of course, it may be of any length where there is indirect contact. We have observed in many experiments that pigs are just as susceptible to TGE in the summer as in the winter and that the infection will spread from pig to pig by contact within a litter within one day regardless of the weather. It is true that more pigs are kept in close contact within herds during the winter, but this alone should not affect the spread of infection between herds. Reasoning from this, the difference between summer and winter must lie in their effect on the virus. In warm weather its survival time is probably very short. In the winter, it may be preserved as long as it remains frozen and it is logical, but not proven, that even if not frozen, the virus will survive longer at temperatures near freezing. The nature of feces of infected pigs also must have some bearing on the transmissibility of the disease. In the active stage of the disease, when virus content is high, the stools are thin, watery, and adhere to almost any surface or penetrate anything absorbent. Such feces would be carried easily from herd to herd by any animate or inanimate object that moves between them. Virus in such fecal material should, however, be very susceptible to the inactivating forces of sunlight, drying, and warm temperatures.

This appears to be a logical explanation for the higher incidence in cold weather but does not explain how the virus is perpetuated from winter to winter. The possibility of reservoirs other than swine has been considered, but there are several other possibilities. First, once a herd is infected, the virus may continue to be present in a few or many of the swine. The disease does not reappear, or if it does, it may be relatively mild because of the presence of immunity in the herd. Animals from such a herd could be a threat to new animals in the herd or any herd to which they were added. This as a factor in perpetuating the disease was postulated by McNutt\textsuperscript{6} and later by Goodwin and Jennings.\textsuperscript{9} It has been proven that this situation exists with MH enterovirus of swine by Wenner, Beran, and Werder.\textsuperscript{20}

Contrary to this, our observations indicate that it is extremely unusual to have continuing TGE infection in a herd. Most outbreaks are terminated in less than three or four weeks. We have observed a number of herds in which relatively mild diarrheal disease occurred in farrowings subsequent to TGE outbreaks and tried to isolate TGE virus from affected pigs without success. We have also observed several herds in which relatively mild
diarrheal disease recurred repeatedly in successive farrowings. We were able, with filtrates or antibiotic treated preparations of intestines from two of these herds, to transmit a very mild and transient gastrointestinal disease. Recovery from this condition left pigs still susceptible to TGE and pigs that recovered from TGE were still susceptible to this agent. In the one herd from which we were able to isolate TGE virus in three successive farrowings in a six month period, the disease was severe in all three outbreaks. As we have indicated, there was considerable movement of animals in and out of this herd and the herd had more than ordinary chance of exposure from outside because of the owners' regular employment at two sales barns. We are reasonably sure that the great majority of herds do not suffer continuing TGE infection once the disease is established.

A second possibility is that the virus is passed from group to group of swine on a rather continuous basis during the summer months. The disease does not become widespread because the forces which inactivate the virus and prevent it from being carried over considerable distances are more active then. There are concentration points to which new pigs are added and from which they are dispersed on a continuing basis. This could provide the conditions for continuous propagation of the virus. It is well known that pigs under these conditions usually develop diarrhea which appears similar to TGE in swine of this age. It is entirely possible that this diarrhea is due to infection with TGE virus among other things. Without proof, it would be unwise to incriminate these establishments as a major factor in the perpetuation of TGE virus, but this should be an important area for exploration.

A third means of preserving TGE virus that could have some significance is frozen storage in the form of pork or even intestines of infected pigs. It is known that a viremic phase exists in baby pigs. This has not been tested in swine of market age, but probably occurs. We have seen many herds in which swine of this age were obviously infected. In some of these herds, the sick hogs were the remainder of groups that had been sent to market a few days previously. Thus it seems entirely possible that under these conditions TGE could be spread through the medium of uncooked garbage. As for storage of intestinal tracts of infected pigs, many herdsmen have seen the development of immunity following the feeding of this material to sows in the face of outbreaks. Some of them are freezing this material to feed to sows to immunize them during gestation in the following years. While this may be effective in preventing another outbreak in the sow herd to which it is fed, the virus in this form is fully virulent and a new focus of infection is established each time this is done.

SUMMARY

Through observation of natural outbreaks, it is shown that transmissible gastroenteritis (TGE) in swine has a highly seasonal incidence. Over 90 percent of the outbreaks in Indiana occurred between late December and early April. It is postulated that the principal means of transmission of this virus between herds is through the medium of feces of infected animals.
either deposited directly or carried on fomites and that the seasonality of the disease is a reflection of the stability of the virus in the cold or frozen state and its lability at warm temperatures. Little or no evidence was found for the existence of swine that carry virus for more than a few weeks or for long continued infection of a herd.

Tests for TGE virus were of the stools of dogs, foxes, cats, mice, and rabbits after oral inoculation. Clinical disease did not appear in any of these animals but dogs and foxes shed virus for one to two weeks. Dogs developed neutralizing antibody following exposure.

Four possible means for the maintenance of TGE virus between seasons are discussed. These are: 1) the virus may be supported by a host other than swine, 2) the virus may be perpetuated in swine by means of long term carriers or continued infection within herds, 3) the virus may survive by spread from group to group of closely associated swine during the summer, and 4) the virus may be kept in the frozen state in the medium of pork or frozen intestinal tracts used as immunizing material.

REFERENCES

Protection of pigs against hog cholera has been attributed to the presence of specific antibody induced either by vaccination or transferred from an immune animal; few pigs, if any, have been thought to be naturally resistant. After a cytopathogenic HC (hog cholera) virus had been found, a test for HC neutralizing antibody was developed and correlation between HC antibody content and immunity to virulent HC virus became possible. It has been found that pigs with specific neutralizing HC antibody, if they had high titers, showed no signs of illness when given virulent HC virus whereas animals became increasingly more susceptible with decreasingly lower titers until practically all pigs died when no titer was demonstrated.

Although newborn pigs were found devoid of measurable HC antibody and, therefore, susceptible to HC virus, shortly after nursing an immune mother they acquired an antibody titer equal to that of their mother and were no longer susceptible to virulent HC virus until the acquired HC antibody titer had decreased almost to extinction. Exact HC antibody titer required for such protection against virulent HC virus has not been determined; it has been found that pigs with titers between two and 10 succumbed although others with these low titers were protected.

HC serum antibodies have been reported to interfere with active immunization against HC virus when a vaccine of rabbit origin was used. Similar interference by maternally transferred antibodies has been well established with other vaccines, especially those for distemper and for infectious canine hepatitis. Because of these experiences and because maternally transferred HC antibodies protected pigs against virulent HC virus, it seemed desirable to study the effect of maternally transferred HC antibodies on response to hog cholera vaccination.

MATERIALS AND METHODS

In these studies, pigs with HC antibodies acquired by nursing immune mothers and pigs without HC antibodies were given various HC vaccines. Antibody response was determined before and after vaccination, and again, before and after a subsequent challenge inoculation of virulent virus.

Pigs with maternal HC antibody. From seven sows that had been immunized against hog cholera by an inoculation of BVD (bovine virus diarrhea) vaccine followed by subsequent inoculation of virulent strain A HC virus, 47 pigs were obtained. In groups of two or three, pigs from these litters

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*This investigation was aided by a grant from the National Institute of Allergy and Infectious Diseases, United States Public Health Service.
were placed in isolation units when they were two to 20 weeks of age. In some of these groups, each pig was inoculated intramuscularly with one ml of attenuated tissue-cultured HC virus, (PAV strain, 75th passage) that titrated approximately $10^3$ TCID$_{50}$, in other groups, each pig was given one ml of 10 percent spleen emulsion from a pig infected with virulent strain A HC virus that titrated $10^4$, while other groups were left uninoculated. As much as possible littermates were distributed to receive both treatments. In all, 24 pigs were given tissue-cultured virus, 13 pigs were given virulent virus and 10 pigs were left as controls. Serums for HC antibody studies were taken from each pig at the time of inoculation or, if uninoculated, when placed in isolation and every two weeks thereafter until the study ended. After pigs reached an age between 19 and 26 weeks, they were tested for immunity by another intramuscular inoculation of one ml of 10 percent spleen emulsion from a pig infected with virulent strain A HC virus. For each pig, age and results are shown in Tables I and II and summarized in Table III.

Pigs without maternal HC antibody. Pigs in these studies were given four types of homotypic hog cholera vaccines as follows: (1) From SPF sows that had not been immunized against hog cholera, nine pigs were obtained when they were six to 10 weeks of age and were placed in isolation units. Each pig was inoculated intramuscularly with one ml of tissue-cultured HC virus (PAV, strain A, 75th passage), and after 28 days each pig was given a test for immunity by an inoculation of one ml 10 percent spleen emulsion of virulent strain A HC virus. Serum was obtained from each pig at the time of vaccination, 28 days after vaccination and again 14 days after test of immunity. (2) Six pigs were vaccinated with porcine origin vaccine (four serums supplied through the courtesy of Dr. Arden H. Killinger) and bled as described above. (3) Six pigs were vaccinated with rabbit origin vaccine as described above except pigs were bled for serum 14 days after vaccination and 14 days after challenge. (These pigs were vaccinated by Dr. Victor Cabasso, Lederle Laboratories, Pearl River, N. Y.) (4) 10 pigs were vaccinated intramuscularly with five ml of crystal violet (CV) vaccine. They were bled three months after vaccination, at the time of exposure to virulent strain A HC virus and again two weeks later.

For comparative purposes, data on heterotypic BVD vaccine have been included. In this study, 29 pigs whose serum contained HC antibody titers and 69 pigs without were inoculated with BVD vaccine when they were six to eight weeks of age. Inoculation with virulent strain A HC virus was made approximately three months after vaccination; serums were obtained before vaccination, again when virulent virus was given and again two weeks later.

Assay of HC antibody. Neutralization tests were performed in primary pig kidney tissue cultures. After inactivation, dilutions, usually five-fold, of each serum were made and each dilution was mixed with an equal amount of undiluted tissue cultured PAV strain HC virus (28th passage) and placed at 4°C for two hours. Then 0.2 ml of a serum-virus mixture was inoculated into each of three or five tubes of tissue cultured swine kidney cells. Virus titration and uninoculated cell controls were included in all tests.
After incubation at 36°C for six days, final readings were made and a 50 percent endpoint was calculated for each serum according to the Spearman-Karber method.

RESULTS

Pigs with maternal antibody. Of the 24 pigs that received tissue cultured hog cholera virus (Table I, 18 pigs had titers greater than 10^{-2} that ranged as high as 10^{-3.88} when they were vaccinated. After vaccination, titers of 17 pigs continued to decline until a median level of 10^{-1.5} was reached. This titer persisted until virulent virus was inoculated. Then the titers of all pigs increased within two weeks to approximately 10^{-3.5}. One pig whose titer was 10^{-3.41} remained unchanged after vaccination and also remained unchanged after inoculation of virulent virus. The remaining six pigs in this study had HC titers below 10^{-2} when vaccinated and either they were unchanged or became slightly elevated afterwards. When subsequently given virulent virus, their titers also were elevated. Only two of these 24 pigs showed elevation of temperature over 104°F after inoculation of virulent virus. This lasted only one and two days. No other clinical signs of illness were noted.

TABLE I

Response of Pigs from Immune Sows to Hog Cholera Vaccine (75th Passage) Neutralization Test Results*

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Age(wks)</th>
<th>Pre.</th>
<th>Post</th>
<th>Age(wks)</th>
<th>Pre.</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>1465</td>
<td>2</td>
<td>3.41</td>
<td>2.45</td>
<td>24</td>
<td>1.50</td>
<td>&gt;3.50</td>
</tr>
<tr>
<td>1466</td>
<td>2</td>
<td>3.88</td>
<td>2.93</td>
<td>24</td>
<td>1.50</td>
<td>&gt;3.50</td>
</tr>
<tr>
<td>1467</td>
<td>3</td>
<td>2.93</td>
<td>2.16</td>
<td>25</td>
<td>1.50</td>
<td>&gt;3.50</td>
</tr>
<tr>
<td>1468</td>
<td>3</td>
<td>3.27</td>
<td>2.64</td>
<td>25</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>1470</td>
<td>3</td>
<td>3.41</td>
<td>2.31</td>
<td>21</td>
<td>&gt;3.50</td>
<td>&gt;3.50</td>
</tr>
<tr>
<td>1471</td>
<td>3</td>
<td>3.41</td>
<td>1.86</td>
<td>21</td>
<td>1.50</td>
<td>3.50</td>
</tr>
<tr>
<td>1472</td>
<td>4</td>
<td>2.94</td>
<td>1.98</td>
<td>26</td>
<td>1.34</td>
<td>4.15</td>
</tr>
<tr>
<td>1473</td>
<td>4</td>
<td>2.46</td>
<td>1.51</td>
<td>26</td>
<td>1.34</td>
<td>4.15</td>
</tr>
<tr>
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<td>3.41</td>
<td>2.64</td>
<td>25</td>
<td>1.50</td>
<td>2.20</td>
</tr>
<tr>
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<td>5</td>
<td>3.41</td>
<td>2.16</td>
<td>25</td>
<td>1.50</td>
<td>&gt;3.50</td>
</tr>
<tr>
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<td>6</td>
<td>2.93</td>
<td>2.45</td>
<td>26</td>
<td>1.50</td>
<td>&gt;3.50</td>
</tr>
<tr>
<td>1477</td>
<td>6</td>
<td>3.12</td>
<td>2.45</td>
<td>26</td>
<td>2.80</td>
<td>3.20</td>
</tr>
<tr>
<td>1453</td>
<td>7</td>
<td>2.93</td>
<td>2.00</td>
<td>19</td>
<td>1.36</td>
<td>2.45</td>
</tr>
<tr>
<td>1454</td>
<td>7</td>
<td>2.93</td>
<td>1.86</td>
<td>19</td>
<td>2.00</td>
<td>2.31</td>
</tr>
<tr>
<td>1452</td>
<td>7</td>
<td>2.45</td>
<td>1.51</td>
<td>19</td>
<td>1.86</td>
<td>1.98</td>
</tr>
<tr>
<td>1401</td>
<td>8</td>
<td>2.45</td>
<td>2.07</td>
<td>20</td>
<td>1.97</td>
<td>&gt;2.93</td>
</tr>
<tr>
<td>1392</td>
<td>9</td>
<td>2.45</td>
<td>1.71</td>
<td>21</td>
<td>1.50</td>
<td>&gt;2.93</td>
</tr>
<tr>
<td>1393</td>
<td>9</td>
<td>2.45</td>
<td>1.50</td>
<td>21</td>
<td>1.00</td>
<td>&gt;2.93</td>
</tr>
<tr>
<td>1490</td>
<td>10</td>
<td>1.98</td>
<td>2.04</td>
<td>26</td>
<td>2.04</td>
<td>&gt;4.15</td>
</tr>
<tr>
<td>1491</td>
<td>10</td>
<td>1.51</td>
<td>1.51</td>
<td>26</td>
<td>2.04</td>
<td>3.98</td>
</tr>
<tr>
<td>1495</td>
<td>10</td>
<td>1.99</td>
<td>1.51</td>
<td>26</td>
<td>2.04</td>
<td>&gt;4.15</td>
</tr>
<tr>
<td>1496</td>
<td>10</td>
<td>1.51</td>
<td>2.04</td>
<td>26</td>
<td>2.04</td>
<td>3.27</td>
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<td>1396</td>
<td>18</td>
<td>1.00</td>
<td>1.00</td>
<td>20</td>
<td>1.00</td>
<td>2.16</td>
</tr>
</tbody>
</table>

*Titters expressed as minus log_{10} of 50 percent endpoint as determined by Spearman–Karber Method.

**Pigs were inoculated intramuscularly with 1 ml of a spleen emulsion containing virulent strain A hog cholera virus.
Of the 13 pigs that were given virulent virus (Table II), six showed titers above $10^{-2}$ when they were inoculated. After inoculation, titers declined in all to a level of about $10^{-1.96}$. Of the remaining seven pigs, five had titers between $10^{-1}$ and $10^{-2}$ and four of these were increased; the two with titers below $10^{-1}$ and one with a titer of $10^{-1.63}$ died. After a second inoculation of virulent virus, titers of all were increased in a manner similar to that found for attenuated tissue cultured HC virus.

**TABLE II**

Response of Pigs from Immune Sows to Virulent Hog Cholera Virus Neutralization Test Results*

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>First Inoculation**</th>
<th>Second Inoculation**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age(wks)</td>
<td>Pre.</td>
</tr>
<tr>
<td>1481</td>
<td>4</td>
<td>2.45</td>
</tr>
<tr>
<td>1489</td>
<td>4</td>
<td>2.46</td>
</tr>
<tr>
<td>1494</td>
<td>4</td>
<td>2.46</td>
</tr>
<tr>
<td>1480</td>
<td>4</td>
<td>2.93</td>
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<tr>
<td>1478</td>
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<td>3.12</td>
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<td>1479</td>
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<td>2.93</td>
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<td>1394</td>
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<td>18</td>
<td>0.48</td>
</tr>
<tr>
<td>1398</td>
<td>20</td>
<td>1.00</td>
</tr>
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</table>

*Titors expressed as minus log$_{10}$ of 50 percent endpoint as determined by Spearman-Karber method.

**Pigs were inoculated intramuscularly with 1 ml of a spleen emulsion containing virulent strain A hog cholera virus.

HC antibody titers in the 10 uninoculated controls declined to zero. When given virulent viruses, all died.

**Pigs without maternal antibody.** As can be seen in Table III, the nine pigs that received attenuated tissue cultured virus had a median HC antibody titer of $10^{-1.97}$ 28 days after vaccination; the six pigs that received hog cholera vaccine of porcine origin had a median titer of $10^{-2.45}$ 28 days after vaccination and the six pigs that received vaccine of rabbit origin had a median titer of $10^{-1.23}$ 14 days after vaccination. After the test for immunity with virulent virus, all pigs showed final titers that ranged from $10^{-2.1}$ to $10^{-2.7}$ with the greatest increase noted in the rabbit origin type.

Of the 10 that were given CV vaccine, six had no HC antibody titer, two had titers of $10^{-0.6}$ and two had titers of $10^{-1.5}$ three months after vaccination; after inoculation with virulent virus, one died and titers increased in the others to a median titer of $10^{-3.5}$. In like manner, but in contrast to HC antibody titers produced by homotypic live virus vaccines, 14 of 98 heterotypic BVD virus vaccinated pigs showed a median HC antibody titer of $10^{-4.8}$ three months after vaccination. The other 84 pigs developed no HC antibody titer. HC antibody titers of all pigs increased to a median titer of $10^{-3.1}$ after inoculation of virulent virus.
<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Maternal HC Protection</th>
<th>Med. Titer* after Vacc.</th>
<th>Med. Titer after V. Virus</th>
<th>No. Surviving Total Inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homotypic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue culture</td>
<td>+</td>
<td>1.50</td>
<td>3.5</td>
<td>24/24</td>
</tr>
<tr>
<td>origin</td>
<td>-</td>
<td>1.97</td>
<td>2.48</td>
<td>9/9</td>
</tr>
<tr>
<td>Porcine origin</td>
<td>+</td>
<td>N.T.</td>
<td>N.T.</td>
<td>0/0</td>
</tr>
<tr>
<td>origin</td>
<td>-</td>
<td>2.45</td>
<td>2.72</td>
<td>6/6</td>
</tr>
<tr>
<td>Rabbit origin</td>
<td>+</td>
<td>N.T.</td>
<td>N.T.</td>
<td>0/0</td>
</tr>
<tr>
<td>(high passage)</td>
<td>-</td>
<td>1.23</td>
<td>2.16</td>
<td>6/6</td>
</tr>
<tr>
<td>Virulent Virus</td>
<td>+</td>
<td>2.04</td>
<td>3.21</td>
<td>10/10</td>
</tr>
<tr>
<td>-</td>
<td>died</td>
<td>--</td>
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<td>--</td>
</tr>
<tr>
<td>Crystal Violet</td>
<td>+</td>
<td>N.T.</td>
<td>N.T.</td>
<td>0/0</td>
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<tr>
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<tr>
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<td>Bovine Virus</td>
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<td>0</td>
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<td>27/29</td>
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<tr>
<td>Diarrhea</td>
<td>-</td>
<td>0</td>
<td>3.00</td>
<td>69/69</td>
</tr>
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</table>

N.T. = none tested
*Titers expressed as minus log10 of 50 percent endpoint as determined by Spearman Karber method.

**DISCUSSION**

From the data presented, it appears that maternally transferred hog cholera antibody does not interfere completely with the immunization of pigs by homotypic live HC virus vaccine or by virulent HC virus. At first, this finding seemed to contradict previous studies in which "serum block" was thought to be responsible for certain "breaks" after hog cholera vaccination. Actually, no interference with active immunization by hog cholera antiserum has been shown except when rabbit origin hog cholera vaccines of low virus titer were used and in the single observation of Van Es who reported that 1.7 ml of hog cholera antiserum per pound of body weight interfered with development of immunity in five of 48 pigs. These five pigs died between the 22nd and 54th day after treatment. The other 43 pigs that lived to be six months of age survived an inoculation of virulent HC virus at that time. In view of subsequent findings that HC virus can persist and establish a chronic form of hog cholera, these five cases may not represent interference with subsequent immunity but could be the result of the initial inoculation. Studies by Weide suggested that maternal HC antibody may interfere with immunization when rabbit origin HC vaccine was used in one day old pigs, however, no distinction was made between interference and the reported inability of the young pigs to produce antibody.

Although no pigs in this study became susceptible after vaccination, optimal antibody production was inhibited by the presence of maternally transferred HC antibody whether attenuated or virulent HC virus was used.
As typically presented in Figure 1, after initial inoculation of attenuated HC virus into pigs with a high titer of maternally transferred HC antibody, the titer continued to decline to a level of approximately $10^{-1.5}$. Then, after an inoculation of virulent HC virus, antibody titers increased quickly to $10^{-3.5}$. A similar result was obtained with virulent virus. In contrast, when no HC antibody titer was present, higher HC antibody titers were produced after inoculation of attenuated virus and these titers were not greatly increased by subsequent inoculation of virulent HC virus.

The difference between the partial interference found here and "serum block" reported by others could be explained by the amount of virus used. In Dunne's work, serum interfered with rabbit origin HC vaccine that contained approximately $10^1$ immunizing doses but not with virulent HC virus which titrated greater than $10^4$ immunizing doses. It was suggested that the lesser amount of virus in the vaccine may have been responsible for this interference. In our studies, the tissue cultured attenuated HC virus contained $10^3$ immunizing doses and the virulent virus contained $10^4$ infective doses. Perhaps $10^3$ immunizing doses were enough to overcome the high titer of maternally transferred HC antibody, to stimulate antibody response and to give protection.

In our studies, response of pigs to the various types of homotypic life-virus hog cholera vaccines and virulent virus appears to be similar.
Pigs that received rabbit origin vaccine produced slightly lower antibody titers but perhaps the shorter period of time between vaccination and collection of serum after vaccination accounts for this difference. Data presented in this paper seem to compare favorably with the serological findings of York on HC antibody titers following vaccination with porcine and rabbit origin vaccines and with virulent virus. But, since the time between vaccination and collection of serum samples was not reported, no comparison could be made.

The absence of HC antibody following a single inoculation CV inactivated HC vaccine or of BVD vaccine indicates that these vaccines create a state of resistance rather than an immunity measurable by HC antibody tests as was found for homotypic live-virus HC vaccines. The rapid appearance of high levels of measurable HC antibody after subsequent exposure to virulent virus, however, suggests that a primary response was initiated by CV and BVD vaccines. Maternally transferred HC antibody did not appear to hinder the response of pigs to BVD virus vaccine; secondary response reactions were evident in BVD vaccinated pigs with or without maternal HC antibody when inoculated with virulent HC virus.

SUMMARY

Hog cholera antibodies acquired by young pigs through ingestion of colostrum did not completely inhibit HC antibody formation following inoculation either with attenuated or with virulent HC virus, but the amount of HC antibody produced in these pigs was lower than that formed by pigs without maternal HC antibody. Furthermore, subsequent inoculation of virulent HC virus produced a secondary response reaction in pigs that showed this partial interference; in contrast, titers in pigs that had been vaccinated with no maternal HC antibody were not boosted substantially by virulent virus inoculation. Protection against virulent HC virus and secondary response antibody reactions also were found in pigs given a single inoculation of either CV vaccine or BVD vaccine. Maternally transferred HC antibodies did not interfere with response to BVD vaccine.

DISCUSSION

Dr. James A. Baker: Dr. Coggins, you have inferred that after invoking secondary response in pigs, they produce HC antibody maximally, i.e., titers between $10^{-3}$ and $10^{-4}$. Have you titrated hyperimmune HC serums and if so, what titers did you find?

Dr. Coggins: Yes, we have. 10 or more serums including the concentrates have been checked and titers of $10^{-3}$ or slightly more were found.

Dr. Baker: Because it requires large amounts of virulent HC virus, five cc per pound of body weight to hyperimmunize a pig, and if hog cholera antiserum of equal quality could be produced with one cc per pig, would it not be better to use less virus?

Dr. Coggins: It would seem so.
Dr. Baker: If I interpreted your last table correctly, then I could produce HC antibody maximally in pigs by any one of four methods:

1. Hyperimmunize by injecting an immunized pig with five ml of virulent HC blood virus per pound of body weight.
2. Vaccinate a pig from an immune mother with live hog cholera virus and subsequently inoculate with one ml of virulent HC blood virus.
3. Vaccinate a susceptible pig with crystal violet vaccine and subsequently infect with one ml virulent HC blood virus per pig.
4. Vaccinate either a susceptible pig or a pig from an immune mother with BVD vaccine and subsequently infect with one ml virulent HC blood virus.

Dr. Coggins: That is correct.

Dr. Baker: In producing secondary response and HC antibody maximally, you used virulent HC virus. Have you tried attenuated HC virus?

Dr. Coggins: No I haven't.

Dr. Baker: Should attenuated HC virus be as good as virulent HC virus, would it then be possible to produce a hog cholera antiserum using BVD vaccine followed by attenuated HC virus?

Dr. Coggins: It should be tested. If it succeeded, then virulent HC virus could be eliminated in the production of HC antiserum.

REFERENCES

REPORT OF THE COMMITTEE ON TRANSMISSIBLE DISEASES OF SWINE


This Committee notes with satisfaction the progress that has been made during the past year by the several States in their development of a Cholera Eradication program and that the Federal Inter-State Regulations governing the movement of swine is now an accomplished fact. We believe that the responsible officials in the Animal Disease Eradication and Animal Inspection and Quarantine Divisions of the Agricultural Research Service and the members of the National Advisory Committee to the Secretary of Agriculture are to be commended for their accomplishment.

It is hoped that swine breeders, market operators, livestock dealers and Livestock Disease Control Officials will work cooperatively with the Federal Agencies in the enforcement of the Inter-State Regulations and in the development of effective laws and regulations for the comparable control of swine movements within their respective States. These should be designed to safeguard swine against excessive exposure not only to cholera but to all contagious and infectious diseases. These likewise should encourage a more direct and less time-consuming marketing process.

There is no longer good reason for any State to remain without adequate and effective laws and/or regulations which provide for the licensing of auction markets and dealers alike and for properly designed facilities which permit the maintenance of maximum standards of sanitation and the humane handling of livestock with a minimum of stress and exposure to disease. All concerned should make every effort to attain such laws and regulations.

The eradication of brucellosis will not be complete and final until the disease is at least eradicated from all domestic animals; therefore, this Committee believes that programs for the control and eradication of the disease in swine should be initiated by every State during the coming year. If eradication on an area basis is not legally possible or economically feasible, then serious consideration should be given to the following measures: (1) the testing of boars and stags at the market and/or slaughtering plant level; (2) a negative blood test on all breeding swine entering, moving, selling or exhibited within the State; (3) the identification of all animals reacting to the agglutination test with prompt movement to slaughter on permit; (4) mandatory reporting by veterinarians when clinical evidence and history indicate the presence of the disease; and, (5) the quarantining of infected herds.

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Diseases of swine have been given too little attention in our overall livestock disease control effort. Economic necessity is causing a greater awareness within the swine industry that the greatest potential for relief is in the more effective control and eradication of disease. Obviously swine producers are needing and seeking assistance from the profession and from their disease control officials. Established control and eradication procedures may not be effective against some of these diseases, or if effective, will be economically prohibitive. Disease control officials must be alert and receptive to new concepts or new and different approaches for their control. An example in mind is the market and slaughterhouse testing or screening procedure as used in our bovine brucellosis eradication program. Mandatory and prompt reporting of all contagious and infectious diseases, better utilization and reporting of postmortem information obtained at the slaughter plant are to be considered. Greater effort and cooperation from the meat inspection services at all levels should be expected; therefore, this Committee recommends that the Meat Inspection Division of the Agricultural Research Service authorize its personnel to cooperate with State Disease Control Officials and special swine producer groups, or organizations, by extending their inspection activity for such special diseases as Tuberculosis, Atrophic Rhinitis, Virus Pig Pneumonia, Erysipelas, Necrotic Enteritis, Cervical Abscesses and Brucellosis and the reporting of such information to the appropriate State Disease Control Official.

The value of a compulsory meat inspection service and the information which such a service can contribute to the overall livestock disease control effort within a State should not be overlooked; therefore, this Committee recommends to those States not presently having such a service, to develop one at the earliest possible date. We believe that the need for meat inspection can and should be justified as being essential to the more effective control of livestock diseases as well as being necessary for the protection of the consumer.

Experience during the past several years points up the inadequacy of design and management of swine testing stations. This Committee strongly recommends that the responsible persons or organization redesign their physical plant to permit strict isolation; higher standards of sanitation and better management practices necessary to prevent the spread of disease within the station and thence to breeding herds.

We wish to call your attention to the 1960 and 1961 reports of this Committee and the recommendations contained in the reports. These, for the most part, are still pertinent and should be reviewed. This Committee reaffirms these reports and recommends their activation where necessary.

Resolution - to Federal Meat Inspection Division of the Agriculture Research Service attached.
BOVINE VIRUS DIARRHEA (BVD) VACCINE FOR PROTECTION OF PIGS AGAINST HOG CHOLERA*


Ithaca, New York

The potential usefulness of bovine virus diarrhea (BVD) virus for the protection of pigs against hog cholera was reported in 1961 following BVD virus inoculation tests both in specific pathogen free (SPF) pigs and in field pigs. Results of these initial tests proved encouraging, but were considered inconclusive because variations in protection were observed for different strains of the BVD virus as well as for different tissue culture passage levels. SPF pigs, inoculated at six to 10 weeks of age with either BVD strain NY1 virus, maintained as infected spleen from a calf, or with strain C24V at the ninth serial passage level in tissue culture, survived a challenge test with virulent strain A hog cholera (HC) virus. They showed only a one or two day moderate rise in temperature as the only signs of illness. Field test pigs, however, inoculated at two weeks of age and with higher passage levels (11th to 31st) of C24V BVD virus showed only partial protection when challenged with virulent strain A HC virus; the response in this case indicated that as passage level increased, the effectiveness of protection decreased and also that possibly two weeks of age is too young for fully effective vaccination. These findings led to the tentative conclusion that maximum protection could be provided by BVD strain NY1 virus maintained at a very low passage level in tissue culture and administered at the age of weaning. To test the effectiveness of a BVD vaccine prepared and administered in this manner, a field study was conducted in swine herds from Florida, using virulent strain A HC virus as a challenge. This was followed by a similar study in New York State using virulent strain Ames challenge. The experimental design and the results of these two studies are presented below.

FIELD STUDIES

Preparation of vaccine. In the preparation of BVD vaccine for the Florida field trial, a 10 percent spleen emulsion from a calf infected with BVD NY1 strain virus was prepared, using fluid from the first tissue cultured passage of BVD strain NY1 virus as diluent. Vaccine was kept frozen at -50 C until used. This vaccine, labeled Batch one, titrated 10^6.5 per ml.

*This investigation was supported by the Office of Naval Research.
**Biometrics Unit, Department of Plant Breeding, Cornell University, Ithaca, New York.
***Veterinary Virus Research Institute, Cornell University, Ithaca, New York.
****Department of Agriculture, Division of Animal Industry, Tallahassee, Florida.
For the New York field trial, the first tissue cultured passage was considered seed virus and a second transfer was made. Fluids were harvested and mixed with equal portions of SPGA stabilizer and used either in the dried form or kept frozen at -50 C until used. This vaccine, designated Batch two, whether frozen or dried titrated $10^{5.7}$ per ml.

Because BVD strain NY1 virus was noncytopathogenic, titration of vaccine for virus content was accomplished by the cellular interference method. Tenfold dilutions of vaccine were prepared and 0.1 ml of a dilution was inoculated into each of five tubes of bovine embryo kidney tissue cultured cells. After incubation at 36 C for four days, the medium was changed and each tube was inoculated with 0.1 ml of a dilution of BVD strain C24V virus that contained 100 TCID$_{50}$. After further incubation for four to six days, tubes were examined and those not showing degeneration were recorded. A 50 percent endpoint was calculated by the Spearman-Karber method.

A standard was arbitrarily set that vaccine would not be used unless it titrated at least $10^4$ per ml. The exact amount of BVD virus required to protect a pig, however, has not yet been determined. In repeated tests, pigs inoculated with BVD infected calf spleen were protected when given 300 tissue culture titrated units. This would seem to indicate that Batches one and two contained more than enough virus to protect pigs. A $10^{-3}$ dilution (30,000 units) of Batch one protected pigs against a challenge test of virulent strain A HC virus.

**FLORIDA TRIAL**

_Vaccination procedure_. On February 8 and 9, 1962, BVD vaccine was administered in two litters of each of 11 swine herds from Jackson County, Florida. All pigs in these litters were bled at the time of vaccination and were identified individually by ear notches. In every litter each of five pigs was vaccinated by intramuscular injection of two ml of BVD vaccine (batch one), while the remaining pigs in each litter were left unvaccinated.

No selection for management or immunization practices was exercised in choosing these eleven herds. However, a good cross-section of management practices was represented, ranging from excellent to below average. This study included pigs from herds in which no vaccination was practiced to herds where annual vaccinations with modified live HC virus plus serum were practiced.

The number of pigs to be vaccinated was arrived at from consideration of the sequential decision rule to be used in the challenge phase of the experiment. Vaccinated pigs were to be challenged in successive lots until the chart in Figure 1 dictated that tests be terminated with either acceptance or rejection of the vaccine. This chart was constructed to test for a standard vaccine efficacy of 90 percent, and its construction incorporated the assumption that without vaccination at least 95 percent of these pigs challenged would develop identifiable signs of hog cholera resulting in death. Any vaccination procedure which exceeds the 90 percent standard efficacy by more than three percent stands at least a 19 in 20 chance of passing this sequential test, and any vaccination procedure poorer than
the standard by more than three percent stands at least a 19 in 20 chance of failing the test.

On the basis of the previous experience with BVD virus inoculation, the vaccine was anticipated to be either highly effective (near 100 percent) or grossly ineffective (below 50 percent). For any specific value of efficacy, the expected number of test challenges required for the sequential experiment can be deduced from the mathematical properties of the decision rule. For example, if the true efficacy is 98 percent, then the expected number of challenges required to reach a decision is 60; if the true efficacy is 95 percent, then the expected number of challenges is 110, and if the true efficacy is below 50 percent then the expected number of challenges is less than 30. Since the higher efficacy was anticipated, the number of pigs to be vaccinated and made available for challenge was set at approximately 100, and the final figure of 110 vaccinates was then chosen to allow an extra margin of safety.

One herd was later eliminated from the study when a pre-challenge serological test revealed that all of the experimental pigs from this herd, both BVD vaccinates and their littermate controls, had developed high titers for hog cholera. In addition, two other BVD vaccinates from separate herds showed high HC titers before challenge and hence were eliminated from the test; one of these, with a pre-challenge HC titer of 7300 was suspected of having been vaccinated for hog cholera and the second, for some unknown reason, had developed a HC titer of 90 at pre-challenge. The total number of usable BVD vaccinates available for the challenge test
was thus reduced from 110 to 98. As the sequential test turned out, it was necessary to challenge only 70 of these 98 in order to reach a decision.

**Challenge procedure.** Beginning on April 24, 1962, two and a half months after vaccination, groups of test pigs were brought in at bi-weekly intervals for challenge at the Florida State Diagnostic Laboratory, Cottondale, Florida. The first lot consisted of a litter of five BVD vaccinates and two littermate controls from each of five herds, and the subsequent two lots were similarly composed. These pigs were bled and inoculated intramuscularly with one ml of a 10 percent spleen emulsion that contained virulent strain A HC virus. For a two week period following challenge daily temperatures were taken, failures to feed were noted, and time of death recorded. A pig was considered to be adequately protected if it continued to feed and showed no signs of illness other than an elevated temperature of short duration. Survival after prolonged illness was considered a protection failure. To confirm that deaths were due to hog cholera, autopsies of all dead pigs were performed by Dr. Donald Nelson, Director of the Laboratory, who also followed the surviving pigs to slaughter one month later and examined them for residual lesions. Further confirmation that deaths were caused by HC virus was sought by histological examinations of brains by Dr. W. Sippel, Diagnostic Laboratories Section, Kissimmee, Florida. Serum samples were taken from all surviving pigs three weeks after the challenge.

**Results.** Experimental results on the individual test pigs are presented in Table I, which shows the HC and BVD antibody titers at pre-vaccination, pre-challenge and post-challenge stages and indicates the clinical response to challenge in terms of duration of elevated temperature and percent protection based upon the ARS appetite scoring procedure. Vaccinated pigs are identified by a V-number in this table and controls are assigned a C-number.

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</tr>
<tr>
<td></td>
<td></td>
<td>V-88</td>
<td>2 0 22</td>
<td>773 3200</td>
<td>7</td>
<td>96</td>
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<tr>
<td></td>
<td></td>
<td>V-89</td>
<td>0 0 22</td>
<td>560 1600</td>
<td>0</td>
<td>100</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>C-85</td>
<td>2 0 0</td>
<td>--- ---</td>
<td>--- died HC</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>V-90</td>
<td>4 0 12</td>
<td>--- ---</td>
<td>8</td>
<td>94</td>
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</tr>
<tr>
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<td></td>
<td>V-91</td>
<td>4 0 32</td>
<td>560 3162</td>
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<td></td>
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<td></td>
<td>V-93</td>
<td>4 0 22</td>
<td>560 3200</td>
<td>3</td>
<td>98</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>V-94</td>
<td>32 0 22</td>
<td>2793 3200</td>
<td>3</td>
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<td></td>
<td></td>
<td>C-99</td>
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<td>--- died HC</td>
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<td></td>
<td></td>
<td>V-100</td>
<td>0 0 2 2</td>
<td>560 3855</td>
<td>3</td>
<td>98</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>V-101</td>
<td>0 0 4 560</td>
<td>2793 3</td>
<td>3</td>
<td>98</td>
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<td></td>
<td></td>
<td>V-102</td>
<td>0 0 4 560</td>
<td>2793 4</td>
<td>100</td>
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<td></td>
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<td>V-103</td>
<td>0 0 12 560</td>
<td>2793 3</td>
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<tr>
<td></td>
<td></td>
<td>V-104</td>
<td>0 22 22 1884</td>
<td>9441 1</td>
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<td></td>
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<td>C-105</td>
<td>0 0 0</td>
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<td>--- died HC</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>C-106</td>
<td>0 0 0</td>
<td>--- ---</td>
<td>--- died HC</td>
<td>0</td>
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<tr>
<td></td>
<td>HC VD HC VD HC VD Fever** %Prot.***</td>
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<tr>
<td>V-110</td>
<td>0 0 -- 560 3200 7 100</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>V-111</td>
<td>0 0 32 2793 320 5 100</td>
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</tr>
<tr>
<td>V-112</td>
<td>0 0 22 2793 3200 4 100</td>
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<td></td>
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</tr>
<tr>
<td>V-113</td>
<td>0 0 -- 111 3200 5 98</td>
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<td></td>
</tr>
<tr>
<td>V-114</td>
<td>0 0 0 22 560 1000 6 98</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>C-115</td>
<td>0 0 -- -- 3200 5 100</td>
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<tr>
<td>C-116</td>
<td>0 0 0 0 died HC</td>
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</tr>
<tr>
<td>V-130</td>
<td>0 0 2 111 560 9441 3 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-131</td>
<td>0 0 22 560 3855 2 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-132</td>
<td>0 0 4 12 1884 2793 2 100</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>V-133</td>
<td>0 0 4 22 2793 3855 2 100</td>
<td></td>
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<tr>
<td>V-134</td>
<td>0 0 22 74 1884 14000 1 100</td>
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</tr>
<tr>
<td>C-136</td>
<td>0 0 0 died HC</td>
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<td></td>
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</tr>
<tr>
<td>C-138</td>
<td>0 0 0 died HC</td>
<td></td>
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</tr>
<tr>
<td>V-140</td>
<td>0 0 0 22 560 3200 2 98</td>
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<td></td>
</tr>
<tr>
<td>V-141</td>
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<td></td>
<td></td>
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<tr>
<td>V-142</td>
<td>0 0 22 2793 3200 0 100</td>
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</tr>
<tr>
<td>V-143</td>
<td>0 0 74 773 3200 3 98</td>
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</tr>
<tr>
<td>V-144</td>
<td>0 0 0 32 560 32000 3 98</td>
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<td></td>
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<tr>
<td>C-145</td>
<td>0 0 0 died HC</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>V-150</td>
<td>0 0 0 22 2793 3200 2 94</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>V-151</td>
<td>0 0 4 12 2793 3200 1 98</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>V-152</td>
<td>0 0 22 2793 6310 2 94</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>V-153</td>
<td>0 0 22 560 15850 2 98</td>
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<td></td>
</tr>
<tr>
<td>V-154</td>
<td>0 0 22 560 3200 1 98</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>C-155</td>
<td>0 0 0 died HC</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>C-156</td>
<td>0 0 0 died HC</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>C-157</td>
<td>0 0 0 died HC</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>V-163</td>
<td>0 0 0 12 --- died HC</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>V-164</td>
<td>4 0 0 4 2793 10000 1 100</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C-165</td>
<td>0 0 0 died HC</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C-166</td>
<td>0 0 0 died HC</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>C-168</td>
<td>0 0 0 died HC</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>V-182</td>
<td>12 0 0 22 1884 3200 0 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-183</td>
<td>1 0 0 22 2793 10000 3 100</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C-185</td>
<td>20 0 0 died HC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Titers expressed as reciprocal of 50 percent endpoint as determined by Spearman-Karber method.

**Defined as the number of days of temperatures above 104 F.

***Percent protection based upon observations on appetites scored according to ARS procedure.
The numbers of survivors among BVD vaccinates and controls are summarized by litter and test lot in Table II. In total, two of the 70 BVD vaccinates died and 31 of the 32 controls died. Among the 68 surviving

**Table II**
Sequential Results in the Florida Field Trial of Pigs Inoculated with Virulent Strain A HC Virus Following Vaccination with BVD Virus

<table>
<thead>
<tr>
<th>Test 1 - April 24, 1962</th>
<th>Herd</th>
<th>Litter</th>
<th>No Animal Surviving</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>1</td>
<td>5/5</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1</td>
<td>5/5</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>1</td>
<td>5/5</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>2</td>
<td>5/5</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>1*</td>
<td>4/4</td>
<td>0/2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>24/24</td>
<td>1/12</td>
</tr>
</tbody>
</table>

*The fifth vaccinate died before challenge from parasitism.

<table>
<thead>
<tr>
<th>Test 2 - May 8, 1962</th>
<th>Herd</th>
<th>Litter</th>
<th>No Animal Surviving</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>J</td>
<td>3</td>
<td>5/5</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>1</td>
<td>4/5</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1</td>
<td>5/5</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>1,2</td>
<td>6/6</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>1</td>
<td>5/5</td>
<td>0/3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>25/26</td>
<td>0/11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test 3 - May 22, 1962</th>
<th>Herd</th>
<th>Litter</th>
<th>No Animal Surviving</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>1*</td>
<td>4/4</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>2</td>
<td>5/5</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>1</td>
<td>2/2</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>2</td>
<td>5/5</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>2,3</td>
<td>3/4</td>
<td>0/3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>19/20</td>
<td>0/9</td>
</tr>
</tbody>
</table>

*One of the vaccinates developed high antibody titer to HC prior to challenge supposedly from mistaken HC vaccination.

<table>
<thead>
<tr>
<th>Vaccinates</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1</td>
<td>24/24</td>
</tr>
<tr>
<td>Test 2</td>
<td>25/26</td>
</tr>
<tr>
<td>Test 3</td>
<td>19/20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>68/70</td>
</tr>
</tbody>
</table>
BVD vaccinates, signs of illness ranged from 100 percent protection with no temperature elevation to 90 percent protection with an eight day period of elevated temperature. The distribution over this range is shown in Table III; a temperature elevation persisted for an average of less than four days and the off-feed period averaged less than one day. All pigs that died showed gross and histological lesions of hog cholera; survivors showed no lesions at the time of slaughter.

**TABLE III**

Summary of Feeding and Temperature Response of Test Pigs

<table>
<thead>
<tr>
<th>Number of Days off Feed</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinates</td>
<td>41*</td>
<td>17</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>69</td>
</tr>
<tr>
<td>Controls</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1**</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration of Temperature Elevation (greater than 10^4°C)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinates</td>
<td>5</td>
<td>9*</td>
<td>11</td>
<td>16</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>1*</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>69</td>
</tr>
<tr>
<td>Controls</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4**</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

*One vaccinate in this class died of hog cholera.
**One control in this class survived hog cholera.

The experiment was terminated after 68 successes and two failures because at this point, as shown in Figure 2, the sequential chart called for a terminal decision to accept the vaccine. A mortality of 31/32 among the controls confirmed our assumption that at least 95 percent of the unvaccinated pigs would succumb to hog cholera, thus confirming the validity of our sequential decision rule.

**NEW YORK TRIAL**

Although efficacy of greater than 90 percent was shown by the Florida field trial in which strain A was used as the test HC virus, other workers felt that another strain of HC virus also should be tested. This seemed an excellent idea, and plans were made to repeat the Florida field trial in New York pigs using the different virus to test efficacy.

*Materials and Methods.* As in Florida, pigs were located on New York farms and, when six to eight weeks of age, each of five pigs in a litter were inoculated with five ml of Batch two BVD vaccine. Two littersmates were retained uninoculated as susceptible controls. One month after vaccination, 10 litters were placed in isolation units at the Veterinary Virus Research Institute and each pig was given two ml of blood that contained virulent strain Ames HC virus (serial 319) prepared at the National Disease Laboratory, Ames, Iowa. (This was furnished through the courtesy of Doctors Johnson and Mott, Animal Disease and Parasite Research Di-
vision, Agricultural Research Service). In an effort to save time, all 10 litters were given a challenge test inoculation simultaneously.

**Results.** As can be seen in Figure 3, after five vaccinates were tested, greater than 90 percent efficacy became impossible. Of 10 litters tested, one showed efficacy; of the 45 pigs in the remaining nine litters, 11 survived and all but one were too ill to be considered acceptable for efficacy. Actually, because most pigs were ill on the third day after challenge, the New York trial was declared "no test."

"No test" is understood to mean that either the pigs were not suitable or else the test virus was not. Because the pigs appeared good subjects for test, it seemed worthwhile to compare some of the features of illness produced by strain Ames with that produced by strain A. The following observations have been made:

<table>
<thead>
<tr>
<th>Features</th>
<th>Ames</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Temperature elevation</td>
<td>1 to 2</td>
<td>2 days</td>
</tr>
<tr>
<td>(Figure 4)</td>
<td>higher</td>
<td>lower</td>
</tr>
<tr>
<td>(a) onset</td>
<td>3 days</td>
<td>5 days</td>
</tr>
<tr>
<td>(b) peak</td>
<td>5 to 6</td>
<td>Absent or terminal</td>
</tr>
<tr>
<td>2. Loss of appetite</td>
<td>Prolonged</td>
<td>Normal</td>
</tr>
<tr>
<td>3. Diarrhea</td>
<td>7 to 12</td>
<td>10 to 12 days</td>
</tr>
<tr>
<td>4. Coagulation time</td>
<td>About 10%</td>
<td>90% efficacy</td>
</tr>
<tr>
<td>5. Death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. BVD Vaccinates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3. A Plot of the Sequential Results of the New York Trial.

Figure 4. Comparison of Temperatures and Appetite Between Ames HC Virus and Strain A HC Virus.
Isolation of RFF (Rabbit Fever Factor). Because of the possibility that test virus may not have been suitable, a search began for an adventitious agent. The following procedures were utilized, all aimed at losing HC virus in order to capture any other agent that might be present. (1) Ames blood virus was serially transferred in rabbits at two-day intervals. Initially a rabbit was given one ml of Ames blood virus intravenously; then, two days later, the rabbit was bled and one ml of its blood was inoculated intravenously into another rabbit. Rabbits in each passage showed an elevated temperature of one to two degrees F; thence the designation of RFF was chosen. After three transfers, one ml of blood from the last rabbit was inoculated intramuscularly into a young pig. (2) In another attempt, Ames blood virus was fed to a pig four weeks of age that had nursed an immune sow. (3) Exposure of a pig in a circumstance that did not permit transmission of HC virus.

All pigs, whether given rabbit material, fed or exposed, showed temperature elevations that began one to three days later and lasted one to three days. They developed diarrhea on the day following initial temperature elevation. None died except those killed for preservation of the infective agent.

Ames blood virus was inoculated into a pig that had recovered from RFF. It continued to eat for five days after inoculation, at which time the pig was bled and its blood inoculated into a SPF pig. At the same time, a littermate was given strain A. Comparison between the responses of these two pigs showed a higher temperature in the A inoculated pig than was produced by Ames. No diarrhea was seen in either pig. Because the pigs were killed for blood collection, comparison beyond seven days was not possible, but the impression was obtained that Ames and A now were very similar. Of course, the most important comparative feature is response of BVD vaccinated pigs to inoculation of HC virus because this was the feature that revealed RFF. Work is in progress at the present time to determine whether RFF was responsible for loss of efficacy. Further reports will be made on this subject.

DISCUSSION

The principle that heterotypic VD virus protects pigs against virulent HC virus was well established by the Florida field trial. Use of only strain A HC virus, however, has left the question of whether BVD virus should be considered as a practical vaccine until it has protected pigs against other strains of HC virus, especially those thought by some to be more virulent than strain A, although strain A itself is lethal. When another strain was tested that produced earlier signs of illness and killed in a shorter period of time, it began to seem that BVD could not become a practical vaccine.

In the present state of our knowledge of virology, propagation and maintenance of a particular strain of virus without impurity is a difficult task and requires considerable testing in order to assure purity. The HC virus used for challenge test purposes ordinarily is produced by taking blood from one pig of unknown status and transferring it to another pig of similar status. This method almost inevitably leads to contaminations,
sooner or later. A good example of similar contamination would be the former methods of maintenance of canine distemper virus by transfer from dog to dog; eventually there would occur a mixture of canine distemper virus (CD) and infectious canine hepatitis (ICH) virus and, if these two viruses then were inoculated simultaneously, severity of illness produced would be greater than by either virus alone. In a method similar to that of BVD-HC in pigs, a dog can be given measles virus for effective protection against subsequent inoculation of virulent CD virus; but neither the measles virus nor the CD virus can protect the dog against ICH virus, which is in a different group. The marked increase in severity of signs of illness following Ames HC virus when compared to the strain A HC virus was reminiscent of the results that had been found with the mixture of CD and ICH viruses. The finding of RFF was not surprising, therefore.

Because distemper and hepatitis occurred so frequently in young puppies, for their better protection, a combination vaccine was produced. Today, the majority of veterinary practitioners use it in preference to anything else and dog owners are aware of its value. If this analogy is to be extended similarly to swine, thought should be given a combination vaccine for swine. Pigs are of considerable economic importance. If further search reveals that HC virus and RFF are occurring together with any frequency or if either HC or RFF are occurring with any other disease-producing agent, suitable combinations might fill the economic need for disease prevention which is greatly desired by swine producers.

Singly or in combination, however, BVD vaccine should be considered in the hog cholera eradication program because it has the following attributes:

1. BVD vaccine can be standardized by laboratory assay.
2. Any amount of BVD vaccine can be made readily because it is a tissue cultured product.
3. Bovine embryos are used for tissue culture of BVD virus, a tissue culture system thought to be free of any contaminating viruses that might be pathogenic for pigs.
4. Hog cholera antibody does not interfere with efficacy of BVD vaccine; hog cholera antiserum can be given before, during or after BVD vaccination if this antiserum is free of BVD antibody.
5. BVD virus has been shown to be safe, because it cannot spread from vaccinated pigs to other pigs nor from vaccinated pigs to susceptible cattle.
6. Because it is not hog cholera virus BVD virus cannot be responsible for any hog cholera outbreak.

ACKNOWLEDGEMENTS

Mr. W. W. Glenn, County Agent, Jackson County, Florida, made the trial in Florida possible. We wish to thank him and his assistants. The cooperation and technical advice provided by Dr. John Hejl, Dr. G. V. Peacock, Dr. G. Davidson and Dr. L. Sinclair of the AIQ Division, Dr. F. Mulhern, Dr. G. Wise and Dr. M. Tillery of ADE Division and Dr. H. Johnson, Dr. L. O. Mott and Dr. M. Zinober of ADP Division of ARS has
been invaluable and our appreciation is expressed herewith. Also, we would like to express our appreciation to Dr. D. B. H. Dalrymple and Dr. R. Fuller, Supervising Veterinarians, New York State Institution Farms, Dr. A. Tice and Dr. G. Kaley, Division of Animal Industry for their assistance in the New York trial. Our appreciation also to Mrs. Fran Barnes for preparation of the charts and to Mrs. Dorothy Edgar for preparation of the manuscript.

REFERENCES

PROGRESS REPORT OF THE EXPERIMENT ON THE ERADICATION OF HOG CHOLERA IN THE FLORIDA PILOT TEST AREA, FISCAL YEARS 1961 AND 1962


The method of operation of the Hog Cholera Research Station at Live Oak, Suwannee County, Florida, was described in reports to this Association in 1958,1 19592 and 1960.3 The present report covers the activities of this station during fiscal years 1961 and 1962.

In fiscal years 1961 and 1962, 60,722 farm pigs in 1936 herds were vaccinated with lapine origin, porcine origin or tissue culture modified live virus hog cholera vaccines simultaneously with a minimum dose of 15 ml. of serum. Table I shows the approximately equal distribution of the vaccinates among the three different types of vaccine used during the two year period and the average serum dose. Table II shows the percentages of swine vaccinated each month of the total swine vaccinated each fiscal year.

Challenges of vaccinated hogs were carried out in the manner described to this Association in 1958.1 The challenge virus used was identified as Hog Cholera Research Station virus serial number 1B, and was prepared in a manner similar to the preparation of Hog Cholera Research Station virus serial number 1 described in the 19581 report. Hog Cholera Research Station virus serial number 1B had an LD50 of 10^-5 per 5.0 ml.

The challenge results of fiscal years 1961 and 1962 are shown in Table III by twelve month periods. The method of preparation of this table is described in the report to this Association in 1960.3 These data are illustrated in Graph 1 by percentages only.

It will be noted from a comparison of the challenge results as reported in 19603 that lapine origin vaccines improved slightly and porcine origin vaccines improved markedly in immunogenic efficacy from fiscal year 1960 to fiscal year 1962 and that tissue culture vaccines declined slightly from fiscal year 1960 to fiscal year 1962.

As a result of the gradual decline in the immunogenic efficiency for all types of modified live virus vaccines during calendar year 1959, reported in 19603 which appeared to be associated with the vaccine storage age at the time of vaccine administration, we recommended the change made in the vaccine age procurement requirements on November 1, 1960, which resulted in the field use of fresher vaccine. This change was accomplished by the Florida Livestock Board, now the Florida Division of Animal Industry, changing the vaccine bid specifications for procurement

TABLE I
Vaccination of Farm Pigs With Modified Live Virus Vaccines in Suwannee County, Florida, During Fiscal Years 1961 and 1962

<table>
<thead>
<tr>
<th>Fiscal Year</th>
<th>Vaccine</th>
<th>Number of Pigs and Herds Vaccinated</th>
<th>Pigs</th>
<th>Herds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Percent of Total</td>
<td>Average Serum Dose (ml.)</td>
</tr>
<tr>
<td>1961</td>
<td>Lapine Origin</td>
<td>10819</td>
<td>35.0</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>Porcine Origin</td>
<td>11851</td>
<td>38.4</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>Tissue Culture</td>
<td>8202</td>
<td>26.6</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30872</td>
<td>100.0</td>
<td>17.8</td>
</tr>
<tr>
<td>1962</td>
<td>Lapine Origin</td>
<td>9307</td>
<td>31.2</td>
<td>19.3</td>
</tr>
<tr>
<td></td>
<td>Porcine Origin</td>
<td>10931</td>
<td>36.6</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>Tissue Culture</td>
<td>9612</td>
<td>32.2</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>29850</td>
<td>100.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Total</td>
<td>Lapine Origin</td>
<td>20126</td>
<td>33.1</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>Porcine Origin</td>
<td>22782</td>
<td>37.5</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>Tissue Culture</td>
<td>17814</td>
<td>29.3</td>
<td>21.0</td>
</tr>
<tr>
<td>Grand Total</td>
<td></td>
<td>60722</td>
<td>100.0</td>
<td>17.9</td>
</tr>
</tbody>
</table>

TABLE II
Number and Percent of Swine Vaccinated Each Month of the Total Swine Vaccinated During Fiscal Years 1961 and 1962 in Suwannee County, Florida

<table>
<thead>
<tr>
<th>Month</th>
<th>Fiscal Year 1961</th>
<th>Fiscal Year 1962</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Pigs</td>
<td>Percent of Total</td>
</tr>
<tr>
<td>July</td>
<td>3328</td>
<td>10.8</td>
</tr>
<tr>
<td>August</td>
<td>2555</td>
<td>8.3</td>
</tr>
<tr>
<td>September</td>
<td>1974</td>
<td>6.4</td>
</tr>
<tr>
<td>October</td>
<td>2617</td>
<td>8.5</td>
</tr>
<tr>
<td>November</td>
<td>2902</td>
<td>9.4</td>
</tr>
<tr>
<td>December</td>
<td>1979</td>
<td>6.4</td>
</tr>
<tr>
<td>January</td>
<td>2413</td>
<td>7.8</td>
</tr>
<tr>
<td>February</td>
<td>3051</td>
<td>9.9</td>
</tr>
<tr>
<td>March</td>
<td>2608</td>
<td>8.4</td>
</tr>
<tr>
<td>April</td>
<td>2764</td>
<td>8.9</td>
</tr>
<tr>
<td>May</td>
<td>2252</td>
<td>7.3</td>
</tr>
<tr>
<td>June</td>
<td>2429</td>
<td>7.9</td>
</tr>
<tr>
<td>Total</td>
<td>30872</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Graph 1. Challenge of Vaccinated Hogs in Suwannee County, Florida
### TABLE III

Results of Challenge of Hogs Vaccinated With Modified Live Virus Vaccine By Twelve-Month Periods During Fiscal Years 1961 and 1962 In Suwannee County, Florida

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Lapine Origin Adequately Protected</th>
<th>Porcine Origin Adequately Protected</th>
<th>Tissue Culture Adequately Protected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>8/1/59- 7/31/60</strong></td>
<td>315/364</td>
<td>153/249</td>
<td>66/72</td>
</tr>
<tr>
<td><strong>9/1/59- 8/31/60</strong></td>
<td>322/372</td>
<td>160/257</td>
<td>78/84</td>
</tr>
<tr>
<td><strong>10/1/59- 9/30/60</strong></td>
<td>304/354</td>
<td>164/267</td>
<td>90/99</td>
</tr>
<tr>
<td><strong>11/1/59-10/31/60</strong></td>
<td>279/333</td>
<td>161/263</td>
<td>87/96</td>
</tr>
<tr>
<td><strong>12/1/59-11/30/60</strong></td>
<td>257/313</td>
<td>146/244</td>
<td>100/107</td>
</tr>
<tr>
<td><strong>1/1/60-12/31/60</strong></td>
<td>223/272</td>
<td>130/226</td>
<td>116/124</td>
</tr>
<tr>
<td><strong>2/1/60- 1/31/61</strong></td>
<td>194/244</td>
<td>128/220</td>
<td>139/148</td>
</tr>
<tr>
<td><strong>3/1/60- 2/28/61</strong></td>
<td>184/235</td>
<td>137/226</td>
<td>155/162</td>
</tr>
<tr>
<td><strong>4/1/60- 3/31/61</strong></td>
<td>166/215</td>
<td>135/225</td>
<td>183/193</td>
</tr>
<tr>
<td><strong>5/1/60- 4/30/61</strong></td>
<td>175/218</td>
<td>137/219</td>
<td>195/207</td>
</tr>
<tr>
<td><strong>6/1/60- 5/31/61</strong></td>
<td>187/220</td>
<td>149/214</td>
<td>192/206</td>
</tr>
<tr>
<td><strong>7/1/60- 6/30/61</strong></td>
<td>194/230</td>
<td>158/221</td>
<td>193/210</td>
</tr>
<tr>
<td><strong>8/1/60- 7/31/61</strong></td>
<td>198/232</td>
<td>159/219</td>
<td>186/204</td>
</tr>
<tr>
<td><strong>9/1/60- 8/31/61</strong></td>
<td>197/232</td>
<td>162/221</td>
<td>180/198</td>
</tr>
<tr>
<td><strong>10/1/60- 9/30/61</strong></td>
<td>191/227</td>
<td>166/217</td>
<td>171/187</td>
</tr>
<tr>
<td><strong>11/1/60-10/31/61</strong></td>
<td>216/250</td>
<td>185/238</td>
<td>185/207</td>
</tr>
<tr>
<td><strong>12/1/60-11/30/61</strong></td>
<td>216/251</td>
<td>228/287</td>
<td>177/203</td>
</tr>
<tr>
<td><strong>1/1/61-12/31/61</strong></td>
<td>240/275</td>
<td>243/297</td>
<td>183/208</td>
</tr>
<tr>
<td><strong>2/1/61- 1/31/62</strong></td>
<td>256/286</td>
<td>260/313</td>
<td>179/204</td>
</tr>
<tr>
<td><strong>3/1/61- 2/28/62</strong></td>
<td>266/293</td>
<td>271/320</td>
<td>168/196</td>
</tr>
<tr>
<td><strong>4/1/61- 3/31/62</strong></td>
<td>280/315</td>
<td>275/327</td>
<td>153/184</td>
</tr>
<tr>
<td><strong>5/1/61- 4/30/62</strong></td>
<td>280/313</td>
<td>285/333</td>
<td>145/174</td>
</tr>
<tr>
<td><strong>7/1/61- 6/30/62</strong></td>
<td>260/295</td>
<td>278/321</td>
<td>165/191</td>
</tr>
</tbody>
</table>

*Numerator – number of pigs adequately protected.
Denominator – number of pigs challenged.
**Results for fiscal year 1961.
***Results for fiscal year 1962.

from not less than 12 months prior to the expiration date to not more than 90 days after the production date. The dramatic effect from using fresher modified live virus vaccines after November, 1960, is illustrated in Graph 1, for lapine and porcine origin vaccine which had the correspondingly longest vaccine age prior to the time of change.

The inverse relationship between age of the three types of vaccine and percentage of adequately protected pigs after challenge in the pilot eradication area is also demonstrable during the entire six year period, 1957-1962, of the operation of our station. These data are presented in Tables IV, V and VI and illustrated in Graphs 2, 3 and 4 for lapine origin, porcine origin and tissue culture vaccines, respectively. There is one exception,
Graph 2. Vaccine Age (Shelf-Life) and Percentage of Pigs Adequately Protected by Lapine Origin Modified Live Virus Vaccine in Suwannee County, Florida
Graph 3. Vaccine Age (Shelf-Life) and Percentage of Pigs Adequately Protected by Porcine Origin Modified Live Virus Vaccine in Suwannee County, Florida
Graph 4. Vaccine Age (Shelf-Life) and Percentage of Pigs Adequately Protected by Tissue Culture Origin Modified Live Virus Vaccine in Suwannee County, Florida
TABLE IV

Vaccine Age (Shelf-Life) and Percentage of Pigs Adequately Protected by Lapine Origin Modified Live Virus Vaccine in Suwannee County, Florida

<table>
<thead>
<tr>
<th>Fiscal Years</th>
<th>Vaccine Age (Shelf-Life) in Days</th>
<th>Adequately Protected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number* of Pigs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percent</td>
</tr>
<tr>
<td>1957</td>
<td>342.6</td>
<td>65/67</td>
</tr>
<tr>
<td>1958</td>
<td>455.0</td>
<td>389/415</td>
</tr>
<tr>
<td>1959</td>
<td>509.2</td>
<td>137/168</td>
</tr>
<tr>
<td>1960</td>
<td>332.9</td>
<td>311/358</td>
</tr>
<tr>
<td>1961</td>
<td>424.0</td>
<td>194/230</td>
</tr>
<tr>
<td>1962</td>
<td>151.6</td>
<td>260/295</td>
</tr>
</tbody>
</table>

*Numerator = number of pigs adequately protected. Denominator = number of pigs challenged.

TABLE V

Vaccine Age (Shelf-Life) and Percentage of Pigs Adequately Protected by Porcine Origin Modified Live Virus Vaccine in Suwannee County, Florida

<table>
<thead>
<tr>
<th>Fiscal Years</th>
<th>Vaccine Age (Shelf-Life) in Days</th>
<th>Adequately Protected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number* of Pigs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percent</td>
</tr>
<tr>
<td>1957</td>
<td>215.9</td>
<td>79/83</td>
</tr>
<tr>
<td>1958</td>
<td>390.3</td>
<td>250/278</td>
</tr>
<tr>
<td>1959</td>
<td>395.3</td>
<td>176/215</td>
</tr>
<tr>
<td>1960</td>
<td>564.4</td>
<td>146/237</td>
</tr>
<tr>
<td>1961</td>
<td>468.9</td>
<td>158/221</td>
</tr>
<tr>
<td>1962</td>
<td>206.3</td>
<td>278/321</td>
</tr>
</tbody>
</table>

*Numerator = number of pigs adequately protected. Denominator = number of pigs challenged.

however, in the inverse relationship. In tissue culture vaccine there was a significant decline in the average age of the vaccines from 471.4 days in fiscal year 1961, to 261.9 days in fiscal year 1962, but the percentage of adequately protected pigs after challenge remained about the same, 87.6 percent in fiscal year 1961 and 86.4 percent in fiscal year 1962.

It should be pointed out that, with the exception noted above, the inverse relationship between the average age of the vaccines and the percentage of adequately protected pigs after challenge is perfect; in most respects, it is also correlative. For example, in lapine origin vaccine there is a decrease in the average age of the vaccine of 272.4 days.
TABLE VI
Vaccine Age (Shelf-Life) and Percentage of Pigs Adequately Protected by Tissue Culture Origin Modified Live Virus Vaccine in Suwannee County, Florida

<table>
<thead>
<tr>
<th>Fiscal Years</th>
<th>Vaccine Age (Shelf-Life) In Days</th>
<th>Adequately Protected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number* of Pigs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1957</td>
<td>319.8</td>
<td>46/47</td>
</tr>
<tr>
<td>1958</td>
<td>455.6</td>
<td>422/450</td>
</tr>
<tr>
<td>1959</td>
<td>553.4</td>
<td>223/257</td>
</tr>
<tr>
<td>1960</td>
<td>394.4</td>
<td>57/63</td>
</tr>
<tr>
<td>1961</td>
<td>471.4</td>
<td><strong>155/177</strong></td>
</tr>
<tr>
<td>1962</td>
<td>261.9</td>
<td>165/191</td>
</tr>
</tbody>
</table>

*Numerator - number of pigs adequately protected.
Denominator - number of pigs challenged.
**Groups of less than 10 pigs per time unit were eliminated in this study which accounts for any difference in numbers and percentage from Table 3.

between fiscal years 1961 and 1962, and a corresponding increase in the percentage of adequately protected pigs of 3.8 percentage points. This is equivalent to a decrease of 0.01 percentage point for each day of increase in the average age of the vaccine between these two fiscal years. During fiscal years 1957, 1958, 1959, 1960 and 1961, there was a change of 0.03 percentage point for each day of change in the average age of the vaccine. In lapine origin vaccines, therefore, this figure varied from 0.01 to 0.03 percentage point, both of which are within the limits of experimental error. In porcine origin vaccine, the range of these figures is from 0.03 to 1.6 percentage points, which are also within the limits of experimental error. In tissue culture vaccine, there is a variation from 0.01 to 0.07 percentage point of change in percentage of adequately protected pigs for each day of change in the average age of the vaccine, both of which are also within the limits of experimental error.

It is significant that the foregoing results were derived from the challenge of 4,073 swine vaccinated with 31 serial numbers of lapine origin vaccine from seven licensees, 26 serial numbers of porcine origin vaccine from six licensees and 28 serial numbers of tissue culture vaccine from two licensees during the period 1957 through fiscal year 1962.

During fiscal years 1961 and 1962, a total of nine serials of serum were used in various combinations with all types of vaccine. Since the average serum dose was closely maintained between 15 and 20 ml. (see Table I), no valid conclusions can be drawn regarding the effect of serum serials on the changes in the immunological picture.

During fiscal years 1961 and 1962, a total of 29 serials of all types of vaccine were used. In fiscal year 1961, the percentage of pigs that were adequately protected by lapine origin vaccine serials ranged from 66.7
percent to 88.2 percent, with porcine origin serials from 58.8 percent to 88.6 percent and with tissue culture serials from 71.9 percent to 95.6 percent. In fiscal year 1962, the percentage of pigs which were adequately protected by lapine origin vaccine serials ranged from 75.0 percent to 91.7 percent, with porcine origin serials from 75.0 percent to 100 percent and with tissue culture serials from 66.7 percent to 98.0 percent.

It is interesting to note that, in fiscal year 1961, there were six serials which protected less than 80 percent of pigs and nine serials which protected more than 80 percent. In contrast to this, in fiscal year 1962, when the average age of the vaccines was much lower (see Tables IV, V and VI) four serials protected less than 80 percent of pigs and 15 serials protected more than 80 percent. There were five serials which were used in both years which accounts for the fact that a total of 34 serials are mentioned above whereas actually only 29 serials were used.

ERADICATION OF HOG CHOLERA

At this time, it cannot be said that hog cholera has been eradicated from the pilot eradication area. Although one 12 month period of freedom from hog cholera was reported to this Association in 1961 by Campbell, there have been 16 cases confirmed since July 1, 1960 (Table VII). Thirteen of these cases were in fiscal year 1961, and three were in fiscal year 1962.

The most salient feature of Table VII is that 88.9 percent of the positive cases were in farm-raised herds compared to only 52.5 percent reported to this Association in 1960. In six of the eight positive cases in vaccinated swine, the outbreaks occurred from five to 17 days following vaccination and in the other two cases the outbreaks occurred more than one month following vaccination. In the nine positive cases in nonvaccinated swine, immunizing viruses were isolated from two of them. In the two cases in which immunizing viruses were isolated, in Case 1, the outbreak occurred 107 days following vaccination of other pigs on the farm. In Case 2, the outbreak occurred about five and one half months following vaccination of other pigs on the farm. Of the other cases, in Case 3, the outbreak occurred within one week following vaccination of other pigs on the farm. In Cases 4, 5, 6 and 7, the outbreaks occurred more than one

<table>
<thead>
<tr>
<th>Categories</th>
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<th>Non-Vaccinated</th>
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TABLE VII
Confirmed Cases of Hog Cholera in Fiscal Years 1961 and 1962 in Suwannee County, Florida
ERADICATION OF HOG CHOLERA

month following vaccination of other pigs on the farm. In Cases 8 and 9, there was no record of any prior vaccination.

One factor contributing to the difficulty of controlling hog cholera in the pilot eradication area is enforcement of the regulation restricting removal from public market premises of nonvaccinated swine not intended for immediate slaughter. These swine constitute a potential source of virulent hog cholera virus and could be foci of outbreaks of hog cholera on farms. The effective policing of any regulation designed to prevent the exercise of this practice would seem to be essential in any effective hog cholera eradication program.

Quarantines were placed on all herds suspected of being infected with hog cholera as quickly as possible by a representative of the Florida Division of Animal Industry. In general, the conditions of these quarantines were carefully and honestly observed by the swine owners.

The control and regulation of movement of swine into the pilot eradication area was performed effectively by border guards of the Florida Division of Animal Industry stationed at five major roads into the county. These stations are manned continuously, day and night.

SUMMARY

Vaccination coverage and challenge results in the pilot eradication area in Florida for fiscal years 1961 and 1962 have been given. During the period, 1957-1962, data collected from challenge of 4,073 swine vaccinated with 87 serial numbers of lapine origin, porcine origin or tissue culture modified live virus vaccines from 11 licensees show an inverse relationship between average age of vaccine at time of use and immunogenicity of vaccine. Hog cholera was confirmed in six vaccinated, farm-raised swine, in nine nonvaccinated, farm-raised swine and in two vaccinated, purchased hogs. The difficulty of enforcing regulations restricting movement of nonvaccinated swine from public market premises has been described. Adherence to quarantine provisions by most swine-raisers and effective enforcement of regulations affecting swine transportation by the Florida Division of Animal Industry have been reported.

REFERENCES

REPORT OF THE COMMITTEE ON THE NATIONWIDE ERADICATION OF HOG CHOLERA


More concerted effort has taken place during the past year in grouping forces for launching an all-out attack on hog cholera than has occurred during the entire preceding 10 years since eradication of hog cholera was first discussed by this Association, as evidenced by:

1. Appointment of a 12-man advisory committee by the Secretary of Agriculture.
2. Appropriation of specific funds by the Congress for the implementation of a national eradication program.
3. Action on the part of an additional four states to prohibit the use of virulent hog cholera virus.
4. Organization of additional state and local hog cholera eradication committees.
5. Increased activity on the part of scientists throughout the country in the development of improved techniques for the diagnosis of hog cholera.
6. Increase in vaccination throughout the country as evidenced by increased sales of hog cholera vaccines, which in fiscal year 1962 were eight percent above 1961 and 28 percent above 1960, to the end that during the calendar year 1961 45 percent of the nation's eligible swine were vaccinated.

Your Committee had an opportunity to meet with the Secretary of Agriculture's Advisory Committee on Hog Cholera in conjunction with Livestock Conservation, Incorporated's Hog Cholera Committee earlier this year to assist in the development of regulations aimed at reducing the spread of cholera through interstate channels. It was the consensus of the group that through consideration of the multiplicity of views expressed at this meeting, the Secretary's Committee was enabled to obtain a broader understanding of the problems faced by industry in the ultimate development of federal interstate regulations. Your Committee recognizes the many problems with which the Secretary's Advisory Committee was faced and wishes to commend the group on its diligent efforts in establishing these regulations which we feel will greatly curtail the spread of hog cholera.

In considering the federal interstate regulations and the standards proposed by the Animal Disease Eradication Division for the state-federal
approval of livestock markets handling swine moved under provisions of Part 76, Title 9 CRF, your Committee recommends that the following standards be adopted by ADED:

Livestock markets approved under Part 76 shall be limited to those operating under cooperative Federal-State supervision where inspection and vaccination of swine is provided to meet the requirements prescribed in the regulation. No livestock market will be approved by the Director of the Division without the joint endorsement and recommendation of the appropriate State livestock sanitary official and the veterinarian in charge of ADE activities in the State that the service is adequate to meet the purpose of Federal regulations to prevent the spread of hog cholera and other communicable swine diseases. (Part 76, Title 9, Code of Federal Regulations).

A market may be removed from the approved list by the Director of the Division when it is determined by the Federal veterinarian in charge of ADE activities in the State that the operators of the market fail to meet the standards mutually agreed on and issued by the cooperating State & Federal officials.

Approval will be for one of the following purposes:

1. To receive and consign interstate shipments of all classes of swine, under the applicable provisions of Part 76.
2. To receive and consign interstate shipments restricted to slaughter swine only, under the applicable provisions of Part 76.

The operators of livestock markets in applying for approval to handle all classes of swine under this regulation shall agree to:

1. Cooperate in obtaining compliance with Part 76, 9 CFR.
2. Permit no swine to be removed from the premises of the market without inspection; and to permit no feeder pigs or breeding swine to be removed from the premises of the market without vaccination, unless accompanied by evidence of official vaccination as required by Part 76.9 (a) (3), Title 9, CFR and health certification by the accredited veterinarian authorized to furnish such services; and in accordance with Federal and State regulations.
3. Permit no slaughter swine to be removed from the premises of the market unless consigned for immediate slaughter to a recognized slaughtering center approved for this purpose in accordance with Federal and State regulations.
4. Maintain well-constructed pens and swine-handling facilities that are clean and in good repair.
5. Provide pens surfaced with impervious material for holding and vaccinating feeder pigs and breeding swine.
6. Provide satisfactory, well lighted facilities for inspection and proper restraint during vaccination.
7. Arrange for refrigeration facilities as necessary for storage of hog cholera biologics.
8. Clean holding and vaccinating pens after each day's use.
9. Clean and disinfect swine-handling facilities as deemed necessary by State and Federal agencies to guard against spread of disease.
10. Arrange for adequate facilities and service at a nominal cost for cleaning and disinfecting cars, trucks, and other vehicles with a permitted disinfectant when this is required under the regulations.

11. Maintain records of origin and destination for swine handled at yard or market, and grant authorized Federal and State inspectors access to such records.

12. Furnish a schedule of sales days.

The operators of livestock markets in applying for approval to handle slaughter swine only under this regulation shall agree to:

1. Cooperate in obtaining compliance with Part 76, 9 CFR.

2. Receive only swine for slaughter; and to permit no swine to be removed from the market unless they are consigned for immediate slaughter to a recognized slaughtering center approved for this purpose in accordance with Federal and State regulations.

3. Maintain well constructed pens and swine-handling facilities that are clean and in good repair.

4. Maintain records of origin and destination of swine handled at the yard or market, and grant authorized State and Federal inspectors access to such records.

5. Furnish a schedule of sales days.

6. Isolate all swine suspected of being affected with or exposed to hog cholera, promptly notify the State or Federal agency, and hold such swine in isolation pending instructions on disposition.

7. Clean and disinfect swine-handling facilities as deemed necessary by State and Federal agencies to guard against spread of disease.

It has been brought to the attention of your Committee that Congress has appropriated two million dollars for hog cholera eradication on a national level during the balance of the present fiscal year. While the amount appropriated was considerably less than that requested, it does bring congressional focus on this problem and will provide for initial federal participation in fields of national responsibility, such as the interstate movement of swine and for the initiation of cooperative state-federal programs.

Your Committee recommends that the Animal Disease Eradication Division establish the following minimum standards for Division participation in cooperative hog cholera eradication programs with the various states:

I. The following standards are established to provide a basis for state and federal participation in cooperative hog cholera eradication programs.

II. INITIATING THE PROGRAM

A. Program action within the State is dependent upon State authority. It is felt that State authorities are necessary as follows:

1. Authority requiring the prompt reporting of hog cholera;

2. Authority to place and maintain quarantines (including quarantine on suspicion of hog cholera);
3. Authority to regulate interstate movement of swine;
4. Authority to require cooking of garbage fed to swine;
5. Authority to perform necessary inspections of swine and involved premises;
6. Authority to require proper disposal of carcasses, as well as infected and exposed animals;
7. Authority to require cleaning and disinfection of premises and all vehicles and equipment on involved premises;
8. Authority to control the type and administration of hog cholera biologics.

B. The Veterinarian-in-Charge and the appropriate State official will provide, through the ADE Regional Assistant Director, a joint statement covering the following points:

1. That necessary State authorities as listed in II A exist. If any of the listed authorities do not exist, the joint statement should explain why they are not felt to be necessary for the program in the State.
2. Plans (in outline) for carrying out steps in III below. If initial level of the program will extend to steps included in IV, V, or VI below, plans (in outline) for carrying out these steps should be included.

C. The Division will either approve the joint statement as a basis for initiating a cooperative program; or will, through discussion and correspondence, participate in modification of the joint statement until mutually satisfactory to the Division and the State; at which time approval will be made.

III. PREPARATORY PHASES OF PROGRAM

A. Information

1. State Hog Cholera Eradication Committee or group acting in that capacity in active operation. Include list of groups represented on committee as members or advisors.
2. County or other local hog cholera committees in active operation. (If none, indicate why they are not felt to be necessary.)
3. Organized distribution of information to swine producers and interested groups concerning the disease and the eradication program.
4. Working relationship with State and local veterinary groups concerning the program.

B. Incidence of hog cholera

1. If deemed advisable by cooperating officials, surveys to determine the incidence of the disease, vaccination practices, and general swine management practices should be conducted, such surveys to include interview with swine producers, veterinary practitioners and other allied groups. (Preferably, a field survey based on personal contact by regulatory personnel and cooperating local groups.)
2. An established system for reporting hog cholera that provides definite channels for reporting and for prompt reporting. (It is felt that prompt reporting is essential if outbreak investigations and quarantine measures are to be effective. Mail reports, unless confirming a previously reported outbreak, do not satisfy this need for promptness.)

3. *Prompt and complete* investigation of outbreaks by regulatory personnel.

4. Standard diagnostic procedure used as a guide by practitioners and regulatory veterinarians, so that each report is confirmed by this procedure or is eliminated as being other than hog cholera.

C. Garbage Cooking

1. Inspection of licensed garbage-feeding premises at least twice a month.
2. Regular cooking equipment checks, including temperature during cooking and recording of results.
3. Prompt quarantine of swine fed raw garbage, and institution of necessary enforcement action for effecting compliance by violators. (The attitude toward enforcement must be that such enforcement is essential to the cooperative program and will be carried out.)

IV. REDUCING INCIDENCE

A. Quarantines

1. All herds suspicious for hog cholera to be quarantined pending confirmation. All infected herds continued under quarantine until officially released following determination that they no longer constitute a threat to the swine industry.
2. All exposed herds quarantined until result of exposure is determined.
3. No movements from quarantined herds except to slaughter under regulatory controls according to State or Federal requirements.

B. Intrastate movement of swine

1. Inspection of swine entering markets and returning to farms, including vaccination if this is necessary as a part of the cooperative program in the State.
2. Maintenance of swine-assembling and handling facilities to guard against spread of hog cholera.
3. Provision for dealer records, available for inspection, that identify origin and destination of swine handled. (If outbreak investigations demonstrate that dealers records are inadequate for traceback purposes, provision should be made to require them to be available.)
V. ELIMINATION OF OUTBREAKS

A. Before Division participation in indemnity payments for hog cholera is instituted, the Veterinarian-in-Charge and the appropriate State official will provide a joint statement, through the ADE Regional Assistant Director, covering the following points:

1. That steps given in III and IV have been carried out.
2. Detailed plans for handling disposal of infected and exposed swine under indemnity procedures.

(Note: Federal indemnities are intended for use as a final stage in the program, after incidence has been reduced; and with disposal of the entire herd. Indemnity for part of the herd puts indemnities into earlier phases of the program. If this is contemplated in any State, thorough evaluation of results of the program in that State will be necessary before consideration by the Division of extending the use of Federal indemnity funds to other than final phases of the program.)

3. If it is proposed to differentiate between vaccinated and unvaccinated swine for indemnity purposes, criteria for recognition of vaccination for indemnity purposes must be given in detail. Consideration should be given to the stage of eradication in which the State is engaged.

4. Plans for inspection of swine surrounding outbreaks and other contact swine that may be revealed during investigations.

5. Plans for cleaning and disinfection of infected premises and swine-handling facilities under immediate supervision of regulatory personnel.

6. Controls over type and administration of hog cholera biologics.

7. A statement that gives the incidence of hog cholera for preceding twelve months, and the number of herds and swine estimated to be eligible for hog cholera indemnities in the first year of the indemnity program.

VI. PROTECTION

A. A State may be listed in Part 76.2 (f) as a hog cholera eradication State when:

1. Steps required in III, IV, and V above are in full operation.
2. All feeding and breeding swine, imported from other than designated hog cholera-free areas or hog cholera eradication areas, are subject to at least 21-day segregation or isolation after arrival and are inspected and found free of evidence of hog cholera at the end of the period of isolation or segregation.

B. A State may be designated by the Division as hog cholera-free when steps required in III, IV, V, and VI A have been carried out, along with standards for hog cholera-free areas that have been or hereafter may be adopted by the USLSA and approved by the Division.

Your Committee has reconsidered its recommendations for the establishment of hog cholera-free areas and herein recommends the
following requirements in the declaration and maintenance of hog cholera-free areas:

Reporting System:

a. Hog cholera must be a reportable disease.
b. An acceptable reporting system must be established and in effective operation at least 12 months before the area can be declared free.

Vaccination:

a. Prohibition of fully virulent virus or live virus vaccines or immunizing agents against hog cholera; provided that the appropriate state and federal authorities may authorize the use of these products in supervised research and biologic production.
b. Vaccination permitted only with inactivated (killed) vaccines. **(Note: Questions may be raised about including the possibility of using live vaccines of alien host origin, or other products which may in the future be developed with non-virulent properties and which can be used with complete safety. If such products should in the future be licensed for production, their use could be considered at that time.)**
c. All vaccination against hog cholera shall be official (requiring identification and reporting).
d. Regulatory authorities in the area should be notified of all shipments of hog cholera immunizing products, including antiserum, going into the area. (The Serum-Virus Control Agency should consider methods by which such information could be furnished to the various hog cholera-free areas.)

Quarantine:

a. Authority to quarantine, including authority to quarantine on suspicion of hog cholera, and to maintain such quarantine for whatever period may be deemed necessary.
b. Authority to quarantine swine fed raw garbage and to maintain quarantine as long as deemed necessary.

Garbage Cooking:

a. Laws or regulations that clearly define cooked garbage, require that only cooked garbage be fed to swine, and provide penalties for violations.
b. Swine found to be fed raw garbage will be placed under strict quarantine, such quarantine to be continued in force under official veterinary supervision for a period of at least 30 days after proper cooking measures have been instituted; provided that quarantine may be removed if no indication of hog cholera has been observed during that period.
c. Personnel sufficient to provide garbage-cooking inspection as frequently and thoroughly as necessary.
Importation of Swine:

a. Importation for purposes other than immediate slaughter must be under permit and either:
   (1) Originate in an adjacent hog cholera-free state and enter on a veterinary health certificate, such swine to be held in strict isolation and quarantine upon arrival at destination for a period of at least 21 days, with inspection and freedom from disease at the end of this period; or
   (2) Have been vaccinated with inactivated (killed) vaccine not less than 21 days nor more than six months prior to shipment and move on veterinary health certificate, with isolation and quarantine upon arrival at destination for at least 21 days with inspection and freedom from disease at the end of this period.

b. All swine imported shall be transported in vehicles cleaned, disinfected and bedded under supervision just prior to shipment.

Hog Cholera-Free Area may be declared if the above requirements are met, and

a. Twelve months have passed without hog cholera being diagnosed within the area;

b. (1) Twelve months have passed after prohibition of fully virulent virus and modified live virus vaccines within the area;
   (2) Twelve months have passed after importation into the area of feeding and breeding swine treated with fully virulent virus and 90 days have passed after importation into the area of feeding and breeding swine treated with modified live virus vaccines;

c. A statistically sound survey of swine within the area fails to reveal any indication of hog cholera.

(Note: Possibilities for accomplishing this purpose include:
   (1) Inspection;
   (2) Slaughterhouse testing as a screening device.)

Maintenance of a Hog Cholera-Free Area shall include the following:

a. If no outbreaks of hog cholera other than primary outbreaks occur, an area may be maintained as hog cholera-free provided that such primary cases are completely eliminated. A primary outbreak is defined as one involving one owner whether or not occurring on one or more premises of said owner. Any spread from such primary outbreak shall result in revocation of the hog cholera-free status.

b. Prompt disposition of infected herds on the farm by supervised burning or burial.

c. Authority and action to inspect premises and conduct traceback investigations.

d. A statistically sound survey of swine within the area fails to reveal any indication of hog cholera.

In its report of last year, it was noted that 40 states had adopted laws or regulations to prohibit the use of virulent hog cholera virus. An additional four states have been added to this list, bringing the present total to
44 states and Puerto Rico. While this figure of 88 percent approaches total elimination of virulent hog cholera virus, your Committee wishes to again emphasize that as long as a single state allows the use of this product in field vaccination, foci exist which represent a hazard to the swine industry.

In response to inquiries from the United States Department of Agriculture, 43 states and Puerto Rico have confirmed their interest in a cooperative hog cholera eradication effort and have also indicated that basic legal authorities for supporting such programs are available. Funds have been appropriated specifically for hog cholera work in several states, with additional states having limited funds available from existing appropriations that are being used in preliminary activities. A few states have actually initiated active eradication programs.

While we have not yet seen the development of a fool-proof diagnostic test, there has been increased research in this field. A sub-committee of clinicians from this Committee is presently working with a group appointed by the Veterinary Laboratory Diagnosticians in a crash program to develop a standard diagnostic procedure to be used as a guide by the various states and the ADED for the confirmation of hog cholera.

Your Committee wishes to again stress two points that have been repeatedly set forth in reports of previous years, but upon which there still exists an apathetic attitude. Upon the first point hinges probably the most basic step in the eradication of this or any other disease; that is, the prompt reporting of its existence. Hog cholera epidemiological studies indicate that investigations started within one week of onset of illness resulted in far greater success in establishing the probable source of outbreaks than investigations delayed beyond this period of time.

The second point is that of subsequent immunity interference through the use of hog cholera anti-serum alone. Your Committee recommends that the various states adopt regulations which would preclude this practice and provide for the use of hog cholera anti-serum only in conjunction with modified live virus vaccines.
DURATION OF IMMUNITY AND SEROLOGICAL PATTERNS OF SWINE CONVALESCENT FROM VESICULAR STOMATITIS

A. A. Holbrook, D.V.M.*, and J. N. Geleta, D.M.V., M.S.**

Although enzootic vesicular stomatitis (VS) in swine has been diagnosed frequently in southeastern United States, information concerning the duration of immunity or serological titers following infection with VS virus is rare. Swine convalescent from VS have been found resistant to the disease following reexposure to virulent virus 52 days after initial infection. Complement-fixation (CF) and serum-neutralization (SN) tests have been used extensively in the study and diagnosis of VS; however, the relationship between serological titers and immunity is unknown.

The purpose of this investigation was to determine (a) the duration of resistance to disease caused by infection with VSV and (b) to observe the CF and SN patterns of swine convalescent from VS.

MATERIALS AND METHODS

Crossbred swine, six to twelve weeks of age, obtained from the Animal Husbandry Research Division, Agricultural Research Service, Beltsville, Maryland, were used. The swine were inoculated intradermally with suspensions of vesicular coverings from swine infected with the New Jersey-type VSV. The rectal temperatures of the swine were recorded prior to inoculation and twice daily during the acute phase of the disease. The swine were also observed twice daily for the development of lesions. The coverings from unruptured vesicles were harvested and stored in a dry-ice chest to be used as source of challenge virus. The immunity of two or more convalescent pigs was then challenged at monthly intervals beginning at the third month. The snouts, plus the coronary bands and interdigital spaces of the two front feet, were scarified, and a thick paste of vesicular coverings was rubbed into the scarified areas. Susceptible swine were treated in the same manner to ascertain the virulence of each inoculum. This method was used to permit interpretation of the results in comparison with earlier work and to provide a method of exposure which would be as severe as any natural exposure.

All experiments were conducted under strict quarantine in isolation units. Precautions were taken to prevent any exposure to VSV before the time of challenge. Blood samples were taken before initial exposure,

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three times a week for four weeks, once a week during the next four weeks, and once a month thereafter. The serums obtained were stored in a dry ice chest until tested. Serums from consecutive bleedings of each of 32 animals (24 of which were reexposed and eight of which were of the same group but not reexposed) were tested for CF and SN antibodies. All serums from any one animal were tested simultaneously.

**Complement-Fixation Test**

A previously described CF technique for serum titration was used. Each serum was diluted one to five in veronal buffered salt solution and heated at 56°C for 30 minutes. Twofold dilutions of the heated serums were made and distributed in 0.25 ml of complement containing 1.9 hemolytic (complete) units, and 0.25 ml of one to five dilution of chicken chorioallantoic membrane antigen was then added. The first set of tubes received New Jersey-type antigen, the second set Indiana type, and the third set normal chorioallantoic membrane. The resulting mixture of serum, complement, and antigen was thoroughly shaken and incubated for two hours in a water bath at 37°C, when 0.5 ml of sensitized sheep red blood cells was added to each tube and the incubation continued for an additional 30 minutes.

Control tubes containing antigens, test serums, anti-New Jersey and anti-Indiana (bovine) serums, complement, and sensitized sheep red blood cells were included in all tests. The results were read visually and recorded in terms of complement fixation from complete hemolysis (-) to no hemolysis (4+). The endpoints were selected as the highest dilution of serum resulting in 2+ or more fixation.

Procomplementary activity of the serums was tested by using 0.25 ml of a one to five dilution of each serum, varying dilutions of complement, and the standard amount of sensitized sheep red blood cells.

**Serum Neutralization Test**

Neutralization tests to measure the virus infectivity-neutralizing potential of test serums were conducted as described previously for horse and cattle serums. The allantoic cavities of eight-day-old embryonating chicken eggs were inoculated with 0.15 ml of a mixture of virus and serum in equal parts. Decimal dilutions of a suspension of the egg-propagated New Jersey-type virus and a one to five dilution of each of the serums were used. The LD<sub>50</sub> endpoint was calculated by the method of Reed and Muench. Virus-neutralizing activities of the serums were expressed as neutralizing titers, which represent the difference between the titers of virus mixed with preinoculation serum and with postinoculation serum.

**RESULTS**

**Immunity to Challenge**

Lesions were observed in all swine (including controls) after initial exposure to VSV. Extensive vesiculation and characteristic hyperthermia occurred within 24 to 48 hours, and secondary lesions were observed in
many cases within four to ten days postinoculation. Results of reexposure at monthly intervals are shown in Table I. Convalescent swine did not develop VS lesions when given immunity challenge between three and nine months after initial infection. On the tenth month, two of seven reexposed swine developed lesions. Two of three were susceptible at the eleventh month. All of the susceptible convalescent swine evidenced a partial immunity; none of them developed secondary lesions, the primary lesions healed more rapidly, and the degree of rectal hyperthermia was lower and of shorter duration than that observed in the control swine.

### TABLE I

Results of Reexposure of Swine Convalescent from Vesicular Stomatitis to Virulent Vesicular Stomatitis Virus at Monthly Intervals

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*Numerator indicates number of pigs susceptible. Denominator indicates number of pigs reexposed.

**Complement-Fixation Tests**

The mean CF titers of consecutive serums collected from 32 swine are shown in Figure 1. Detectable antibodies appeared within five to six days postexposure and increased rapidly to a peak 12 to 18 days post-exposure. After attaining the peak, the titers declined gradually until most became undetectable at two to four months postexposure. One serum taken before initial exposure had a titer of 1:40 with New Jersey-type antigen, 1:20 with Indiana-type antigen, and 1:10 with normal antigen; however, the subsequent serums in that series followed much the same pattern as the other serums. In two series of serums in which specific titers had disappeared between two and four months, low titers to all three antigens reappeared between eight and ten months postexposure.

In those serums taken while the titer was rising (from immediately after infection until the peak titer), a marked prozone (increased hemolysis) was observed in the lower serum dilutions. Cross reactions with Indiana-type antigen were also observed in many of these serums. Shortly after the titers reached a peak the prozoning and cross reactions disappeared in most instances. A check of the serums for procomplementary activity by the complement dilution test showed that those serums taken while the titers were rising had more procomplementary activity than did those taken before inoculation and after the titers had reached a peak.

**Serum Neutralization Tests**

The mean SN titers of the consecutive serums are shown in Figure 1. All preinoculation serum samples were negative when examined for neutralizing activity. Detectable SN antibodies appeared within five to eight days postexposure and increased rapidly to a plateau within three to four
Figure 1. Mean complement-fixation and serum neutralization titers of 32 series of swine serums following experimental infection with New Jersey-type vesicular stomatitis.

weeks. After reaching a plateau, a neutralizing antibody level of approximately four logs of virus neutralized was maintained. The fluctuation was less than two logs throughout the entire test period.

DISCUSSION

The immunity pattern conferred by VS infection in swine is different from the patterns of vesicular exanthema (VE) and foot-and-mouth disease (FMD) infection in swine, and the pattern of VS infection in horses and cattle. Mott reported that the 50-percent duration of immunity from B-51-type VE virus was about 20 months in two separate experiments. Further study in our laboratory proved that this was also true for ten other types of VE virus. Information pertaining to resistance to intradermal reexposure of swine convalescent from FMD is not known; however, Culiffe reported that one of five swine convalescent 128 days from FMD which were reexposed by contact to pigs actually infected with FMD was susceptible and developed lesions on three feet. Although the results of this study showed that the duration of immunity to intradermal challenge was ten months or about one-half the duration of VE immunity, the immunity conferred by both diseases is of the solid type as indicated by absence of primary lesions and of hyperthermia. This is in contrast to the methods used in evaluating immunity following FMD infection in which
secondary lesions are the determining factor. In the work in our laboratory over the years, it has been found that horses and cattle convalescent from VS may be immune in intradermal challenge from 60 days to nine or ten months; however, in most instances the duration is much less than the ten months determined for swine.

Most of the difficulties such as interference, procomplementary activity, and cross reactions—usually encountered when swine serums were used in the CF test—were also encountered in these series of serums. The preinoculation serum and the two series in which a titer reappeared after having disappeared between two and four months, were considered to be due to fixation of complement by antigen-antibody complexes other than VS. Although in these series of serums there were only three examples of this phenomenon, as many as 25 percent of other groups of swine serums which we have tested in our laboratory have shown positive titers to VS-infected chorioallantoic membrane antigen. These swine had never been exposed to VS and were susceptible to exposure to VS virus. The groups showing the most positive reactions had recently recovered from some other disease such as pneumonia or enteritis. The prozones observed in the lower serum dilutions from immediately after infection until the titers had reached a peak are consistent with the findings of Rice and McKercher. The procomplementary activity was increased during this period; however, it was not of a sufficient amount above that of the subsequent serums which did not exhibit any prozoning to account for the total effect. From our studies with this type of serums we have concluded that substances (probably lipoproteins) are present in these serums which interfere with the fixation of complement by the antigen-antibody complex. Treatment of these serums by such procedures as lowering or raising the pH, ion-exchange resins, and addition of aniline, pyridine, and ethedtin have removed some of the interference, but no procedure has been found which has proved satisfactory for all swine serums. When these serums are serially diluted, the activity of the procomplementary and interfering substances is diluted out before the complement-fixing antibody, permitting observation of a CF titer. The serological patterns of swine, horses, and cattle are very similar—when individual differences and numbers are considered.

The relationship between immunity and serological tests is important in the study and control of a disease. The results of this study indicate that the time following infection is a factor in evaluating the correlations between resistance to reinfection and CF or SN titers. For the first two months following infection, a positive correlation exists for swine are immune to reinfection and exhibit CF and SN circulating antibody. Between two and four months, a less positive correlation exists, since during this period some swine continue to show circulating SN antibodies and insusceptibility to reinfection but fail to show circulating CF antibodies. After four months, there is a negative correlation between CF titer and immunity, while a positive correlation between SN titer and immunity remains until the 10th month when convalescent swine become susceptible to reinfection.
SUMMARY

At monthly intervals beginning three months after primary exposure of swine to the New Jersey-type vesicular stomatitis virus, the immunity of two or more of the convalescent swine was challenged. A paste of virulent stomatitis virus was rubbed into scarified areas of the snout and forefeet. All reexposed swine were resistant to the challenge dose until the tenth month when two of seven challenged swine were susceptible. By the eleventh month two of three were susceptible.

Circulating complement-fixing antibodies were detected in swine within five to six days after exposure to the virus and reached their highest concentration 12 to 18 days after exposure. Within two to four months, the antibodies were no longer detectable in most of the animals. Virus-neutralizing antibodies appeared in the blood of swine five to eight days after exposure and increased in concentration to a high level within three to four weeks. This level was maintained for 11 months, the duration of the experiment. No absolute correlation existed between presence of circulating antibody and immunity.

REFERENCES

FIELD TRIAL OF LIVE VIRUS VACCINATION PROCEDURE FOR PREVENTION OF VESICULAR STOMATITIS IN DAIRY CATTLE

I. PRELIMINARY IMMUNE RESPONSE

Lloyd H. Lauerman, Jr., D.V.M., M.S., Merle L. Kuns, Ph.D., and Robert P. Hanson, Ph.D.

Madison, Wisconsin

The distribution of vesicular stomatitis (VS) antibodies has recently been described for Panama by Kuns. Antibodies were found with the greatest frequency in livestock in the tropical moist forest and subtropical humid forest regions. Epizootics of economic significance have occurred primarily in heavily grazed portions of the tropical dry forest or savanna on the Pacific coastal plain. The virus appears to be introduced into a few animals in the herd and to be spread throughout the herd on the hands of the milkers. The animals go off feed, lose body weight and drop in milk production.

The manager of one of the largest dairy herds in Panama considers VS his major disease problem and requested assistance in controlling the disease. On this farm animals with clinical VS were separated from the herd and their milk was not used as long as open lesions were present. The lesions on the teats usually persisted for two to six weeks, probably due to secondary infection. Dairy cattle affected during the latter half of lactation generally went dry. The reports of Camargo, Meyer, et al. and Stozzi and Ramos-Saco give an indication of the disease importance in other American countries.

A vaccination procedure, using live virus vaccine, had been outlined for use in dairy herds in Wisconsin in the event of an epizootic. Since the disease had not appeared in Wisconsin during the last thirteen years, there had been no opportunity to field test the procedure. The following program was proposed as a means of evaluating the vaccination procedure in dairy cattle that are exposed to VS one or two months of each year.

MATERIALS AND METHODS

The field trial is being carried on in a dairy herd located near Aguadulce in Cocle Province of the Republic of Panama. Heifers are held in pastures near El Rincon on the Río Santa Maria until freshening. Half of the cows are kept at the main barn located in the savanna between Aguadulce and Nata during the first years of production. All older and some young cows not kept in the main herd are located on a farm in the dry tropical forest region near the Río Chirubi, approximately three miles northeast of Nata. The milking herd on the two farms, totals approximately 1,300 animals. Approximately 600 heifers are located at El Rincon. Clinical vesicular stomatitis has occurred in the milking herds during December and January of each year with the exception of 1957 and 1961.
The serological results from 22 serum samples collected from the milking herd at random five months prior to the field trial indicated that approximately 40 percent of the animals ranging in age from three to 12 years were immune to VS New Jersey and less than five percent were immune to Indiana. All animals with a history of clinical VS were carrying virus neutralizing antibodies to VS New Jersey, whereas, only 14 percent of these animals were immune to VS Indiana serotype.

The vaccine was a lyophilized preparation consisting of equal volumes of allantoic fluids containing VS virus New Jersey serotype and 0.2 percent bovine serum albumin in 0.02 M phosphate buffered water, pH 7.2. The tubes were sealed under dry nitrogen. The allantoic fluids were harvested from 10 day embryonated chicken eggs that were infected 29 hours previously with 15 chick embryo LD₅₀ of virus per 0.1 ml of inoculum. The bovine serum albumin was fraction V powder prepared by the Nutritional Biochemicals Co., Cleveland, Ohio. The vaccine virus was a high egg passage Wisconsin isolate of VS virus New Jersey serotype. Identity of the virus was determined by virus neutralization (VN) tests in nine-day old chicken embryos and tissue culture colorimetric neutralization test.² The VS virus preparation, the serum albumin solution and the final lyophilized vaccine were tested for bacterial and fungal contaminants by streaking one-tenth ml of each material on blood agar and nutrient agar plates or by inoculating thioglycollate medium. These preparations were devoid of bacterial and fungal contaminants. With the use of high allantoic sac passage virus such bovine pathogens as rabies, pseudorabies, virus diarrhea and mucosal disease viruses, if present in the original isolation material, would have been diluted beyond the point of extinction. The vaccine was safety tested by intramuscular inoculation of two bovine males seven months of age with five times the vaccination dose to be used in the field trial. Blood was drawn and body temperature recorded from both animals every eight hours or at more frequent intervals for three days. From the twelfth to the twenty-fourth hour after virus inoculation blood was collected and body temperature was recorded at two hour intervals. Saliva was collected from both animals 18 and 48 hours after virus inoculation. The saliva was mixed with an equal quantity of diluent consisting of medium 199 plus 10 percent calf serum, 1,000 units of penicillin and 1,000 micrograms of streptomycin per ml. Each blood sample and saliva diluent mixture was inoculated into the allantoic cavity of six nine-day old chicken embryos in an attempt to detect virus. Serum samples were collected at four-day intervals for a period of three weeks to study the serological response of the animals. The oral mucosa and coronary bands were examined for vesicles once a day for the first three days. The animals did not have a significant temperature rise, vesicles were not observed nor was virus detected in the blood or saliva. Eight days after vaccination VS New Jersey VN antibodies were detected in the sera of these animals.

The colorimetric neutralization test described by Kuns² was used to screen animal sera for VN antibodies. The colorimetric test is based on differences in the pH of virus infected and uninfected HeLa cell cultures replicated in plastic panels. The principal advantage of the colorimetric method is economy of materials and the rapidity with which large numbers of sera can be screened for VN antibodies.
Five hundred heifers eight to 36 months of age selected for incorporation into the dairy herd were the subjects of the vaccination program. The older half of these animals were in all stages of pregnancy. Seventy-five percent of these animals (375) were vaccinated and the remainder (125) held as nonvaccinated controls. Serum samples were obtained from each vaccinated and nonvaccinated animal prior to and two weeks following vaccination. Sera will be collected from these and animals that are subsequently incorporated into the study at yearly intervals to determine duration of immunity and the nonimmune to immune conversion rate. The efficacy of the vaccine and vaccination procedure is being evaluated both on the basis of serological response and the freedom of the vaccinated animals from clinical disease. Each vaccinated animal received one ml of reconstituted vaccine, containing approximately 100 CELD_{50} of VS New Jersey virus, intramuscularly in the dorsal region of the pelvic limb. The vaccination took place in July during the interepizootic period.

Fifty tubes of lyophilized VS New Jersey vaccine virus packed in leak-proof metal-lined shipping containers were personally carried into Panama under importation permit #2243 issued by the director of the Department de Sanidad Animal, Ministerio de Agricultura, Comercio e Industrias, Republica de Panama. During the transportation of the vaccine to Panama and into the field, the shipping containers were exposed to tropical temperatures on five different occasions for varying periods of time ranging from eight to 18 hours before animal vaccination was complete. At the time of vaccination the vials and syringes were emersed directly into a solution of quaternary ammonium compound. After completion of the program all of the equipment which came in contact with the VS vaccine virus and the remaining vials of vaccine were autoclaved at 15 pounds for 30 minutes. The 50 vials of vaccine were accounted for after being autoclaved. Since the vaccine remaining after the heifers were vaccinated was destroyed in Panama to conform to the terms of the importation permit it was not possible to determine the stability of the product after field exposure. An experiment was designed to simulate the numerous wide variations in temperature five to 37 C. Two vials of vaccine from the same stock as used in Panama were subjected to temperature variations that would be equivalent to the most severe experienced by the vaccine during transportation. The temperature variation schedule was as follows: 5 C storage, 18 hours at 37 C, 96 hours at 5 C, 10 hours at 37 C, 48 hours at 5 C, 12 hours at 37 C, 12 hours at 5 C, 12 hours at 37 C, 12 hours at 5 C, 12 hours at 37 C. The lyophilized product was reconstituted and inoculated into nine-day old chicken embryos. Infective virus was not detected in a volume equivalent to the vaccinating dose.

Data from 298 paired sera from animals on the vaccination field trial are summarized in Table I. Five nonvaccinated and 11 vaccinated animal sera possessed VS New Jersey VN antibodies before vaccination and retained their titer. The 60 nonvaccinated animals remained free of antibody. Half of the vaccinated animals developed VN antibody within the two weeks between vaccination and bleeding.
TABLE I
Vesicular Stomatitis New Jersey Virus Neutralizing
Antibody Data on Paired Sera

<table>
<thead>
<tr>
<th>Serological Reaction</th>
<th>Before Vaccination</th>
<th>Post Vaccination</th>
<th>Vaccinated Group</th>
<th>Nonvaccinated Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>110</td>
<td>60</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>112</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>11</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total Paired Sera</td>
<td>233</td>
<td>65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Clinical vesicular stomatitis has occurred annually in the savanna and tropical dry forest areas of Cocle Province during the December to February period. Five percent of the herd replacement heifers possess antibodies to VS. Forty percent of the lactating animals, age range of three to 12 years, had had previous experience with the disease. The percentage of animals possessing antibodies increases on the average eight percent for each lactation period. A majority of clinical cases had been recorded for animals during the first or second lactation. These observations support the theory that virus is spread by the milking process.

Clinical vesicular stomatitis is seldom observed in the enzootic tropical moist and subtropical humid forest regions. Greater than 50 percent of the cattle in these areas possess VS New Jersey VN antibodies before lactation. If 50 percent or more of the replacement heifers in the savanna and tropical dry forest regions were immune to VS before first calving, it is anticipated that clinical VS would not occur in the milking herd. Half of the vaccinated dairy heifers developed demonstrable VN antibodies in the two week period following inoculation. Demonstrable antibodies might have been produced in a larger percentage of the animals had the serum samples been collected three weeks following inoculation. However, the results of the simulated temperature variation experiment indicate that the vaccine could well have lost 99 percent of its activity during transportation and it is very possible that some of the animals did not receive infectious virus. Signs of disease, such as salivation, vesicle formation or anorexia were not observed during the two week observation period following inoculation. Vaccination did not adversely effect or accentuate any latent disease condition present in the animals. Virus was not shed in the experimental animals nor did the vaccinated animals shed virus during the field trial as substantiated by the negative contact controls.

In view of the failure of many individuals to respond to the field trial and the demonstrated thermal instability of the vaccine, further studies should be conducted on means of improving vaccine stability and on the use of a larger vaccinating dose.
REFERENCES

THE USE OF THE FLUORESCENT ANTIBODY TECHNIQUE FOR DETECTING CATTLE CONVALESCENT FROM FOOT-AND-MOUTH DISEASE

Greenport, New York

INTRODUCTION

Demonstration of antigen of foot-and-mouth disease virus (FMDV) by fluorescent antibody technique has been reported by workers in Europe, and South America. Studies on the application of fluorescent antibody techniques to research on foot-and-mouth disease (FMD) have been underway at the Plum Island Animal Disease Laboratory for several years. Preliminary observations were made with the direct method, using fluorescein conjugated bovine and guinea pig anti-FMDV serums on calf kidney tissue culture monolayers infected with FMDV. However, more definite microscopic evaluations could be made with the indirect method using rabbit anti-FMDV serum and fluorescein conjugated sheep anti-rabbit serum. Consequently, work on the indirect method was continued.

Development of information on the indirect method revealed that cross reactions occurred between the various types of FMDV thus rendering the method unsatisfactory for typing viruses or antisera. Attempts were made to eliminate the cross reactions by preparing antisera devoid of antibodies to the products of cellular destruction resulting from infection of calf kidney cells with FMDV. This was accomplished by inoculating rabbits with centrifuged (pelleted) virus produced in swine kidney tissue cultures. However, when these antisera were used on infected bovine kidney cells, no microscopically visible fluorescence was seen despite the fact that the antisera had neutralization indices ranging from 6.8 to 7.6. This led to the presumption that the mass of viral antigen in the system was too small to produce microscopically detectable fluorescence; therefore a combination of both viral and cellular antigens with viral and cellular antibodies would be necessary for practical results with the fluorescent antibody technique. Accordingly work was directed toward determining what could be done with the relatively crude immunofluorescence method herein described.

MATERIALS AND METHODS

Tissue Culture

Primary monolayers of bovine calf kidney cells were grown on 12 mm. square No. 1 coverslips in Leighton tubes. The tissue culture was prepared with media and by methods previously described.

Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Greenport, Long Island, New York.
*Department of Pathology and Parasitology, Auburn University, Auburn, Alabama.
Viruses

Foot-and-mouth disease virus, type A, strain 119, passaged 10 times in primary calf kidney cell culture was used routinely. The cultures in Leighton tubes were infected by covering them with two ml. of medium containing approximately 100 plaque forming units of virus per ml. An amount of virus was chosen that would produce well marked cytopathic effect in about one-third of the cells in the monolayers in a period of 18 hours. In several instances FMDV, type 0, strain M11, passaged eight times in calf kidney cells or type C, strain 149, passaged 23 times in calf kidney cells were used.

Vesicular stomatitis viruses (VSV) Indiana and New Jersey types each passaged eight times in calf kidney cells also were used.

Serums

The serums used in this report were prepared during the course of various Plum Island studies. Some had been stored at 4°C. in fluid form, some had been lyophilized and others had been held frozen at -10°C. For purposes of description, the serums were divided into six groups as given in Table I. In addition to these, other serums also were examined, as will be reported later. The type and strain designations of the viruses used to produce the various serums are given in Table I.

Group A. Serums from normal control cattle were taken shortly after the animals were received and before they had any known contact with FMDV.

Group B. Serums from FMD convalescent cattle were taken from cattle inoculated on the tongue with specific types of FMDV of bovine tongue tissue origin. All cattle in this group developed signs and lesions of FMD within 48 hours postinoculation and had both tongue and foot lesions. All seven types of FMDV were used.

Group C. Serums from vesicular stomatitis (VS) and virus diarrhea (VD)* convalescent cattle were used as controls on the specificity of the fluorescent antibody reactions. The VSV was of bovine tongue tissue origin and was inoculated into the tongue epithelium of cattle.

Group D. Serums from FMD immunized cattle were obtained from cattle used in virus detection tests and vaccine trials. Serums 34 through 40 were from cattle immunized by virus-detection-test materials. Details regarding the tests and these serums and those of group E (43-47) are given elsewhere by Cottral, et al.4

Serums 41 and 42 were from cattle immunized with an experimental vaccine prepared from formalin-treated FMDV A-119 grown on bovine kidney cell cultures. Blood samples for test serum were obtained 224 days after vaccination.

Group E. Serums from cattle negative to FMDV-detection-tests were also of two experimental groups. Serums 43 through 46 were from cattle that had been used on tests to detect FMD virus in various cattle tissues. The animals did not develop signs or lesions of FMD from the test

*Obtained through courtesy of A. J. Kniazeff and P. D. DeLay.
TABLE I
Fluorescent Antibody Reactions of Cattle Serums with Foot-and-mouth Disease Virus as Related to Specific Virus Experiences of Hosts and to Serum Log Neutralization Indices

<table>
<thead>
<tr>
<th>No. Serum</th>
<th>Host Virusesa</th>
<th>DPI Bledb</th>
<th>FMD NICc</th>
<th>FA Reactionsd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A - Normal Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>0</td>
<td>1.5</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>N</td>
</tr>
</tbody>
</table>

| Group B - FMD Convalescent |
| 8 | A-119 | 23 | 6.3 | P |
| 9 | A-119 | 14 | 6.5 | P |
| 10 | A-119 | 11 | 6.2 | P |
| 11 | O-M11 | 43 | 5.7 | P |
| 12 | O-M11 | 28 | 5.8 | P |
| 13 | C-149 | 30 | 5.8 | P |
| 14 | C-149 | 17 | 5.9 | P |
| 15 | C-149 | 30 | 5.5 | P |
| 16 | SAT-1, RV-11 | 22 | 6.8 | P |
| 17 | SAT-1, RV-11 | 22 | 6.7 | P |
| 18 | SAT-1, RV-11 | 15 | 6.7 | P |
| 19 | SAT-2, RHO-1 | 14 | 4.4 | P |
| 20 | SAT-2, RHO-1 | 13 | 5.2 | P |
| 21 | SAT-2, RHO-1 | 17 | 5.6 | S |
| 22 | SAT-3, RV-7 | 13 | 4.6 | P |
| 23 | SAT-3, RV-7 | 20 | 4.0 | S |
| 24 | SAT-3, RV-7 | 11 | 4.2 | P |
| 25 | Asia-1, PAK-1 | 9 | 5.2 | P |
| 26 | Asia-1, PAK-1 | 11 | 5.6 | P |
| 27 | Asia-1, PAK-1 | 13 | 5.5 | P |

| Group C - VS and VD Convalescent |
| 28 | VSV-N.J. | 31 | 0 | N |
| 29 | VSV-Ind. | 27 | 0 | N |
| 30 | VSV-Ind. | 27 | 0 | N |
| 31 | VSV-N.J. | 24 | 0 | N |
| 32 | VDV-Ore. C-24V | 42 | 0 | N |
| 33 | VDV-Ore. C-24V | 42 | 0 | N |

| Group D - FMD Immunized |
| 34-37 | A-119 | 13-18 | 3.1-5.6 | N |
| 38-40 | O-M11 | 13-18 | 3.1-5.2 | N |
| 41-42 | A-119 | 224 | 4.6-4.9 | N |

| Group E - FMDV-Detection Test Negative |
| 43-46 | A-119 | 13-14 | 0-1.8 | N |
| 47-48 | A-119 | 15 | 1.8-2.7 | N |
TABLE I (continued)

<table>
<thead>
<tr>
<th>No. Serum</th>
<th>Host Viruses&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DPI Bled&lt;sup&gt;b&lt;/sup&gt;</th>
<th>FMD NT&lt;sup&gt;c&lt;/sup&gt;</th>
<th>FA Reactions&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group F - FMD Multi-infected Convalescent&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>2 types FMDV</td>
<td>19</td>
<td>5.7-6.0</td>
<td>P</td>
</tr>
<tr>
<td>50</td>
<td>3 types FMDV</td>
<td>20</td>
<td>5.6-6.6</td>
<td>P</td>
</tr>
<tr>
<td>51</td>
<td>3 types FMDV</td>
<td>21</td>
<td>5.6-6.1</td>
<td>P</td>
</tr>
<tr>
<td>52</td>
<td>4 types FMDV</td>
<td>21</td>
<td>4.4-6.8</td>
<td>P</td>
</tr>
<tr>
<td>53</td>
<td>6 types FMDV</td>
<td>22</td>
<td>4.6-5.8</td>
<td>P</td>
</tr>
<tr>
<td>54</td>
<td>7 types FMDV</td>
<td>730</td>
<td>3.7-4.9</td>
<td>P</td>
</tr>
<tr>
<td>55</td>
<td>7 types FMDV</td>
<td>730</td>
<td>2.9-5.3</td>
<td>N</td>
</tr>
</tbody>
</table>

<sup>a</sup>The viruses of vesicular stomatitis (VSV) and virus diarrhea (VDV) are indicated, all others are the seven types of FMDV.

<sup>b</sup>Days postinoculation bled for test serums.

<sup>c</sup>Neutralization index per ml of undiluted serum.

<sup>d</sup>Fluorescent antibody reaction: N = negative, P = positive and S = suspicious. FMDV type A, strain 119, in bovine kidney cell cultures was used for all FA reactions listed in table.

<sup>e</sup>Different types of FMD viruses inoculated 20 to 30 days apart. DPI is dated from last virus used. The two NI figures represent extremes for specific virus types given hosts. FMDV A-119 was one of viruses used for all except No. 51.

materials; however, unlike those animals of Group D, they were susceptible to challenge. Serums 47 and 48 were from cattle that has been used in a safety test of an experimental vaccine prepared from formalin-treated bovine tongue tissue FMDV A-119. The cattle did not develop signs or lesions of FMD within 15 days when blood samples were obtained for test serum.

Group F. Serums from cattle convalescent from more than one FMDV infection were from a group of animals that were inoculated on the tongue with FMD viruses of tongue tissue origin. The viruses were inoculated at intervals of 19 to 30 days. Serum 49 was from an animal inoculated with FMD viruses Asia-1 and A-119, serum 50 was from the same animal after SAT-1 had been given. The animal furnishing serum 51 received SAT-3, Asia-1, and C-149 while serum 52 represents use of Asia-1, A-119, SAT-1, and SAT-3. The two animals furnishing serums 54 and 55 had been inoculated with all seven types of FMDV. The days postinoculation on which all animals were bled was dated from last virus inoculation.

Neutralization Tests

The neutralization tests employed a constant serum dilution with varying 10-fold dilutions of virus. The test animals were Rockefeller H strain Swiss suckling mice seven to nine days old. Ten animals were used for each dilution. For normal cattle serums and for VS and VD convalescent serums, FMDV A-119 was used. In all other tests, the specific types of FMDV given to the hosts were used.
Conjugate

Commercial fluorescein conjugated rabbit anti-bovine globulin, globulin was used.* The counter stain employed was rhodamine bovine albumin.**

Technique of Staining

Infected monolayers of calf kidney cells on coverslips were taken for staining when one-third to one-half of the cells showed microscopic evidence of cytopathic effect (approximately 18 hours or less after infection). The coverslips were rinsed in phosphate buffered saline*** (PBS) pH 7.3 and the cells fixed for five minutes in two changes of methyl alcohol. All of the reagents were used at room temperature. The coverslips were held in Chen, Type B staining racks**** for rinsing and fixing.

After fixation, the coverslips were rinsed four times in PBS to remove the methyl alcohol. The preparations were not allowed to dry after fixation or at any other time to avoid diminution in the intensity of the staining reaction. The coverslips then were laid, cells up, in a horizontal position on a staining rack consisting of two tightly stretched fine steel wires placed seven mm. apart. (A staining rack made of linen thread can be used but has the disadvantage of absorbing reagents that overflow from the coverslips; also it vibrates when struck accidentally by forceps.) Upon placing each coverslip on the staining rack, it was covered immediately with serum to be tested. The test serums were diluted 1:10 with cold PBS and 0.4 ml of each diluted serum was mixed with 0.1 ml rhodamine bovine albumin counterstain. Two coverslips were treated with each serum sample and a like number of control coverslips were treated with normal serum. Five-tenths ml serum-rhodamine mixture placed dropwise upon two coverslips with a Pasteur pipette with a rubber bulb was sufficient for adequate coverage. Care was taken throughout, not to let the monolayers dry during the manipulations. Sufficient diluted normal control serum was mixed with an appropriate amount of rhodamine bovine albumin to cover the required number of control coverslips. It was found by experience that addition of rhodamine in the first step greatly enhances the contrast between preparations made with positive and negative serums.

*Sylvana Chemical Co., 22 E. Willow St., Milburn, New Jersey
**Microbiological Associates, 4813 Bethesda Ave., Bethesda 14, Md.
***Formula for PBS: NaCl 8.0 g.
KCl 0.2 g.
Na2HPO4·7H2O 2.172 g.
KH2PO4 0.2 g.
MgCl2·6H2O 0.1 g.
CaCl2 0.1 g.
Penicillin 100,000 units
Dihydrostreptomycin sulfate - crystalline 0.1 g.
Aerosporin
Polymyxin B sulfate 100,000 units
Distilled water 1,000 ml.

After the monolayers had been in contact with the serums to be tested for 45 minutes, they were rinsed repeatedly in PBS. This was done carefully to prevent one test serum from coming into contact with others. Coverslips from each case to be tested were rinsed twice, individually, before putting them together in a Chen rack for further rinsing. After four rinses for a total of 10 minutes, the coverslips were again placed on the wire rack and covered with a mixture of conjugate one part, rhodamine bovine albumin two parts, and PBS seven parts. All coverslips were covered with the same conjugate mixture.

After the monolayers had been in contact with the conjugate mixture for 45 minutes all the coverslips were placed together in Chen racks, rinsed six times in PBS for a total period of 10 minutes and mounted with cells down upon microscope slides using as mounting medium a mixture of glycerin nine parts and PBS one part. A control coverslip treated with normal serum was mounted on each slide next to the coverslip treated with a serum to be diagnosed. Both coverslips on a slide were then pressed down firmly (with forceps) and rinsed gently with a fine stream of distilled water. The coverslips will withstand this rinsing without floating loose if they are pressed down firmly enough upon the microscope slide. They were then dried with bibulous paper and labelled. A small drop of mounting medium was then placed at the edge of each coverslip to prevent introduction of air bubbles.

Identity of the coverslips was maintained by keeping them in order, working from back to front in the Chen racks and from left to right on the wire rack. When an experiment was set up, a diagram of the procedure to be followed was prepared and followed carefully throughout.

Microscopic Equipment

The preparations were examined with a Leitz Ortholux - UAM Universal Double Lamp Research Microscope for Fluorescence Microscopy. The light source was a Philips CS 150 UV burner, and the light was passed through a heat-absorbing filter and ultraviolet transmitting filter two mm. UG 1. A dark field substage condenser was used and the eyepieces protected with UV absorption filters "Euphos" 2.5 mm. Microscopic examination was made with 16 mm. and four mm. objectives. Pictures were recorded on 35 mm. high speed Ectachrome film using a two-minute exposure time and protecting the film from ultraviolet rays by interposing a UV absorption filter, "Euphos" 2.5 mm. between the coverslip and the camera. Developing time in the first developer was increased three minutes.

Interpretation of Results

Some experience in microscopy is required. Infected cells treated with convalescent serum showed significant greenish-white fluorescence of the cytoplasm (Figure 1). Control preparations treated with normal serum showed unaffected cells with dim orange fluorescence; but the cells shrunken from effects of the virus exhibited a brilliant orange to orange-white fluorescence that did not constitute a positive reaction. Furthermore,
Figure 1. Photomicrographs illustrating contrast between infected calf kidney monolayers treated with normal bovine serum and conjugate (left) and one treated with FMD convalescent bovine serum and conjugate (right). The black and white photograph does not show the greenish-white cytoplasmic fluorescence upon which diagnosis is based, but does illustrate the approximate degree of microscopic contrast between positive and negative preparations. All preparations contained occasional cells with spurious greenish fluorescence. However, control preparations and those treated with convalescent serum were sufficiently distinct for diagnostic interpretation.

OBSERVATIONS AND INTERPRETATIONS

Exploratory work with the above technique began by testing two samples of serum taken from cattle 21 days after infection with FMDV A-119. In each instance, significant greenish-white fluorescence of infected cells (Figure 1) was seen in the tissue cultures treated with the convalescent serum as opposed to a negative appearance of the control cultures. Each serum was tested three times on three different days with consistently positive results. One of the serums was then tested twice on a series of tissue cultures infected with FMD viruses including types A, O and C. In all instances significant fluorescence of infected cells was seen, indicating that the test might be specific for FMDV but, would not differentiate between the various types. A final preliminary test was made with one of the serums on a series of tissue cultures infected with viruses including A, O, and C types of FMDV and New Jersey and Indiana types of VSV. Significant fluorescence was seen in the tissue cultures infected with A, O or C types FMDV but those infected with either type of VSV could not be distinguished from control preparations.
In order to determine the time required for development of antibodies on which the test depends, two cattle were infected with FMDV A-119 by tongue inoculation. Serum samples were taken from each animal before inoculation of the virus and on one, two, three, four, five, six, seven, eight, 10, 11, 14, 16, 18, 21, 23, 25 and 29 days thereafter. Antibodies demonstrable by the fluorescent antibody test were found in the serum of each animal beginning on the sixth day after infection in one instance, and on the seventh day in the other. The positive reaction persisted without diminution through the 29th day after infection.

At this point, serums from the various groups listed in Table I were studied. They were coded so that the microscopist would not know the actual identity or history of the serums and could make an unbiased interpretation of the fluorescent antibody (FA) reactions.

The first observation was that serums from normal cattle consistently gave negative FA reactions, Group A. The second observation was that serum from cattle convalescent from FMD infection gave positive or suspicious FA reactions, Group B. Only two suspicious reactions were found in this group, one with SAT-2 and another with SAT-3 FMD viruses. It also was demonstrated with the results from this group, as well as from the preliminary observations, that the FA reaction did not distinguish between types of FMDV. Another point established with Groups A and B and also with F was that serums stored at 4°C., either fluid or lyophilized, and serums frozen at -10°C. could all be used for FA studies without fear of obtaining false reactions. Furthermore, some of the serums were stored for as long as two years. It also was determined that heat inactivation of serums (56°C. for 30 min.) was neither harmful nor necessary, and that merthiolate added as a serum preservative had no effect on results.

Group C serums indicated that the FA reaction was specific for FMDV, because negative results were obtained with VS and VD convalescent serums. This group also indicated that tongue lesions per se were not a factor in FA reaction in the absence of FMDV.

Serums from immunized cattle, Group D, all gave negative FA reactions in spite of the fact that all of the serums had significant neutralization indices, and some were higher than certain FA positive ones in Group B. Some of these negative reactions were peculiar in that the monolayers exhibited a vague increased fluorescence as compared with the control. However, in such instances the contrast was not sufficient or characteristic enough to be useful in microscopic evaluation of the preparations. It is presumed that the vague increase in fluorescence was caused by combination of viral antigen and antibody, but that the mass of the reaction was insufficient to permit definite and reliable interpretation by human observation. There was no difficulty in classifying them as negative reactions when compared with the positive or suspicious reactions.

Two cattle, represented by serums 42 and 43, received an experimental vaccine. Their postvaccination serums, as given in Table I, were negative for FA reaction. However, their 14-day postchallenge serums gave a positive FA reaction and the neutralization indices were raised.
Both animals had small tongue lesions following challenge inoculations which did not generalize to the feet or to other areas of the mouth. This, in absence of FMD lesions, positive FA reactions were not found even though cattle had previous virus experience.

Group E cattle were negative to FMDV-detection-tests and were susceptible to challenge inoculation. Their serum neutralization indices were not considered significant, and the FA reactions of their sera were negative. Water-soluble cellular components and waste products from FMD lesions probably were present in some of the test materials inoculated into animals of this group. Thus, the results indicated that such materials, at the levels given (or formalin treated), do not stimulate antibodies necessary for FA reaction. Group D also contributed to this point.

Positive FA reactions were found with all sera of group F, except serum from one animal from which blood samples were taken two years after it received the last of seven successive inoculations of FMDV (all types represented). Another animal similarly treated still gave a positive FA reaction. The FA reactions with sera from the FMDV multi-infected animals were no more vivid than those from group B that received only one virus inoculation.

Two sera from a European brown fallow deer (raised in this country) gave a positive and a suspicious FA reaction. At the time sera were collected (21 and 220 days after the last inoculation) the deer had been inoculated in succession (similar to cattle) with all seven types of FMDV. Guinea pig FMDV antiserum (A-GB and C-GC) had negative FA reactions. The deer and guinea pig sera were not listed in Table I.

**SUMMARY**

An indirect fluorescent antibody (FA) technique for foot-and-mouth disease (FMD) evaluation of cattle sera was studied. The reacting system included commercial fluorescein-conjugated rabbit anti-bovine globulin, calf kidney cell cultures infected with foot-and-mouth disease virus, rhodamine bovine albumin and test cattle serum. Sera from 55 cattle were evaluated for FA reaction and the results were related to FMD virus experiences of animals and serum neutralization indices.

Sera from cattle that had developed lesions of FMD consistently gave positive or (in two instances) suspicious FA reactions. All seven types of FMD virus were used to infect cattle and the FA reaction did not distinguish between types. The FA reaction was detectable in two such sera as early as six days and for as long as two years after inoculation of cattle. One animal bled two years after inoculation gave a negative serum FA reaction.

Sera from cattle that had not developed lesions of FMD consistently gave negative FA reactions. This included cattle in the following groups: normal controls, vesicular stomatitis and virus diarrhea convalescent cattle, animals negative to FMD virus-detection tests and animals immunized by FMD virus test materials or an experimental vaccine.
REFERENCES


VESICULAR DISEASE - CURRENT THREAT

The presence of foot-and-mouth disease (FMD) South African Territory I (SAT I) outside the African Continent poses a serious threat to Europe and consequently to the western hemisphere. The rapidity of its spread through the near East from the first reported case on the Island of Bahrain in the Persian Gulf, confirmed in January 1962, is of immediate concern to all countries in southern Europe. The disease spread rapidly to the north and in a matter of weeks confirmed cases were reported from Iraq, Israel, and Syria. Simultaneously, widespread outbreaks occurred in Lebanon, Libya, and Jordan. Though untyped at the time these outbreaks were subsequently confirmed as being caused by SAT I Type FMDV. In June 1962 the first confirmed cases were reported from Turkey, By the middle of August nine provinces in Eastern and Southern Turkey were infected. The outbreak of SAT I FMD on August 21 on the European side of the Bosporus within the city of Istanbul, substantiated the early concern of authorities that Europe could be in grave danger. The latest confirmed case places SAT I FMD 100 Kilometers west of Istanbul.

The limited worldwide supply of vaccine against SAT I to effect immediate control measures and the complete susceptibility of the exposed livestock population presents a very grave picture. International organizations are presently establishing new production facilities in Turkey to meet the demand for vaccine to establish a buffer zone to protect the livestock population of Europe.

In addition to the occurrence of the type SAT I in the middle east and its spread in a westerly direction, there has been a costly epizootic of type C FMD in swine in the Netherlands. The epizootic has also occurred in Belgium and West Germany, however, it apparently has not been as costly in these two countries as in the Netherlands. Through strict control measures, the disease is presently quiescent and it appears that the control measures may have been effective. It is understood that this one outbreak has cost the Dutch more than $12,000,000.

The recent reports of outbreaks of FMD in South America brings the threat even closer to home. In March 1962, FMD was confirmed in the Choco Department of Columbia which borders on Panama. In April 1962 vesicular stomatitis was diagnosed in two horses in the Cochobamba Valley of Bolivia. This led the investigator to question whether the disease afflicting the cloven-hoofed animals in the area was FMD. It was not until three months later that bovine tissue specimens submitted to the Pan American Foot-and-Mouth Disease Center in Brazil confirmed the disease
to be Type O FMD. In August the National Livestock Exposition in Lima, Peru was cancelled due to serious outbreaks of FMD in six Departments in that country.

**VESICULAR STOMATITIS**

Although the outbreak of vesicular stomatitis (VS) in 1962 has not been as extensive as the one experienced in 1961, the disease is still a serious problem in the Southeastern States. Increased concern by the livestock owners was noted, particularly by those whose herds were infected for the second consecutive year.

The first 1962 case was confirmed by the laboratory in January and occurred in Tallapoosa County, Alabama. Another case was confirmed during February in Horry County on the Atlantic Coast in South Carolina. Tissue and/or serum samples from 171 premises in fifteen states was submitted for laboratory test. Positive results were obtained on 43 from Georgia, 29 from Alabama, eight from North Carolina, eight from Louisiana and two from South Carolina for a total of 90 infected premises thus far in 1962.* These figures represent laboratory confirmed cases only and may not be a true indication of incidence.

Several farmers with confirmed cases in their herds during 1962 reported having observed a similar but unreported condition in their herds last year. One investigator reports that during August 1962, some cases of VS were not being reported to livestock regulatory officials. This information was received from a farmer who had reported a case in his herd. He was later contacted by neighbors to determine what could be done for a similar but undiagnosed condition in their animals. Upon learning there was no effective treatment and that 30 day quarantines were being applied, they ended their call with the request that they not be reported. It would be impossible to determine the number of unreported cases any one of which could have been FMD.

During the summer Dr. Derl L. Brooks, Entomologist, National Animal Disease Laboratory, attempted to collect additional field data relative to the possible transmission of VS by insect vectors. His efforts were initiated in Louisiana since the first confirmed case of the anticipated summer enzootic occurred in Livingston Parish in May. No new cases of VS occurred during the period he was in Louisiana from June 22 through July 10 although numerous insect collections were made. Insects representing six genera were shipped to the National Animal Disease Laboratory, Ames, Iowa. Insects most prevalent at the time were the horsefly *Tabanus*, the hornfly *Siphona irritans* and mosquitoes. Although blood-sucking insects were very numerous, further cases of VS were not reported to regulatory officials in Louisiana. Virus isolation attempts from insect pools using embryonated eggs, suckling mice and swine kidney tissue cultures were negative.

With several cases of VS breaking in Carroll, Coweta and Heard counties during early July, insect collection operations were transferred to

*Through October 24, 1962.*
this area of Georgia on July 12. In contrast to the situation in Louisiana, insect populations in Georgia at this time were very low. Diligent effort and application of several methods of collection failed to produce significant numbers of insects. The investigator resorted to exposing himself from sundown until after dark and suffered only two mosquito bites. Interviews with farmers in the area indicated that they had experienced the most dense population of horseflies they could recall in recent years, however they had largely disappeared by the second week in July. Though an exhaustive search was conducted for the elusive sandfly, *Phlebotomus*, not a single specimen was recovered during the 1962 operation.

A review of the epizootiology of the cases of VS in 1962 fails to disclose significant information pointing to common factors contributing to the spread of the disease.

These reports of extensive outbreaks of FMD together with expanding international trade and foreign travel of United States citizens, demands increased vigilance by all parties with responsibility to the livestock industry. The threat of FMD is further magnified by the annual occurrence of VS in the enzootic areas of the United States. An unreported case, or one treated complacently, in this area could mask an outbreak of FMD and allow the disease to become established over a wide area. This would not only compound the difficulties encountered in applying control and eradication measures, but would also be an extremely embarrassing situation for those responsible for its control. To reduce our vulnerability to this threat, the Committee recommends that this Association urge all segments of the livestock industry to direct concerted efforts toward the following points: (1) The early reporting of all suspect vesicular conditions to eliminate the possible masking of FMD by cases of VS. (2) The strict enforcement of existing State laws and regulations controlling the feeding of garbage to swine. (3) The study of the epizootiology of VS on a regional basis by those states located within the enzootic area. This regional approach should be aimed at the development of comparable and comprehensive epizootioclocal information. A compilation of the information obtained and its evaluation relative to research and field trials already completed would provide direction for necessary additional research. (4) Additional research to disclose the vector, or vectors, of vesicular stomatitis virus (VSV) and the reservoir host or hosts in the enzootic area. This recommendation is of utmost importance in view of cases of VS confirmed in isolated herds.

**RESEARCH OF VESICULAR DISEASES**

*Vesicular Exanthema*

Since the disease was officially declared eradicated in the United States by the United States Department of Agriculture on October 22, 1959 research with the virus has been limited to work at the Plum Island Animal Disease Laboratory and the Naval Biological Laboratory at the University of California. Work at the Plum Island Animal Disease Laboratory was completed during 1960.
**Vesicular Stomatitis**

Work is continuing at several locations on the method of spread of vesicular stomatitis virus (VSV) and there are indications that some species of vectors may play an important role. Species of horseflies, deer-flies, and mosquitoes have been shown to transmit vesicular stomatitis virus from infected to noninfected embryonating chicken eggs. However, the mode of transmission has been determined to be mechanical because of the rapid loss of ability of the insects to transmit the virus. VSV has been isolated from mosquitoes trapped in Panama and there have been repeated isolations of VSV from sandflies. Attempts to isolate virus from insects collected during an epizootic in Texas including biting gnats, deer-flies, horseflies and mosquitoes, were fruitless. It has been reported by Mussgay that VSV multiplies in *Aedes aegypti* mosquitoes and that the mosquitoes are capable of transmitting the virus to one-day old mice in laboratory studies. Transmission was observed at five, seven and 15 days after the mosquitoes had fed on infected fluids. Transmission did not occur at four days after such feeding trials. It has not been determined whether this species of mosquito plays a role in the transmission of VSV in nature.

The duration of VS immunity patterns of compliment fixation and serum neutralization in sera from convalescent swine has been compared with immunity by virus challenges and reported at this meeting.

Field trials of live virus vaccination procedures for the prevention of VS in cattle were conducted in Central America. A preliminary report on the results of these trials have been reported at this meeting.

**Foot-and-Mouth Disease Virus**

Research is in progress in several laboratories, to develop a modified FMD vaccine, using tissue culture virus. Workers at the Virus Laboratory at Tubingen, Germany, have attenuated types O, A and C FMDV by passage in calf or calf embryo kidney cell cultures. Various types of virus have been attenuated following as few as 141 passages for some types and as many as 558 for other types. While the workers appear optimistic about these modified virus strains, they also caution that much work remains to be done prior to use in the field.

Work is also continuing in South America on adaptation and attenuation of FMDV in embryonating chicken eggs and in young rabbits. Not all types of FMDV have been adapted to these two hosts, and the work has not progressed sufficiently to permit field use of those types which have been adapted.

In work conducted in Germany with polyvalent modified FMDV, it has been concluded that strains, when attenuated by passage in cultures of renal epithelial cells give good results when used in monovalent vaccines. However, in tests where the strains have been combined, they have behaved differently. Workers have observed an increase in virulence when two or three attenuated strains are used simultaneously. Following such vaccination, each of the strains may cause FMD or the immunity may not develop to one or more types of the virus. Symptoms of the disease in these cases, is mild and cannot be compared with those found following natural
infection, however, the signs also cannot be ignored, and their occurrence indicates that the strains, when used as living bivalent or trivalent vaccines cannot be considered as entirely innocuous. Similarly, potency tests have shown that when a vaccine is used simultaneously, the degree of immunity which follows does not approach that which results from the vaccines which are given one at a time. More intensive experimental research is necessary to solve this problem.8

For several years, British workers have been using modified live attenuated vaccines in which the pathogenicity for cattle has been reduced by passage, first in unweaned mice and, secondly, in adult mice. The vaccines consist of a filtrate prepared from infected mouse carcasses and equal parts of glycerine. During the past year, products prepared in this manner have been used extensively in field trials in South Africa and while the results have been generally satisfactory, in that vaccination has been attributed as being a major factor in control of the epizootic, there have been some disappointments in the trials. There have been reports that when the product was used in sheep and goats, some untoward results have followed. However, it has not been conclusively shown that deaths in these species was directly attributable to the vaccine. There were also reports that losses occurred in young stock, that is, lambs, piglets and young calves, however, these losses could not be directed as due to the vaccination. British workers indicate that more work is needed on the effects of these live vaccines in pigs, goats, and sheep, than has thus far been possible to do.9

A South African Type 1 of FMDV has been attenuated for cattle by passage in suckling and adult mice. Considerable quantities of the vaccine have been used during the past year for control of this type of infection in the Middle East. Generally, the results have been satisfactory, and in countries where it has been applied extensively, it has probably assisted in controlling the infection. As observed in the use of modified vaccine in South Africa, similar experiences were encountered in the Middle East where there were deaths among the young of some species. Work is continuing at Pirbright on these modified strains of FMD vaccine.10

Using the complement fixation technique developed by Brooksby, Davy, of the Research Institute, Pirbright, has examined a selection of virus strains which have caused epizootics in the field and which have been used for vaccine production in various parts of the world; these strains have been compared with the established subtype strains and classified into subtype groups. In many cases, they have shown close relationship between strains of viruses, but in some cases, the relationship has not been so closely defined. The significance of such antigenic differences must be correlated with the differences which can be demonstrated in vaccination trials. There is evidence the vaccines may be improved sufficiently to protect against the kind of heterologous challenge normally encountered in the field. The limited range of vaccines may be sufficient to protect against all the subtypes corresponding with one immunological type. In the subtype studies, 10 type O, 15 type A, six type SAT-1, three type SAT-2, and three type SAT-3 subtypes have been identified. Studies with Asia I and type C virus have not yet been completed.11
It has been known for sometime that the virus of FMD is made up of two specific complement fixing components. The infectivity of this suspension appears to be confined to the larger 20 millimicron particle. When this component is heated or when the pH is permitted to drop below seven, there is a corresponding drop in infectivity, with the simultaneous production of a component apparently identical with the smaller seven millimicron noninfective component found in virus suspensions. This close relationship between the two components has now been emphasized by the discovery that infective ribonucleic acid is released at the same time as the seven-millimicron component. From this evidence, the particles may be viewed as being composed of the ribonucleic acid core, combined with seven-millimicron protein subunits, a view which is in agreement with the general structure of small viruses postulated by Watson and Crick in 1956. The development of the phenol method for extracting infective ribonucleic acid virus infected cells enables studies to be made of methods by which viruses multiply. The multiplication of FMDV has been studied by a variety of workers and F. Brown has reviewed this work and has shown that the following distinct stages may be observed: 1) adsorption, 2) penetration, 3) release of RNA from the virus, 4) synthesis of viral RNA and protein, 5) combination to form complete virus. Adsorption and penetration have been shown to be complete within one minute, and the nucleic acid is also quickly released from the adsorbed virus. If these cells are incubated, new virus begins to appear after about 1-1/2 hours. By extracting the cells at intervals with phenol, it may be shown that new infective ribonucleic acid is produced in the cells shortly before complete virus. So far, there is little evidence regarding the formation of viral protein, so it is not yet possible to build a complete picture on the way in which the complete virus particles are produced.12

Workers at Pirbright have recently shown that a line of tissue culture cells originating from baby hamsters designated BHK-21, is capable of supporting virus growth of all seven types of FMDV.13 These cells were isolated in the Institute of Virology, Glasgow, and have been described by McPherson and Stoker.14 In addition to supporting growth of the virus, the monolayers may be used for plaque assay techniques. Preliminary investigations with this cell line indicate that it will be of considerable value in FMD research. In addition to its use for virus assay purposes, it is likely that these cells may be of value in the preparation of both inactivated and live attenuated virus vaccines.13 Cunliffe has studied the antibody response in a group of swine after infection with FMDV. Virus neutralizing antibody and immunity after infection with FMDV was studied for 128 days. Antibody first appeared at three days, rose to peak levels between seven and 10 days, and regressed to a plateau by 28 days. After 28 days, there was little change in mean antibody titers.

An attempt to reinfect 10 swine at 28 days was not successful. At 128 days, the immunity status of four convalescent swine neutralized more than four logarithms of virus in an in vitro titration. However, in another group of five convalescent swine, one developed vesicular lesions when exposed to infective swine. Efforts to demonstrate latent virus in one pig 128 days after infection were not successful.15
Fellowes has studied the antibody response of chickens to FMDV. Following inoculations, lesions suggestive of infection were observed in or on the mouth, combs, and feet of chickens inoculated with the Type A, 119 virus, propagated in tissue cultures. A high titer neutralizing antibody were produced in adult chickens by inoculation of one and two ml of either infective or noninfective Type 119 virus, grown in tissue and cultures. This virus also produced a good response for complement fixing antibody in chickens as tested by a modified direct complement fixation test. No evidence of infection was found in normal chickens held with infected birds in the same cage. This species may be useful in evaluating vaccines.11

Results of studies relative to the detection of FMD recovered cattle by the fluorescent antibody technique have been reported at this meeting.19

REFERENCES

At the 63rd Annual Meeting of the United States Livestock Sanitary Association, the Committee on Biologics and Pharmaceuticals (Proc. 63rd Ann. Meet. USLSA, 1959: 30-32) recommended the appointment of a Viral Research Committee. It further recommended that such a Committee concentrate its efforts on collecting information on "new viruses," development of an animal virus classification and cataloguing system, and eventually a central repository for viruses. In 1960 the new Committee reported progress on two phases of its directed charter, (1) the development of a protocol for reporting new viruses, and (2) the development of a standard protocol for identification and classification of new viruses. (Proc. 64th Ann. Meet. USLSA, 1960: 351-353).

At the 65th Annual Meeting (Proc. 65th Ann. Meet. USLSA, 1961: 413-421) further progress was reported. Protocols developed by the Committee for recording data on viruses, and for serological identification of animal viruses were incorporated as part of the report. A resolution setting forth the future plans of the Committee was adopted by the 65th Annual Meeting. This resolution included a plan for establishing a central repository of virus information at a recognized impartial institution to be financed by a grant from a non-profit institution. Authority was sought and granted for the Committee to act in the name of the United States Livestock Sanitary Association in the financing and operation of the project (Proc. 65th Ann. Meet. USLSA, 1961: 422). A change of the name of the Committee as reflected in the present report was also recommended and adopted.

Initial efforts to obtain permanent financial support for the committee were unsuccessful. Other sources of support are being actively pursued. Temporary support to expedite committee work has been offered by the World Health Organization (WHO) of the United Nations on a matching fund basis.

Progress has been made on locating a site for the proposed repository and cataloguing center if funds do become available. An internationally known Veterinary Scientist has tentatively agreed to act as Principal Investigator of the Project. In the meantime, the Committee has made some progress in getting the Virus Classification Protocols circulated to
Veterinary virologists with a request that they be filled out and returned to the Committee. The completed protocols are being filed for processing and cataloguing as soon as a Center is established. It is recognized that revisions of the 1961 Protocols will be needed to fit future needs. To this end a sub-committee on revision has been appointed.

Several members participated in a World Health Organization Informal Meeting on Comparative Virology held in New York City, March 9, 1962, by invitation of its chairman, Dr. Martin Kaplan. The subject of discussion of this conference was "Animal (Veterinary) Virus Classification—an International Problem." The WHO conference accepted in principle the United States Livestock Sanitary Association, Animal Virus Classification Committee report of 1961 and recommended that a similar committee be set up under WHO sponsorship for European and African participation. A request was made to the United States Livestock Sanitary Association group to cooperate with WHO and to include South and Central America in its scope. Plans were tentatively made to proceed on a WHO-sponsored classification and cataloguing system using the United States Livestock Sanitary Association Committee's protocols for recording data and serological identification. The Foot-and-Mouth Disease Research Institute at Pirbright, England (Dr. Ian Galloway), has agreed to act as the center for this WHO activity in the Eastern Hemisphere. Dr. A.O. Betts, Cambridge, England, was added to the 1962 United States Livestock Sanitary Association Committee to aid in coordination of future plans along the lines suggested by the Conference.

The Committee wishes to express appreciation to Dr. R. M. Taylor, Chairman, Arthropod-Borne Virus Information Exchange, University of California, Berkeley, California, for loan of protocols used as patterns by the Committee in its 1961 report. It is the continuing policy of this Committee to recognize the need for close liaison with the various groups in human medicine concerned with virus classification and cataloguing. We plan to establish official liaison with these various organizations.

It is recommended that the Committee be continued with the same authority vested in it by the Association at its 65th Annual Meeting in 1961.
CONSTITUTION AND BY-LAWS

OF THE

UNITED STATES LIVESTOCK SANITARY ASSOCIATION

ARTICLE I—NAME

The name of this Association shall be "The United States Livestock Sanitary Association."

ARTICLE II—PURPOSE

The purpose of this Association shall be the study of livestock sanitary science, milk and meat hygiene, and the dissemination of information relating thereto, the unification so far as possible of the laws, regulations, policies and methods pertaining to milk and meat hygiene, and to the prevention, control and eradication of transmissible livestock diseases; to maintain co-ordination among the various livestock regulatory organizations, and to serve as livestock sanitary science clearing house between this Association and the following: The livestock owner, the livestock sanitarian, the milk and meat hygienist, the veterinary practitioner, the transportation and stock yard companies, the milk and meat producing and distributing companies, and various other interested agencies. The word "livestock" as herein used shall be understood to include poultry.

ARTICLE III—MEMBERSHIP

There shall be three kinds of members—Official and Individual and Non-Voting Junior.

OFFICIAL MEMBERSHIP

The livestock sanitary departments of each state also the United States, and the Canadian, Cuban and Mexican governments, Puerto Rico, the Virgin Islands and Los Angeles County, California shall be eligible to official membership in this Association and be represented on the Executive Committee by the livestock sanitary executive official.

INDIVIDUAL MEMBERSHIP

Any person engaged in livestock sanitary work for Federal, provincial, state, county or municipal governments and any other person interested in livestock sanitation or milk and meat hygiene may be elected to individual membership.

JUNIOR NON-VOTING MEMBERSHIP

Students in agriculture, medicine, veterinary medicine, vocational agriculture or any 4-H Club member as well as future farmers under 21 years of age are eligible to election as junior non-voting members.
ARTICLE IV—MEETINGS

The meetings of this Association shall be annual and special.

ARTICLE V—OFFICERS

The officers of this Association shall be: President, President-Elect, First Vice-President, Second Vice-President, Secretary-Treasurer, and an Executive Committee.

EXECUTIVE COMMITTEE

The Executive Committee shall be composed of the executive officer representing the livestock sanitary departments of the various States, the Director of Livestock Regulatory Programs of the United States Department of Agriculture, the Veterinary Director General of Canada, the executive regulatory officer of Cuba, Mexico, Puerto Rico, the Virgin Islands, Los Angeles County, California, the elective officers of this Association and eight delegates at large representing the livestock industry including poultry.

No more than two delegates from each of the four districts of the United States shall be elected. Said districts shall be known as the Northeast; consisting of the States of Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island and Vermont; the North central, consisting of the States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin; the Southern, comprising 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. It shall be the duty of the Committee on Nominations to canvass the membership of this Association and select eight (8) nominees for delegates at large. Said nominees must be selected from and represent the livestock industry, including poultry. No more than two (2) delegates at large shall be elected from each of the four designated areas or districts, nominations from the floor of the convention may be made for additional nominees by districts and shall be bona fide residents of the respective district for which they are nominated. Such delegates shall be elected at the time and place as are the elected officers of this Association.

The Executive Committee shall constitute the administrative body of this Association and shall determine its activities and policies. All recommendations and reports of officers and committees shall be referred for consideration to the Executive Committee.

The President-Elect shall be ex-officio chairman of the Executive Committee.

The Executive Committee shall elect yearly a Secretary-Treasurer for the Association. The Secretary-Treasurer shall receive such salary and allowance as may be fixed by the Executive Committee.
The Executive Committee shall cause to be audited annually or oftener if deemed necessary, the receipts and disbursements of the Secretary-Treasurer, and shall have authority to hear and determine all complaints filed before it in writing relative to the conduct of any member; and shall have authority to accept or reject applications for individual membership properly placed before them. Three negative votes shall disqualify for such membership.

That, with the exception of a change in the name of this Association, upon the dissolution of this corporation or the termination of activities thereof, all remaining assets thereof shall be contributed for utilization in the advancement and research of diseases of animals, and no part of the net assets shall inure to any person or group of persons for private gain.

ARTICLE VI—PROGRAM COMMITTEE

The President, the Chairman of the Executive Committee and the Secretary-Treasurer and the Chairman of the respective committees shall constitute the Program Committee. It shall be the duty of the officers of the Program Committee to make the necessary arrangements and provide the program for the annual and special meetings.

ARTICLE VII—DUTIES OF OFFICERS

1. President: It shall be the duty of the president to preside at all meetings of this Association; to appoint all committees excepting the Executive and Officer faction of the Program Committee; to call special meetings of the Association whenever he considers the holding of such meetings necessary for the good of the livestock industry or upon the written request of five members of the Executive Committee. The president shall be an ex-officio member of all committees.

2. President-Elect: The president-elect shall be chairman of the Executive Committee. In the absence of the president, he shall preside at the meetings of the Association. In the event of the absence, disability or resignation of the president he shall perform all duties of the president. He shall be an ex-officio member of the Executive and Program Committees.

3. First Vice-President: The first vice-president shall assume the duties of the president in the event of the absence, disability or resignation of the president and president-elect. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability or resignation of the president-elect. He shall be an ex-officio member of the Executive Committee.

4. Second Vice-President: The second vice-president shall assume the duties of the president in the event of the absence, disability or resignation of the president, president-elect and first vice-president. He shall assume the chairmanship of the Executive Committee in the event of the absence, disability or resignation of the president-elect and first vice-president. He shall be an ex-officio member of the Executive Committee.
5. Secretary-Treasurer. The Secretary-Treasurer shall keep an accurate record of the proceedings of the Association. Whenever authorized so to do by the Executive Committee he shall publish said proceedings and distribute them to the members of the Association. The Secretary-Treasurer shall also keep an accurate record of the proceedings of the Executive Committee and shall furnish a copy to each member of said Executive Committee. He shall forward to each Executive Committee member a copy of each regulation approved by the Association.

He shall keep an accurate account of all Association moneys received and disbursed. He shall also present to the Chairman of the Executive Committee a list giving the name, occupation and address of each applicant for individual membership for the approval of the Executive Committee. He shall perform such other duties as may be authorized and prescribed by the Executive Committee. He shall be ex-officio secretary of the Executive Committee, also an ex-officio member and secretary of the Program Committee. He shall be bonded for not less than ten thousand dollars.

ARTICLE VIII—AMENDMENTS

The constitution of this Association may be amended by a two-thirds vote of the members of the Association 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 and further provided that the amendment has received the approval of the Executive Committee.

BY-LAWS

ARTICLE I—ORDER OF BUSINESS

Registration.
Call to Order.
Report of Secretary-Treasurer.
President's Address
Reading of Papers.
Committee Reports.
Discussion.
Unfinished Business.
New Business.
Nomination and Election of Officers and eight members to Executive Committee.
Adjournment.

A suspension of the By-laws may be made by a two-thirds majority 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. The Application shall give the name,
occupation and address of the applicant and shall be accompanied by a fee of five dollars ($5.00), which amount shall include the membership dues for one year. Applications shall be presented in proper form to the Secretary-Treasurer, who shall in turn submit them to the Executive Committee.

An individual member may be expelled for cause by the Executive Committee.

ARTICLE III—MEETINGS

The annual meetings shall unless otherwise determined not less than thirty (30) days in advance by a majority of the members of the Executive Committee, be held at Chicago, Illinois, during the time of the International Livestock Exposition. The place for holding the meetings in Chicago as well as the duration of said meetings shall be determined by the Officer Members of the Program Committee of the Association.

The place for holding special meetings shall be determined by the President with due regard to the wishes of the members of the Executive Committee, the subject matter to be considered, accessibility, and the information to be obtained. The notice of time and place of holding a special meeting shall be mailed to the members at least thirty days prior to the date fixed for the special meeting.

ARTICLE IV—QUORUM

Twenty-five members of the Association shall constitute a quorum.

Twenty members of the Executive Committee shall constitute a quorum.

ARTICLE V—DUES

The dues for individual membership in this Association shall be five dollars ($5.00) per annum, payable in advance (on or before January 1st of each year) to the Secretary-Treasurer of the Association.

The dues for non-voting junior members shall be three dollars ($3.00) per annum, payable (on or before January 1st of each year) to the Secretary-Treasurer of this Association.

The dues for official memberships shall be one hundred dollars ($100.00) each per annum, payable in advance (on or before January 1st each year) to the Secretary-Treasurer of this Association.
OFFICERS, CONFERENCE OF VETERINARY LABORATORY DIAGNOSTICIANS FOR 1963

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Chairman

E. P. POPE
Secretary
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THE DIAGNOSIS OF COCCIDIOIDOMYCOSIS

Keith T. Maddy, D.V.M., M.P.H.*

Coccidioidomycosis has been diagnosed in a number of species of mammals. In certain highly endemic areas, it is probable that significant portions of the populations of many, if not most, species of mammals become infected.

Besides man, animals which have been found infected include the following: Cattle, dogs, horses, burros, sheep, swine, domestic cat, llama, monkey, gorilla, tapir, coyote, chinchilla, and desert-living rodents—pocket mice (*Perognathus baileyi*, *P. penicillatus*, *P. intermedius*, and *P. formosus*); kangaroo rat (*Dipodomys merriami*), grass-hopper mouse (*Onychomys torridus*), and the antelope ground squirrel (*Citellus lecurus*).

EPIDEMIOLOGY

In considering a diagnosis of coccidioidomycosis, various aspects of the epidemiology must be carefully considered.

*Mode of Acquiring Infection*

The usual mode of infection of man and animals is by the inhalation of air-borne arthrospores from mycelia which grow in the soil. Inhalation of mycelial fragments can also result in infection.

Sporangia are not often released to the outside from the infected person or animal, and this form of the fungus is not usually considered infectious. It is believed that due to the large size of the sporangia, they cannot be inhaled into the pulmonary alveoli. Also, sporangia would not often be expected to be air-borne. When sporangia fall on soil or other suitable media, they can sprout mycelia with production of arthrospores in a few days. This has been observed to occur from some dogs with disseminated disease. The feces, urine, saliva, and wound exudate of such animals could occasionally contaminate a yard for persons or animals. It is not common, however, for persons or animals to shed massive quantities of sporangia.

Neither sporangia nor arthrospores are infective via the digestive tract.

At one time it was believed that man often acquired infection via a skin injury. It is now known that almost all skin lesions are the result of dissemination, and few result from skin wound contamination. A few cases of primary infection of skin lesions have been observed in dogs.

*Most of Dr. Maddy's research on coccidioidomycosis was conducted while assigned to the Communicable Disease Center. At present he is with Extramural Programs of the Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda 14, Maryland.
Natural Exposure

With the fungus growing in the soil, the more dust that is inhaled, the more likely infection is to occur. Infection can occur at any time of the year when desert soil is dug up. When only surface dust is blowing about, infection is more likely just after rains which have been preceded by a dry period of several months. Reduction of exposure to dust results in fewer infections of man and animals.

Fomite Exposure

Dust laden fomites can be a source of infection outside the endemic area, but this does not appear to occur often.

Geographic Distribution

Diagnosis of this infection in persons or animals which have not been in an endemic area should not be very seriously considered, although infection via fomites is a remote possibility.

The etiologic agent *Coccidioides immitis* is believed to propagate only in the Western Hemisphere in the arid and semi-arid areas where the summers are hot and the winters are mild. Reports of cases indicating exposure outside these areas are considered with considerable skepticism.

Infections have occurred in Mexico, Paraguay, Argentina, Venezuela, and possibly also in Peru, Honduras, Ecuador, and Bolivia. In the United States the endemic areas are: western Texas, southern New Mexico, southern and western Arizona, southwestern Utah, southern Nevada, and southern and central California.

Merriam in 1898 classified the United States into several biologic life zones, one of which is the Lower Sonoran Life Zone. Since that time there have been numerous modifications and refinements of the life-zone concept. These zones were first divided according to the sums of the degrees of temperature above a given degree for a year, added to the mean temperature for the warmest six weeks. This classification was based on the knowledge that plants grow only when the environmental temperature is above a certain point. These zones were subdivided into their humid and arid provinces, and the plant and animal life was studied as to its distribution within the zones. These life zones are useful as broad geographic areas in aiding general ecologic studies of plant and animal life (Figure 1). It has been pointed out that for analyzing the ecology of the area of smaller geographic subdivisions, a study of plant and animal associations is more useful. Slope exposure, air currents, streams carrying cold water, evaporation from moist soil, proximity to large bodies of water, influence of lingering snow banks and of glaciers, changes in vegetative covering, rock surfaces, and other miscellaneous local influences can alter the characteristics of a life zone in a local area.

The life-zone concept is empirical, and biologists do not agree on the number of zones or the divisions. Biologists do not agree as to the value of the life-zone concept; some prefer to use a biotic province concept.

The Lower Sonoran Life Zone is characterized by arid and semi-arid climates, with hot summers and few winter freezes, low altitude and
Figure 1. The Lower Sonoran Life Zone of the United States. The endemic area for coccidioidomycosis is within this zone and covers most of it.

alkaline soil. Only certain types of plants and animals can survive in this environment. Only this environment seems to permit *C. immitis* to exist in nature as a soil organism.

Within the Lower Sonoran Life Zone certain areas are more endemic than others. In the northern part of the Western Hemisphere the July mean temperatures of areas of high infectivity are above 80°F. Some infection has occurred in areas with July mean temperatures as low as 77°F, but seldom where it is lower. The January mean temperature is above 45°F in areas of high infectivity. Some infection occurs where the January mean temperature is as low as 35°F, but rarely below this. The annual rainfall varies from five to 20 inches in the more endemic areas. As rainfall decreases to less than five inches, infectivity of the area drops. It appears that infections do not occur in areas with more than 20 inches of rainfall unless there are particularly high temperatures to reduce precipitation effectiveness.
A few arthrospores of *C. immitis* remain viable in desert top soil in spite of high soil temperatures. These periods of high temperature have a semi-sterilizing effect upon the soil and then when rain moistens the soil *C. immitis* appears to propagate more rapidly than some of the other soil microflora. The arthrospores develop extensive mycelia which then develop more arthrospores when dryness returns. The organism grows most abundantly in and around rodent burrows, sometimes around bits of rodent feces.

**Immunology**

Infection of man and animals appears to confer a good degree of lifelong immunity against further attacks. A diagnosis of a second infection should be made with considerable care. There is evidence that an overwhelming exposure can break through an acquired immunity. Most infections produce only the less severe primary infection of the respiratory system. Once immunity is established, there appears to be little danger of a progressive generalization of the infection. Most disseminations of infections throughout the body occur within a few months after infection is acquired.

The immune response can be measured by three tests: skin, precipitin, and complement-fixation.

The skin-test agent most widely used at present is produced by growth of *C. immitis* on a modified asparagine formula prepared by the Smith technique. It will remain stable at room temperatures for a number of years unless it becomes contaminated. In man it is most often used at a 1:100 dilution. A 1:10 dilution is used when low, and a 1:1000 when high, sensitivity is suspected. Coccidioidin is used undiluted in animals.

The coccidioidin is injected intradermally using 0.1 ml. A positive reaction is represented by an induration of five mm. or more in diameter. Man and the dog are most likely to show a positive reaction 48 hours after injection. Cattle are most likely to show a positive test at 96 hours (Figure 2). More studies are necessary on other species to determine the best time to measure their reactions.

Sensitivity to the skin test usually develops in man a few days after symptoms develop. Coccidioidin sensitivity persists for a number of years in man, cattle, and dogs. The duration is unknown in other species. Sensitivity is sometimes lost when generalization of the disease occurs in man and in the dog.

There is not cross sensitivity between tuberculin and coccidioidin, but there is between coccidioidin and histoplasmin, haplomycin, and North American and South American blastomycins.

Coccidioidin can be used in a precipitin test on a patient's sera. Precipitins appear in man a few days or weeks after skin sensitivity develops, and then they usually disappear in a few days or weeks. Not much is known about precipitins in the sera of infected animals except that they seem to have somewhat the same time pattern of appearance and disappearance in the dog as they do in man. Infected cattle seem to develop few precipitins.

Coccidioidin can also be used in a complement fixation test. Complement-fixing antibodies appear later than precipitins or skin sensitivity,
Figure 2. Positive coccidioidin skin test in cervical area of a cow—96 hours post-inoculation.

and then only in the more severe infections. They persist for several months in some primary infections. With dissemination, they may remain as long as the patient lives and may rise to high levels. The complement fixation test with coccidioidin will sometimes give positive results in low titers with sera from patients affected by histoplasmosis. Blastomycin will give some false positives with serum from a patient with coccidioidomycosis. Dogs with disseminated coccidioidomycosis develop high complement fixation titers.

PATHOGENESIS AND PATHOLOGY

How Infection Spreads

Arthrospores contact the mucosa of alveoli and bronchi. The arthrospores round out and increase in size with endospores visible internally. Four to seven days after the arthrospores enter the lungs, mature spherules can be found on the bronchial mucosa. Tiny granulomas develop around the infection sites in eight to 21 days. These may lie along the bronchi or well in the pulmonary parenchyma. Early small lesions may consist of an alveolus filled mostly with spherules and polymorphonuclear leukocytes. Some small lesions of this type seem to heal almost completely. Others may be larger and involve an entire lobe. There may be suppurition, hemorrhage, necrosis, and then granuloma formation (Figure 3). This may be followed by hyalinization, fibrosis and, occasionally,
Figure 3. Two lobes of a dog's lung six weeks post-infection. Note the development of numerous granulomas.

Figure 4. A cow's lung with a cut through the bronchial lymph nodes showing several granulomas. An incision has also been made into a granuloma in the lung tissue.
calcification (Figures 4 and 5). In man, a lesion occasionally cavitates. Usually these cavities heal rapidly, but a few epithelize and persist indefinitely.

The hilar and mediastinal lymph nodes may enlarge and contain small granulomas following pulmonary infection.

When endospores are released from a spherule in tissue, neutrophiles enter the ruptured spherule and attempt to phagocytize the spores. The spores are destructive, killing the neutrophiles and damaging blood vessels. As the endospores grow into sporangia, mononuclear cells may engulf them and become giant cells. Other mononuclear cells may surround the engulfed spherule. When the spherule ruptures, the giant cell dies and more neutrophiles repeat the cycle. If the resistance of the tissue is good, granulomas form and the infection is walled off. When suppuration continues without adequate granuloma formation, dissemination can occur. This may be due to poor local tissue resistance or to an overworked body trying to suppress numerous sites of infection following a massive exposure.

The endospores that escape from the suppurative pulmonary sites are carried by the lymphatic system to the pulmonary lymph nodes, where another attempt is made to destroy them. Usually this is as far as the spores get. If they survive beyond this point in the lymphatic system, they soon enter the blood stream and become disseminated throughout the body (Figure 6).
Figure 6. Some tissues from dog which died of coccidioidomycosis. Note the granulomas in the lungs, liver, spleen and carpal joint area.

**Tissues Affected**

Tissues other than the lungs that are often affected in the disseminated form of the disease are: liver, spleen, kidneys, and bones. In man, dermal and subcutaneous tissues are almost always affected in disseminated disease. Often the meninges of man are involved in disseminated cases. In numerous dog and human cases, it has been observed that almost every organ and tissue can become involved, although the gastrointestinal tract is the one most frequently spared.

Bone lesions often develop at the epiphyses; if they become extensive, nearby joints may become involved. Bone lesions of man may be destructive, while those of the dog often are proliferative.

The disseminated lesions develop much as do the pulmonary ones already described. They tend, however, to be more suppurative, with less tendency to form granulomata.

**Spectrum of Infection**

Smith pointed out the desirability of considering the various clinical entities of coccidioidal infection of man as part of a merging spectrum. At the one end is the completely inapparent infection, and at the other end is the disseminated form ending in death. However, due to the marked
differences in prognosis, it is best to make at least two groupings: the primary types and the disseminated types.

**Primary Types**

Probably most mammals are susceptible to primary infection. Practically all primary infections are acquired via the pulmonary route and only in rare instances by subcutaneous inoculation. Many of these infections are undoubtedly asymptomatic. It appears that all infections of cattle are asymptomatic. In human adults, three-fifths are asymptomatic. It seems that a majority of the primary canine cases are also without signs. Little is known of this aspect of coccidioidomycosis in other species of animals.

There may be only transient malaise, cough, and pleural pain in some human cases. These same symptoms may be present, but of greater severity, in the more serious cases. There also may be fever, headache, backache, night sweat, anorexia, rash, sore throat, erythema nodosum, erythema multiforme, arthritis, and weight loss. In a few patients, a cavity may develop in one or more of the pulmonary lesions. Cavitation may be responsible for some pain, a productive cough, and hemoptysis.

Primary disease may be manifested in the dog by an acute or a chronic cough of variable intensity; there may be shortness of breath due to pressure on the trachea and bronchi by enlarged, hilar lymph nodes. The body temperature may remain high with possible reduction by salicylates but not by administration of antibiotics. The appetite may be poor and weight loss considerable. The animal may also be stiff and disinclined to move.

Signs and symptoms in man and the dog may subside within a week, but they can persist for weeks or months. A number of days during this time may be free of illness only to be followed by a repetition of previously manifested signs.

**Disseminated Types**

Progressive or disseminated coccidioidomycosis has been determined to occur in the following species: man, dog, horse, sheep, llama, monkey, gorilla, chinchilla, coyote, domestic cat, and seven species of wild rodents. No doubt other species will be added to this list.

Dissemination from primary sites usually occurs in man and animals and signs and symptoms vary greatly, depending on the tissues or organs involved. In man, a single lesion in the skin, a bone, a lymph node, or evidence of meningitis may be the first clue of dissemination. Many of the signs and symptoms in dogs during primary illness may become accentuated, but the best clear evidence of spread beyond the thoracic cavity in the dog is the development of a bone lesion. If it is in a leg bone, lameness may soon become quite marked (Figure 7).

Experimental infection of dogs with *C. immitis* has furnished exact data on the clinical course of the disease. A number of the observations are quite applicable to man.
Figure 7. Lesion of coccidioidomycosis in left carpus of a dog. Note also the cachexia.

MAKING THE DIAGNOSIS

A careful consideration of signs, symptoms, and epidemiologic circumstances may result in an accurate clinical diagnosis; however, special tests and studies often are required to confirm the diagnosis. Roentgenograms of the lung may reveal single or multiple parenchymal lesions. There may be evidence of involvement of an entire lobe. Hilar enlargements may be detected. X-rays of bone may also reveal suspected lesions. The leukocyte count may become elevated. Frequently, there is an eosinophilia and the sedimentation rate is elevated.

The skin test should be applied as soon as the infection is suspected. It may be negative or minimal in size in the very early stages. When repeated a few weeks later, a conversion to a positive test or an increase in size of the local reaction may permit a definite diagnosis.

Precipitin tests are of particular value in detecting primary infections because of the transient nature of the antibody response. Complement fixation tests are also of value. Low titers usually indicate a primary stage of infection, and high titers point toward its dissemination.

Fluorescent antibody techniques may prove of some value as diagnostic aids.

The best diagnosis usually involves the isolation and demonstration of both the saprophytic and parasitic forms of the organism.
Demonstration of the organism is sometimes possible in sputum, exudate of draining surface lesions, or in biopsied tissues. These may be examined unstained or stained for sporangia. The mycelial (saprophytic) phase of the organism can be demonstrated by placing some suspected material on any of a number of laboratory media. Sabouraud's dextrose agar phase can be demonstrated by intraperitoneal inoculation of mice and intratesticular inoculation of guinea pigs. Fluid from the testis is examined for sporangia in four to six days. The lungs, liver, spleen, and peritoneum of the mice are examined for sporangia when the mice die, or upon killing them, usually at 30 days post-inoculation. Careful identification includes demonstration of both the parasitic and saprophytic phases of each isolate. Laboratory procedures that result in the production of arthrospores must be carried out with extreme care because of the ease with which these spores can become airborne and be a source of infection.

The saprophytic form is apparently the natural form of the fungus. It grows as a branched, septate mycelium. Young hyphae are two to four micra thick and have septa at regular intervals. Some of the aerial hypha produce specialized side branches known as conidiophores, which are about twice the diameter of the hyphae from which they arise. Emmons first reported seeing these conidiophores on Sabouraud's agar cultures. These side branches may branch further. In these larger branches, the protoplasm condenses at intervals, and arthrospores (rectangular spores) are formed with alternate empty spaces between them. Transverse dimensions of spores are two to 10 μ, but occasionally they are as long as 20 μ.

Figure 8. Stained smear of mycelial phase of *Coccidioides immitis*, showing arthrospores within the mycelia. X 970
Arthrospores develop in aerial hyphae when they get older, particularly as the media dries. If air is prevented from reaching the fungus, both growth and sporulation are depressed. Dry air seems to stimulate sporulation. It only takes slight air movement to break off arthrospores which rupture and leave tags on the ends of the spores (Figure 8).

The parasitic form probably is not the natural form of the fungus but an adaptation which occurs when bits of mycelia or spores come in contact with suitable mammalian tissue. This form is often called a spherule and

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**Figure 9.** Sporangium of *Coccidioides immitis* in lymph node tissue section. Note the well-defined double wall. X 970

**Figure 10.** Sporangium of *Coccidioides immitis* in section of lymph node tissue. Note the endospores inside the sporangium. X 970
is believed to be a sporangium. The diameter usually ranges from 10 to 80 µ but sometimes is much greater. The doubly refractile, smooth wall is about 2 µ thick (Figure 9). Inside the spherule are spherical endospores usually about 2 to 5 µ in diameter and rarely as much as 40 µ. Young mature spherules are small with clear cytoplasm. Small nuclei form and the cytoplasm divides by cleavage to form endospores (Figure 10). The spores fill in from the wall toward the center. The wall of the mature spherule ruptures, and each endospore has the potential of developing into a spherule. Arthrospores or mycelial fragments convert to sporangia when they contact suitable mammalian tissue. In the case of the arthrospore, the conversion looks like a fattening up of the rectangular spore into the round spore which is indistinguishable from the sporangium.

SUMMARY

Although a careful consideration of signs, symptoms and epidemiological circumstances often results in an accurate clinical diagnosis, special tests and studies are usually required to confirm the diagnosis. Skin, precipitin and complement fixation tests are quite useful in this regard but the best documented diagnosis entails isolation of the organism and the demonstration of both its saprophytic and parasitic forms.

REFERENCES

At the border of the area generally considered to be the domain of medical mycology lies the order Actinomycetales. This order, which spans the interest of both bacteriologist and mycologist, is comprised of three families of medical interest, the Mycobacteriaceae, the Actinomycetaceae and the Streptomycetaceae. The mycologist's interest usually includes the families Actinomycetaceae and Streptomycetaceae; the former is comprised of two genera of pathogens, namely Actinomyces and Nocardia.

The recorded history of nocardiosis stems from Prof. Edmund Nocard's isolation in 1888 of an aerobic actinomycete from cattle afflicted with "Farcin de boeuf;" this organism was named Nocardia farcinica. In 1891, Eppinger described Nocardia asteroides isolated from a brain abscess of man. In subsequent years, two species, N. asteroides and N. brasiliensis, have been reported frequently in medical literature as the causes of local and systemic infections of man and animals. The original species, N. farcinica, is currently considered in synonymy with N. asteroides.

Affected Species

Nocardial infection has been found to be the basis of a wide variety of animal diseases. Cattle appear to be the most frequent animal host. Nocardial mastitis has been the predominant infection reported in cattle; this condition has been recognized in the United States, Canada, Australia, Great Britain, and Germany. All of these were infections by *N. asteroides* although one isolate was erroneously reported as *N. brasiliensis*. Bovine farcy, abortion, pulmonary and generalized infections also have been described in cattle.

Nocardiosis of the dog has been reported frequently and constitutes a major portion of the recognized infections in animals. A review of the disease in dogs indicates that cutaneous and subcutaneous, thoracic and abdominal infections are most frequent. Infrequently the infection in dogs has resulted in lesions of oral, nervous, and osseous mandibular tissues. The nomenclature of nocardias isolated from dogs has been greatly confused; one author lists 14 names for the actinomycetes of canine origin. Certainly the majority of those cases recognized in recent years were caused by *N. asteroides*.

Cats apparently do not contract nocardiosis frequently. While the disease has been observed in cats in the United States, it has been reported...
far less frequently in cats than in dogs despite the relatively equal veterinary attention given these two species. It is interesting to note that the only authenticated infection of an animal by *N. brasiliensis* occurred in a cat in California.\(^2\)

Nocardiosis in other animal species has been reported infrequently. Infection has been recognized in the goat,\(^1,15\) in the horse\(^1,3\) and in domestic and wild rodents.\(^6,24\) No reports have been made of nocardial infection in sheep or swine although the latter species has been susceptible to experimental infection.\(^32\)

**Pathogenesis**

Nocardial infection, as is the case with most of the systemic mycoses, is a disease contracted from the environment and is not considered to be directly transmissible from animal to animal or to man. Both *N. asteroides* and *N. brasiliensis* have been isolated from their customary saprophytic habitat, the soil.\(^19,28\)

Systemic nocardiosis of man is most frequently the result of pulmonary infection.\(^13,38\) A review of 40 cases of human nocardiosis disclosed that the lungs were involved alone or in combination with other organ systems in 33 instances.\(^13\) Among domestic animals pulmonary infection is recognized most frequently in the dog. Such infection apparently results from inhalation of Nocardia-laden dust. Pre-existing or intercurrent debilitating diseases may predispose to systemic nocardiosis but are not necessary to the establishment of disease. Canine distemper has been recognized concurrently with canine pulmonary nocardiosis.\(^36\)

Localized nocardial infections of man, actinomycotic mycetoma, are usually relegated to tumorous swellings and sinus tracts of the extremities.\(^28\) Similar distribution of localized nocardiosis is frequent in dogs and cattle, although tissue changes typical of actinomycotic mycetoma may be lacking in these instances. Infection apparently results from direct introduction of the organism through flaws in the skin and has been reported to follow cat bite wounds.\(^2\)

Nocardial mastitis of cattle appears to be a clinical intermediary between systemic nocardiosis and localized actinomycotic mycetoma. While usually localized to the mammary glands, this infection has been reported to result in severe systemic illness.\(^29,30\) Cattle infected with *N. asteroides* of high virulence have been observed with temperatures exceeding 107°F. Systemic metastasis of the infection and death may occasionally result. The usual course is one of moderate febrile reaction, swelling and fibrosis of the involved mammary glands and occasionally the development of draining sinus tracts. Predisposition to nocardial mastitis is unknown but the infection has been shown to result from unsanitary teat infusion techniques used during routine mastitis therapy programs.\(^31\)

**Pathology**

The lesions of systemic nocardiosis are most often chronic processes characterized by suppuration, abscess formation, fibrosis, and extensive macrophage infiltration of the surrounding tissues. Giant cells are numerous in some lesions but are not a necessary feature of the tissue
response. The degree of fibrosis, the extent of granulomatous response and tubercle formation depend largely upon the host species, the organ infected and the duration of infection. In the lungs of man, minimal fibrosis and granulomatous response are described. In pulmonary nocardiosis of dogs, abscessation, granulomatous lesions of the pleura and exudative pleuritis have been described.

In the mammary glands of cattle, infections of one month or greater duration have resulted in extensive fibrosis, granulomatous reaction and tubercle formation. Poliferative reaction of the ductile epithelium of infected mammary glands has been marked in some cases. Extensive necrosis of the mammary parenchyma is also seen with extension of the process into adjacent glands, to the exterior surface of the udder or resulting in vascular erosion with subsequent hematogenous metastasis. The reaction in the mammary gland is one that combines both erosive and proliferative properties.

Actinomycotic mycetomas of nocardial origin are typified by swelling, fibrosis, suppuration, and the formation of sinus tracts. Typically, these lesions contain well defined granules comprised of mycelial masses closely surrounded by accumulations of organic material—a combination of organism, metabolite and exudate. Such mycetoma formation in man is most often the result of infection by N. brasiliensis although infrequently the process results from N. asteroides infection. The greater proteolytic abilities of most strains of N. brasiliensis may facilitate the formation of sinus tracts and the separation of discrete granules from the infected tissue.

In animals, granulomatous swellings and sinus tracts resulting from N. asteroides infection are seen frequently on the extremities of dogs. Well defined grains are seen in the exudates from sinus tracts of infected mammary glands of cattle and in the pleural exudates of dogs. These grains, approximately two mm. in diameter, may be more aptly termed micro-colonies as their major component appears to be mycelial masses with varying amounts of loosely attached organic debris. However, in cases where the material surrounding a mycelial mass is more discrete and sharply defined, the differentiation between mycetoma granule and microcolony becomes rather diaphanous.

The branching filaments of Nocardia can be demonstrated in abscesses and granulomatous lesions by most bacterial stains. The author prefers Gram-Weigart stain for demonstrating the organism in tissue. The organism will appear as beaded, gram-positive branching filaments 1 μ or less diameter. In granulomatous lesions, the organism is seen to be closely applied to and infiltrating the inner epithelioid ring. In tissue it is often difficult to demonstrate the alleged acidfastness of either N. asteroides or N. brasiliensis when conventional acidfast stains are used; recently a technique has been described for this purpose.

Mycology

When studying the genera Mycobacterium, Nocardia and Steptomyces it is often difficult to locate reliable criteria of differentiation and impossible to locate unanimity of nomenclature. Perhaps this is best explained
by considering the nocardias as transitional forms occurring between the acid-fast, bacillary forms of Mycobacterium and the non-acid-fast, spore-bearing, branching filaments of Streptomyces.

Many criteria have been devised for the identification of Nocardia species and for their separation from the genera Streptomyces and Mycobacterium. Some of these have demonstrated minor differences between strains resulting in a divergent system of classification. This group is currently under investigation for possible reclassification, but the final taxonomic scheme is not yet available. For the purposes of identifying the usual pathogenic species, \textit{N. asteroides} and \textit{N. brasiliensis}, the following criteria have been found helpful.

1. Growth on Saboraud's dextrose agar occurs aerobically and is comparatively slow, producing colonies five to 10 mm. in diameter after two weeks' incubation. The colonies are usually heaped and folded, have a mycelloid edge and are adherent to the medium. An aerial mycelium may be present imparting a chalky white surface to the colony; where an aerial mycelium is lacking, the glabrous colony is tan to orange in color.

2. Morphology of the organisms is quite variable. Branching filaments 1 \(\mu\) or less in diameter are typical but are best demonstrated on slide culture. Fragmentation of these filaments results in numerous rod and coccoid forms.

3. Staining characteristics include strong Gram positivity and partial acid-fastness. The acid-fast characteristic must be demonstrated in the absence of alcohol in the destaining reagent. Kinyoun's acid-fast technique using aqueous \(\text{H}_2\text{SO}_4\) sulphuric acid for destaining is preferred.

4. Animal pathogenicity tests are helpful when a positive result is obtained. Intraperitoneal inoculation of guinea pigs with suspensions of \textit{N. asteroides} and \textit{N. brasiliensis} usually results in development of demonstrable lesions or death of the animal in from one to three weeks. The inclusion of five percent gastric mucin in the inoculum may be used to enhance the pathogenicity of the isolate. While the identification of \textit{N. asteroides} and \textit{N. brasiliensis} rests primarily on morphological and biochemical criteria the author prefers to include the demonstration of animal pathogenicity whenever possible.

5. Biochemical reactions of wide variety have been used to differentiate nocardial species. Among the most useful for differentiating \textit{N. asteroides} from \textit{N. brasiliensis} are the demonstration of gelatin liquefication and casein hydrolysis by \textit{N. brasiliensis}; the less proteolytic \textit{N. asteroides} gives negative results in both tests.

\textit{Immunology}

The immunologic reactions of nocardiosis of animals have been studied in cattle. These reactions were found to be of diagnostic value in detecting infected cattle in large dairy herds. A culture filtrate antigen prepared from \textit{N. asteroides} of bovine mammary origin, was used to demonstrate
cutaneous hypersensitivity and complement-fixing antibodies in naturally and experimentally infected cattle.

Skin tests, performed in the cervical region, revealed the presence of delayed hypersensitivity by the second to fourth week after experimental infection. Reactions were read 48 hours after injection of the antigen. A positive reaction was one in which there was a swelling 1/2 inch or greater in diameter and two or more times the normal skin thickness. Cutaneous hypersensitivity persisted after spontaneous clearance of experimental infection. Cross reaction of the nocardial antigen in tuberculin-sensitive animals or reaction of animals infected with *N. asteroides* to injections of tuberculin were not apparent.

Complement-fixing antibodies were demonstrated in the sera of cattle two weeks after experimental infection. Titers in cattle infected for various periods of time ranged from 1:64 to 1:1024. Following spontaneous clearance of experimental infection the complement fixation titer fell to nonsignificant levels. Complement-fixing antibodies were not found in significant titers in animals reacting to the tuberculin skin test.

Agglutinin titers of low magnitude were demonstrated in the sera of both normal and infected adult cattle but not in the sera of calves under four months of age.

When large dairy herds are examined for nocardial mastitis, the entire herd should be skin tested; reactors to this test should then be examined serologically and culturally to determine their infection status.

Summary

Nocardiosis of animals is a chronic infection resulting from soil-borne organisms of the genus Nocardia. *N. asteroides* has been isolated frequently from animal infections while *N. brasiliensis* infection has been recognized rarely. The disease is contracted from the environment and is not considered to be transmissible from animal to animal or to man.

The infecting organisms are aerobic actinomycetes with the properties of a fine, branching, fragmenting mycelium which is partially acid-fast.

Lesions combine both proliferative and erosive qualities. Suppuration, abscessation, fibrosis and macrophage infiltration are relatively constant histological findings while the presence of giant cells, granulomata and tubercle formation are variable. Localized lesions of the extremities and subcutaneous tissues cause swelling, fibrosis and sinus tracts resembling actinomycotic mycetoma. The presence of microcolonies and mycetoma granules in these exudates is discussed.

The immunologic reactions of cattle infected with *N. asteroides* have been used diagnostically. Both cutaneous hypersensitivity and complement-fixing antibodies have been demonstrated in infected cattle.

REFERENCES


Cutaneous Streptothricosis

Cutaneous streptothricosis is an infectious disease of cattle, horses, and goats characterized by an exudative dermatitis. Typical lesions present tufts of matted hair entrapped within crusts of exudate on the superficial epithelial surface. The disease is caused by an aerobic actinomycete, *Dermatophilus congolensis*. Two diseases of sheep, mycotic dermatitis and strawberry foot rot, are caused by closely related organisms.¹

Cutaneous streptothricosis has been widely recognized in cattle in Africa and has been reported from Australia and England. Until 1961, cutaneous streptothricosis had not been recognized in the United States. During 1961, three separate reports of the disease in three different animal species were made in this country. Reports of naturally occurring infection in two calves in Texas,³ a deer⁴ and six horses⁷ in New York served to indicate the presence of the disease in this country. Recently the infection was recognized in a herd of 17 Iowa cattle.⁵

The causative organism has been classified as a Nocardia by some authors⁸ but more acceptable placement appears to be the creation of a new family Dermatophilaceae for the genus *Dermatophilus*.²

The organism grows aerobically on mediums containing blood or serum and forms small, 1 to 2 mm., tan to brown, adherent colonies after 72 hours incubation. A multiplicity of forms are produced. A slender, one µ or less, branching mycelium fragments transversely at intervals until bars one µ wide are formed; subsequent longitudinal divisions create packets of spores along the original axis of the mycelium. Motile spores are produced which eventually germinate to form a new mycelium. Capsules are
produced. The organism is strongly proteolytic but relatively inactive on sugar mediums.

Definitive diagnostic forms are seen in Geimsa-stained smears prepared from the crusts of lesions and appear as spore packets arranged in typically vermiform configurations. Histologic sections show numerous forms of the organism at the surface of the exudative crusts and in the lumen of hair follicles.

The disease can be experimentally transmitted to domestic livestock and laboratory animals and transmission to man has been reported.4

REFERENCES


THE FLUORESCENT ANTIBODY TECHNIQUE
AND FUNGAL DISEASES
William Kaplan, D.V.M. and Leo Kaufman, Ph.D.*

A valuable serological tool for the rapid detection and identification of microorganisms as well as for the demonstration of serum antibodies became available with the introduction of the Coons fluorescent antibody (FA) technique.\(^4\) The FA technique is essentially an immunochemical staining procedure which permits the microscopic observation of an antigen-antibody reaction. Visualization of the reaction is accomplished through the use of antibody conjugated to a fluorescent dye. The fluorochrome-antibody complex is termed labeled antibody or conjugate. The union of the labeled antibody and its homologous antigen results in a product that fluoresces when viewed under the fluorescence microscope. The essential components of a fluorescence microscope are a very bright light source which yields a powerful flow of energy especially in the short wave length region of the light spectrum, selective filters for the control of the fluorescence excitation and emission radiation, and a standard microscope fitted with a cardioid darkfield condenser.

The most commonly used fluorochromes are derivatives of fluorescein, a dye that fluoresces with a yellow-green color. Fluorescein isothiocyanate\(^{27}\) is at present the derivative most widely used as a labeling agent. Fluorochromes of other colors, such as rhodamine and its derivatives which fluoresce with a reddish color, have also been employed as labeling compounds.

The simplest FA procedure, termed the direct method, involves the application of solutions of labeled antibody directly on smears of cultures or clinical materials. One then examines the treated preparations for stained organisms. A commonly employed modification of this basic procedure is the indirect method. In this system an antigen-antibody reaction is made visible by the addition of a conjugate directed against globulins of the animal species producing the initial antibody. The indirect method can be employed as a tool for the rapid detection of antibodies or for the identification of antigens. For example, \textit{Histoplasma capsulatum} antibodies in human serum may be detected by first reacting the serum with smears of \textit{H. capsulatum}. Then the preparation is treated with conjugated anti-human globulins. If the serum contains antibodies for \textit{H. capsulatum}, the cells of this fungus will be stained. If \textit{H. capsulatum} cells in smears of cultures or clinical materials need to be detected, the smears are first reacted with a known human or rabbit anti-\textit{H. capsulatum} serum. Then the preparation is treated with labeled anti-human or anti-rabbit globulins. If \textit{H. capsulatum} is present, the cells will fluoresce with the color of the fluorochrome. Another variation that is especially valuable for the detection of

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antibodies is the FA inhibition method. This procedure involves the blocking of the reaction between antigen and conjugate by saturating antigen sites with homologous unlabeled antibody. For example, to detect *H. capsulatum* antibodies in a serum sample, one first treats a smear of *H. capsulatum* cells with the serum. Then the preparation is treated with labeled *H. capsulatum* antibodies. If staining is inhibited, this implies that the test serum contained *H. capsulatum* antibodies. The inhibition test can also be carried out as a one-step test. This requires a single treatment of smears of *H. capsulatum* cells with a mixture of equal parts of conjugate and unlabeled test serum. Because of the speed with which a one-step inhibition test can be performed, it is preferred by the CDC Mycology Unit.

Because of the extensive occurrence of common antigens among the fungi, difficulties due to common antibodies may be encountered when one attempts to use the indirect FA test for the demonstration of antibodies. These difficulties may be avoided through the use of the FA inhibition technique with species specific conjugates.

An extensive description and discussion of FA methods may be found in the manual prepared by Cherry and his associates, in the review of Beutner and in the textbook by Nairn and his associates.

The FA technique has a number of important advantages over conventional diagnostic methods. First, fluorescent antigen-bearing organisms with distinct morphological characteristics can be observed. Second, fluorescent cells, even when few in number or contaminated with other organisms, can be readily detected. Also, antigen-bearing organisms need not be viable for detection and identification. Another advantage of the FA procedure is its rapidity. The time required to carry out conventional isolation, purification, and identification procedures is made unnecessary.

A review of the literature shows that in the past studies on the application of the FA technique to the area of medical mycology have lagged behind those carried out in other fields of microbiology. This apparent neglect was the result in part of the observation by earlier investigators that hyphae and spores of some fungi were autofluorescent. Due to improper filters such elements of some fungus species autofluoresced with a greenish color simulating that of fluorescein. Thus it was difficult to distinguish autofluorescent from fluorescent antibody stained elements. It was subsequently found that this problem could be overcome by using proper filters. The authors have found that a combination of a 5113 Corning glass barrier filter, three mm in thickness (passes blue violet light) and a Wratten 2A ocular filter (UV barrier) is satisfactory for use with fungi. Additional studies may disclose that other filter combinations are preferable.

In recent years there has been a marked increase in interest in ascertaining the applicability of FA techniques to medical mycology. A number of investigators both in this country and elsewhere have carried out studies to evaluate this procedure as a tool for the rapid detection and identification of fungi and for the demonstration of fungal antibodies in sera. While to date such studies have been essentially preliminary in nature, the findings have been uniformly favorable.
The present paper reviews the significant FA contributions that have been made to date in medical mycology.

DETECTION AND IDENTIFICATION OF FUNGI

Cryptococcus neoformans

In 1957 Eveland and his associates employed the FA technique in a study of the distribution of Cryptococcus neoformans and its polysaccharide breakdown products in formalin-fixed tissues. The procedure permitted these workers to detect this yeast and its polysaccharide antigens in the tissues.

Recently, Kase and Marshall evaluated the FA technique for the identification of C. neoformans cultures. Of 92 isolates of this fungus tested, 91 reacted with labeled rabbit anti-globulins prepared against strains of C. neoformans identified as Evans A, Neill 1, and Neill 5. The one isolate that did not react belonged to Evans' C. neoformans serotype C. Kase and Marshall were able, however, to stain this one strain with conjugates produced from antiglobulins against either Evans' C. neoformans serotype B or C. All of the conjugates used in these studies showed capsular staining. These workers further reported that their conjugates stained four isolates of C. neoformans var. innocuous, which is considered by many workers to be a distinct species, C. diffluens. However, no other cross-reactions were noted when their conjugates were applied to smears of 23 species of heterologous yeasts belonging to genera other than Cryptococcus.

Candida albicans and other Candida species

Several studies have been carried out on the application of the FA technique to the detection and identification of members of the genus Candida. In 1958 Gordon described in detail the production and use of two fluorescein isocyanate labeled rabbit anti-C. albicans globulins. Both conjugates stained cells of C. albicans in clinical materials and cultures. However, one of Gordon's conjugates, in addition, reacted with the cells of C. tropicalis, C. stellatoidea, C. parakrusei, C. krusei, C. pseudotropicalis, and C. guilliermondii, and also with elements of five species of heterologous yeasts representing genera other than Candida. Adsorption of this reagent with cells of C. parakrusei removed all of the aforementioned cross-reactions except that with C. tropicalis and some strains of C. stellatoidea. The second conjugate stained both C. albicans and C. tropicalis, and showed little or no reaction with other Candida species.

In a second paper, Gordon reported that it was possible to differentiate C. albicans from C. stellatoidea by means of the FA technique. Differentiation was accomplished by using labeled anti-C. albicans globulins that had been adsorbed with cells of C. parakrusei. Adsorption of the reagent with elements of C. stellatoidea apparently eliminated too much of the reactivity for C. albicans. This C. parakrusei-adsorbed reagent stained cells of 60 isolates of C. albicans at an intensity rated as three to 4+ and two isolates at a 2+ intensity. However, this adsorbed conjugate also reacted with elements of three isolates of C. stellatoidea at a 1+ intensity, three at
2+ intensity and one at a 3+ intensity. A second conjugate was reported not to require any antibody adsorption to remove cross-reacting factors. This conjugate stained cells of 13 isolates of *C. albicans* at a 4+ intensity. In contrast, six isolates of *C. stellatoidea* reacted at a 0 to 1+ level and one isolate with a 2+ intensity. Similar staining reactions were obtained with a conjugate from *C. tropicalis* rabbit antoglobulins.

Kaplan and Kaufman\(^{14}\) also applied the FA technique to the rapid detection and identification of *C. albicans* and other *Candida* species. Although their studies are preliminary, several interesting findings can be recorded. Adsorption of fluorescein isothiocyanate labeled rabbit anti-*C. albicans* globulins with cells of *C. stellatoidea* revealed the existence of two distinct serological groups of *C. albicans*. One group is serologically similar, if not identical to, *C. stellatoidea*; the second group is serologically similar to *C. tropicalis*. These findings obtained through the use of the FA technique are in accord with the observations of Hasenclever and Mitchell\(^{11}\) who reported the existence of two such serological groups of *C. albicans* on the basis of agglutination tests. Kaplan and Kaufman\(^{14}\) have also found that it is possible to differentiate *C. tropicalis* from *C. albicans* and *C. stellatoidea* through the use of fluorescein isothiocyanate labeled *C. albicans* antibodies adsorbed with cells of *C. tropicalis*. However, this adsorbed conjugate still exhibited low level cross-staining for other species of *Candida*.

In a recent report\(^{10}\) Gordon stated that he found that a conjugate prepared from rabbit anti-*Torulopsis glabrata* globulins adsorbed with *C. albicans* cells reacted with *C. tropicalis*, but not with elements of *C. stellatoidea* or *C. albicans*. In addition, this worker reported that a recently produced lot of labeled rabbit anti-*C. albicans* globulins diluted 1:10 intensely stained cells of *C. albicans* and *C. tropicalis*. In contrast the reagent showed variable reactivity for pure cultures of *C. stellatoidea* (90 percent of the cells did not react; 10 percent of the cells showed two to 4+ staining). Other members of the genus *Candida* did not react with this reagent. On the basis of these findings Gordon suggested the possibility of using the two reagents i.e. the adsorbed anti-*T. glabrata* conjugate and the diluted anti-*C. albicans* reagent concurrently, to identify three species of *Candida* (*C. albicans*, *C. tropicalis* and *C. stellatoidea*) individually.

Several workers have investigated the possibility of using FA reagents for the rapid detection of *C. albicans* in clinical materials. Kunz\(^{21}\) investigated the possible relationship of *C. albicans* to pneumocysts of interstitial plasma cell pneumonia. Through the use of fluorescein isocyanate labeled rabbit anti-*C. albicans* globulins, this worker demonstrated the presence of *C. albicans* in the lungs of two of three premature infants who had died of interstitial plasma cell pneumonia. In no case did he observe a reaction between conjugate and pneumocyst. In another study, Kunz\(^{12}\) demonstrated the capacity of his conjugate to detect *C. albicans* in tissues of experimentally infected mice.

Kemp and Solotorovsky\(^{19}\) employing lissamine rhodamine B 200 and fluorescein isothiocyanate labeled rabbit anti-*C. albicans* globulins demonstrated the presence of the organism in frozen kidney tissue sections of experimentally infected mice. In addition, these workers applied the
fluorescent antibody to study the pathogenesis of experimental *C. albicans* infections in mice. 

*Sporotrichum schenckii*

Kunz labeled rabbit anti-*Sporotrichum schenckii* globulins with fluorescein isocyanate and stained the cells of this fungus in culture, in frozen sections, and in smears from experimentally infected animals. None of 18 heterologous fungus species tested by this worker reacted with the reagent.

Kaplan and Ivens also carried out studies on the application of the FA technique to the identification of *S. schenckii* cultures and its detection in clinical materials. Diluted fluorescein isothiocyanate labeled rabbit anti-*S. schenckii* globulins were shown to stain both the yeast and mycelial phase of the fungus. Furthermore, the diluted conjugates did not stain any of 47 strains of 21 heterologous species representing 12 different genera.

These workers also demonstrated the presence of *S. schenckii* cells in smears prepared from infected mouse testes, and in sections of formalin-fixed tissue from an experimentally infected mouse and rat.

FA reagents for *S. schenckii* also permitted the rapid detection of *S. schenckii* cells in smears prepared directly from lesion exudates of 24 out of 27 culturally confirmed human cases of sporotrichosis.

*Histoplasma capsulatum*

Gordon stained yeast phase cells of *Histoplasma capsulatum* with fluorescein isocyanate labeled anti-*H. capsulatum* globulins. The labeled antibodies also stained the small buds of *H. duboisii* and the buds of an aberrant strain of *H. capsulatum*. In addition, some strains of *Blastomyces dermatitidis* treated with his labeled antibodies showed variable fluorescence. Gordon attempted to eliminate the cross-reacting factors by adsorption of the conjugate with cells of *B. dermatitidis*. This resulted in the conjugate's loss of staining reactivity for both the homologous and heterologous organisms. However, Gordon did report that dilution of the non-adsorbed conjugate (1:4) resulted in a reagent that showed three to 4+ staining of *H. capsulatum* yeast cells, the buds of *H. duboisii* and *Paracoccidioides brasiliensis*, and the cell walls of some strains of *B. dermatitidis*.

Several workers have investigated the possibility of employing the direct FA technique for the rapid detection of *H. capsulatum* in human sputum. In their studies Lynch and Plexico used labeled rabbit anti-*H. capsulatum* globulins that had been adsorbed with *Candida* yeast cells to eliminate cross-reactivity for *Candida* species. On the basis of their work with 84 sputum specimens from 28 patients with proved or suspected chronic (cavitary) pulmonary histoplasmosis, Lynch and Plexico suggested that the FA technique can be used as a rapid screening procedure for the presence of *H. capsulatum*. Carski, Cozad and Larsh also used labeled rabbit anti-*H. capsulatum* antiglobulins in their studies. One of three lots of conjugate showed a high degree of cross-reactivity for *C. albicans*. This was eliminated by a single adsorption with cells of the latter fungus. All lots of reagents used cross-stained *B. dermatitidis*. This cross
reactivity for some strains of *B. dermatitidis* was removed by adsorption with the latter fungus. On the basis of their findings these workers concluded that the direct FA procedure can be used to advantage as an adjunct to a conventional cultural and clinical diagnostic procedures for the diagnosis of pulmonary histoplasmosis. Because of persistent cross reactions with some strains of *B. dermatitidis* and several possible false positive results, Carski et al. urged caution in attempting to use FA technique as the sole criterion for the diagnosis of this disease.

Recently Kaufman and Kaplan\textsuperscript{17} carried out studies designed to develop a conjugate specific for the yeast phase of *H. capsulatum*. In their work they labeled anti-*H. capsulatum* globulins with fluorescein isothiocyanate. Their reagent, in addition to staining yeast phase cells of *H. capsulatum*, exhibited strong cross-staining of *B. dermatitidis* and numerous other heterologous fungus species. Adsorption of the labeled antibodies twice with cells of *B. dermatitidis* or twice with cells of *Coccidioides immitis*, and once with cells of *B. dermatitidis*, resulted in an FA reagent specific for yeast phase cells of *H. capsulatum*. The specific reagent, however, did not stain elements of the mycelial phase of this fungus.

In limited studies, these workers showed that the specific conjugates could be used to readily detect *H. capsulatum* cells in tissue smears made from exudates and organs of experimentally infected mice.

Recently Procknow, Connelly and Ray\textsuperscript{26} successfully applied the FA technique to a study of the pathogenesis of experimental histoplasmosis in the mouse. They found this technique most satisfactory for this purpose.

* Blastomyces dermatitidis

Rabbit anti-*Blastomyces dermatitidis* globulins were labeled with fluorescein isocyanate by Gordon.\textsuperscript{7} This conjugate stained yeast cells of *B. dermatitidis* and *C. albicans* but showed little or no cross-reaction with *P. brasiliensis* or *H. capsulatum*.

Kaplan and Kaufman\textsuperscript{15} have also labeled rabbit anti-*B. dermatitidis* globulins with fluorescein isothiocyanate. All lots of such conjugates produced to date have stained brightly yeast and mycelial phase elements of *B. dermatitidis*. The conjugates, however, have also reacted strongly with elements of yeast and mycelial phases of *H. capsulatum* and cells of numerous other heterologous fungi. Adsorption of one lot of conjugate twice with yeast phase cells of *H. capsulatum* and once with cells of *Geostrichum candidum* rendered the conjugate specific for the yeast phase of *B. dermatitidis*. A second lot of reagent required three adsorptions with *H. capsulatum* yeast phase cells and one adsorption with *G. candidum* elements to be made specific for *B. dermatitidis* yeast phase cells. It is of considerable interest to note that the reagents that had been rendered specific for the yeast phase of *B. dermatitidis* did not react with the mycelial form of this fungus. These workers did find that the yeast-phase specific conjugate did reveal the presence of *B. dermatitidis* in smears of lesion exudates from several animal and human cases of North American blastomycosis.
Detection of Fungal Antibodies

Vogel and Padula\textsuperscript{28} carried out a preliminary study to determine the adaptability of the indirect FA method for the detection of fungal antibodies in human sera. For the second stage reaction they used fluorescein isocyanate labeled rabbit anti-human globulins. Serum samples from one patient with cryptococcal meningitis were tested. Antibody activity was indicated by the following results: One undiluted early sample resulted in the \textit{C. neoformans}-antiserum complex being stained brightly. A diminished reaction occurred when this serum sample was applied at a 1:2 dilution. No reaction was detected when the serum was diluted 1:4.

Vogel and Padula also tested three other sera from patients with clinical manifestations of mycotic disease. One of these sera had a complement-fixation (CF) titer of 1:256 for blastomycosis, a second gave a CF titer of 1:128 for histoplasmosis, and a third agglutinated \textit{C. albicans} antigen at a dilution of 1:256. These serum samples were reported to give identical titers with the indirect FA method.

Kaufman, Schubert and Kaplan\textsuperscript{18} recently used the FA inhibition test to detect antibodies against histoplasmin and \textit{H. capsulatum} yeast cells in sera from suspected and proven human cases of histoplasmosis. The antibody content of these sera was characterized by the complement-fixation test. The FA inhibition test proved to be simple and effective for the rapid detection of antibody against whole yeast cells. However, the test was ineffectual for the detection of antibody to histoplasmin.

DISCUSSION

The results of studies carried out to date clearly demonstrate the value of the FA technique for the rapid detection and identification of fungi as well as for demonstration of fungal antibodies in sera. The potential value of this technique in medical mycology transcends the obvious diagnostic applications. Immunofluorescence can be profitably used as a research tool in a diversity of investigative studies. This technique can serve admirably for investigating antigenic relationships among fungi. Immunofluorescence can also be used to great advantage in studies concerned with the pathogenesis of mycotic diseases. For use in such specialized research situations crude, non-specific reagents may be satisfactory. However, for performance of routine diagnostic work, species-specific reagents are required, if definitive results are desired. Due to the spread of common antigens among fungi the production of species-specific reagents is frequently not a simple matter. Extensive cross reactions, and also non-specific reactions, have to be eliminated by adsorption procedures, dilution techniques, or a combination of the two. Such procedures may be complex. Poor antigenic qualities on the part of some fungi also make the production of high titered specific FA reagents difficult. Therefore, additional basic investigations in the preparation of better fungal antigens for immunization purposes and schedules for their administration are required.
At present, FA procedures are being applied on a limited scale in specialized mycology laboratories. Until FA techniques have been fully evaluated and a pool of trained personnel and standardized reagents become available to the diagnostic laboratory, immunofluorescence will of necessity continue to be employed on a limited basis.

SUMMARY

This paper reviews the significant fluorescent antibody contributions that have been made to date in medical mycology. The review covers the progress made in the development and application of fluorescent antibody reagents for the detection and identification of the causal agents of cryptococcosis, candidiasis, histoplasmosis, sporotrichosis, and North American blastomycosis, both in clinical materials and in culture. The progress made in the application of immunofluorescence to demonstration of fungal antibodies in sera is also covered.

The paper also discusses the basic principles of the fluorescent antibody technique, problems encountered in carrying out the tests and potentialities of immunofluorescence in medical mycology both for diagnostic purposes and research.

REFERENCES

MYCOTOXICOSES IN ANIMAL AND HUMAN HEALTH
Joseph Forgacs, B.S., M.S., Ph.D.*

HISTORICAL

Mycotoxicoses are poisonings of a host manifested in various ways following entrance into the body of fungal metabolites. This group of diseases affects both animals and human beings. Toxicoses caused by ingestion of scab-infected (*Fusarium graminearum*) grains, ergotized (*Claviceps purpurea, C. paspali*) grain or grass, or poisonous basidiomycetes, such as mushrooms (*Amanita verna, A. muscaria*) and ustilagenous fungi which long have been known to exist fall within the definition of a mycotoxicosis. This presentation, however, is concerned primarily with those toxicoses associated with ingestion of foods, feedstuffs, fodders, forage and other substrata on which fungi heretofore considered only as harmless and often times beneficial saprophytes have grown as contaminants, but which have metabolized a toxin (s).

A review of published scientific findings reveals that Soviet scientists long have been active in mycotoxicoses research and since 1940 have made some valuable contributions.¹⁻⁸ On the other hand, publications in North America up to about 1953 indicate that although toxicoses of unknown etiology had occurred in livestock, that fungi could have played a role in at least some of these cases is at variance among scientists. Whereas, some reports indicate that fungi were indeed responsible for the outbreaks studied,⁹⁻¹¹ there exist others which would indicate that fungi are harmless to animal health¹²,¹³ or of minor economic significance, an opinion present even today.¹⁴ Still others maintain that moldy substrata actually may be beneficial to animal health.¹⁵,¹⁶ Some workers are of the opinion that moldy feed may be harmful with certain reservations.¹⁷ These discrepancies in toxicity of fungi, probably due to improper methods used by various workers for isolating and testing the fungal isolates for toxicity, left the field of mycotoxicoses unexplored. In addition, since the mycotoxicoses are frequently accompanied by many varied symptom-complexes, establishing a diagnosis of a mycotoxicosis frequently requires that other etiologic factors be first excluded.⁸ Nevertheless, a careful examination of many of the toxicoses of unknown etiology which occurred in the past, in retrospect, indicated that many had characteristics of the mycotoxicoses as they are known today.

According to Sippel,¹⁸ the published findings of Carl¹ et al¹⁹ and Forgacs and co-workers²⁰ in 1954 created an impetus to re-evaluate the role of mycotoxicoses in poisonings of unknown etiology. These workers first investigated the role of toxigenic fungi in selected cases of bovine hyperkeratosis, or X-disease.

In 1953, Sippel and co-workers²¹ described a unique disease primarily in swine, although cattle also were affected, that foraged moldy corn in the

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field. In 1957, Burnside et al. reproduced this toxicosis in swine using a toxic strain each of *Aspergillus flavus*, Link and *Penicillium rubrum*, Stoll. Although in the field outbreaks, moldy corn was the primary source of the toxin(s), these workers stated that cases had been reported in which moldy stale bread, oats, and peanuts (groundnuts) were responsible for this toxicosis.

In 1955, Forgacs and Carl isolated several toxic fungi, including a toxic strain of *A. flavus*, Link from feed and litter collected from areas where poultry hemorrhagic syndrome was enzootic. Using these fungi, these workers reproduced under laboratory conditions many of the clinical and pathologic manifestations of field cases of poultry hemorrhagic syndrome. These results were later reproduced in older birds and subsequently, the syndrome was reproduced in broilers under field conditions.

During the past five years, facial eczema in sheep and cattle in New Zealand was found to be associated with ingestion of pasture containing dried grass infected with the saprophytic fungus, *Sporodesmium bakken*. A strikingly similar disease in cattle (cattle sunburn, photosensitization) has been reported in the United States. In the latter, a fungus, *Periconia minutissima* was the predominating fungus on dried toxic Bermudagrass and clover. Efforts to establish this fungus as the cause of the toxicosis by the use of pure culture studies to date have been unsuccessful.

During 1960, a unique disease with high mortality occurred in turkey poults in England. Losses occurred in poults primarily from two to four weeks old although in some cases death occurred within two to four days of hatching and even affected birds eight weeks of age. This disease, because of its early unknown etiology was named turkey X-disease. According to Alcroft et al., Blount concluded that the toxicosis appeared to be associated with certain lots of Brazilian groundnut meal (peanut meal) which was used as a protein supplement in the turkey rations. In September 1960, Dr. P. K. C. Austwick of the Central Veterinary Institute examined a sample of toxic Brazilian groundnut meal and found, upon histologic examination, that approximately 20 percent of the cotyledonous tissues were invaded by fungal hyphae. Since the fungus had invaded the tissue prior to expelling of the oil, the heat generated during expelling apparently destroyed the fungus but not the toxin. Doctor Austwick therefore suggested that the toxin present in the groundnuts might be of mycologic origin—a mycotoxin. Subsequently, Sargeant et al. reported that of eight fungal isolates from a toxic lot of groundnut meal, when cultured on Czapek's solution agar and extracted with chloroform, one fungus *A. flavus*, Link showed a fluorescent material Rf 0.7 in n-butanol-5 percent acetic acid. Day-old ducklings that consumed a similar extract prepared from the lot of toxic groundnuts died and showed the characteristic gross and histopathologic lesions of toxic groundnut poisoning. The *A. flavus*, Link, when cultured on heat-sterilized, nontoxic groundnuts, produced a similarly-toxic substance likewise toxic to day-old ducklings.

Although most mycotoxicoses so far described in the literature affect animals, there are some described that also affect man. Stachybotryotoxicosis, a mycotoxicosis chiefly affecting animals can also affect man. A toxicosis, entitled, "Otravlenie pianym khlebom" ("Intoxication due to
drunken bread") affects man and is caused by ingestion of food prepared from grain infected with a species of *Fusarium graminearum*. This fungus apparently is similar to that responsible for disturbances in animals that consume scabby grain. Of all of the mycotoxicoses, both animal and human, none, however, has received as much study and can compare in severity to alimentary toxic aleukia (commonly referred to by scientists as ATA) in man.3,4,5,7

In the sections to follow are described briefly some of the more commonly known mycotoxicoses. Others occur that are not described here and, undoubtedly, there exist others yet to be investigated. For detailed descriptions of the more common mycotoxicoses, the interested reader is referred to other sources.3,4,6,7

Some of the difficulties in studying the mycotoxicoses include a suitable method for isolating mycotoxic fungi and proper methods for culturing the isolates. In order to stimulate much needed research in this somewhat neglected but fruitful field, a section is summarized on some useful methods not only for isolating the fungi, but for testing the fungal isolates for toxicity as well. Details of these methods are available elsewhere.7

Since some of the mycotoxic fungi may be pathogenic, a few words of caution also are given to the investigator who selects to study this intriguing group of diseases.

**ANIMAL MYCOTOXICOSES**

*Strachybotryotoxicosis*. This toxicosis was first reported in the Ukrainian SSR and was one of the earliest and most intensively studied animal mycotoxicoses. The fungus *Stachybotrys atra* associated with this toxicosis is a saprophyte which grows on damp straw and other fodder and forms a lethal toxin. As with most other mycotoxicoses, this disease is seasonal, the first cases occurring in the Fall when the animals are stabled and stall feeding of fodder commences and ceases with the advent of pasturing in the Spring. In the past, the horse was considered the only animal susceptible to the toxin. In 1958, Forgacs and co-workers found that sheep, calves, and swine are also affected by the toxin; however, the calf appears more resistant to the toxin than the horse. In 1959, Fortuskny et al. reported a field outbreak of stachybotryotoxicosis in cattle. More recently, other field outbreaks in cattle have been reported by Soviet scientists. People exposed to the aerosols from toxic substrata also are affected by this toxicosis.

*Stachybotrys atra* is distributed world-wide, is considered by most scientists to be saprophytic, proliferates at temperatures ranging from two to 40°C, and at a relative humidity as low as 30 percent, and is found in the soil and on substrata rich in cellulose such as hay, straw, plant debris, clothing, and paper. Both toxic and nontoxic strains occur. Ethyl ether extracts prepared from substrata on which toxic strains had proliferated for 10 days or longer, when applied to the skin of a denuded sensitive rabbit, produce a pronounced hyperemia, subsequent hyperemia and necrosis. The toxin appears to be highly potent and is soluble in many organic solvents, is stable to heat (120°C.) and acids and has been reported to...
have been crystallized and its chemical structure determined.\textsuperscript{50,51} The toxin apparently is not secreted in the milk, since foals suckling mares affected with the toxicosis remain normal.\textsuperscript{49} Age, type, sex, physical condition and level of feeding of the host do not influence susceptibility to stachybotryotoxicosis. No immunity exists to this toxicosis. Animals that have recovered from this disease have developed the syndrome within the same year upon resuming consumption of toxic fodder.\textsuperscript{49,52}

Stachybotryotoxicosis occurs in two forms, the typical which is divided into three stages and the atypical or shocking form which has one stage.

The typical form is most commonly observed in the field and develops during prolonged ingestion of small amounts of toxic feed. During the first stage the animal salivates excessively, the submaxillary lymph nodes become greatly enlarged, the mucous membranes of the eyes and oral cavity become injected, and cracks develop at the mucocutaneous junction of the lips followed by formation of deep fissures. As the inflammatory process progresses, edema of the underlying tissue causes the lips to swell so that the animal assumes a hippopotamus-like appearance. This stage appears within two to three days following ingestion of toxic feed and may persist for eight to 30 days.

The second stage in most cases is quiescent clinically; however, hematologic examination of the peripheral blood indicates a leucocytosis followed by a progressive thrombocytopenia, leucopenia, agranulocytosis, and prolonged prothrombin and clot-retraction time. The second stage may last from five to 50 days and is followed by a rapid onset of symptoms of the third stage.

During the third stage, body temperature becomes elevated (106.7°F.), diarrhea and dehydration occur, the pulse weakens, and arrhythmia occurs. Thrombocytes and leucocytes continue to decrease to a very low level. Total leucocyte count may fall to 100 or even less per cubic millimeter of whole blood. Thus with the depletion of the protective mechanisms of the host, opportunist infections frequently occur. Local lesions, when present, consist of necrotic areas on the mucous membranes of the cheeks, gums, frenum of the tongue, on the hard and soft palate, and on the lips. Duration of the third stage is from one to six days and usually terminates fatally.

The atypical or shocking form follows ingestion of large quantities of toxic roughage and is characterized primarily by neurologic disorders. The affected host may exhibit hyperirritability or may show pronounced depression, in the latter case the animal often leaning against an object for support. Symptoms which almost always terminate fatally are observed within 72 hours, but may occur as early as six hours followed by death within 17 hours.

The pathologic picture of equine stachybotryotoxicosis is characterized by profuse hemorrhage and necrosis in many tissues and is essentially the same for both the typical and atypical forms. Variations observed are limited only in intensity. The subcutis and skeletal musculature, the serous and mucous membranes, and certain parenchymatous organs are primarily affected.
Necrotic processes on the mucous membrane appear along practically the entire length of the digestive tract and are quite characteristic of this toxicosis. The most typical are observed in the large intestine where, macroscopically, two types of lesions are observed. One type consists of small, yellowish-grey papules on the surface of the mucosa and the other of large, deep-seated lesions extending into the submucosa and muscularis.

The lymph nodes are enlarged and hemorrhagic, those of the pharynx showing the greatest change.

Changes in the liver, kidneys and heart muscle are in the nature of circumscribed degenerative processes of the albuminous or adipose-dystrophic type.

The lungs are intensively congested, edematous, and frequently contain hemorrhages.

The pathologic alterations of stachybotryotoxicosis are unique in that they have no zone of demarcation around the necrotic foci. The tissue appears to be in a nonreactive state.

Aspergillustoxicosis. This is a mycotoxicosis which has been reproduced experimentally in calves using a strain each of *Aspergillus chevalieri*,¹⁹ *A. clavatus*²⁰ and *A. fumigatus*⁶¹ and which resemble some field cases of bovine hyperkeratosis.⁶² Both the *A. chevalieri* and the *A. clavatus* had been isolated from feedstuffs responsible for field cases of the hyperkeratosis, but which apparently did not contain highly chlorinated naphthalene compounds (which produce bovine hyperkeratosis⁶³). The term aspergillustoxicosis tentatively has been assigned to this mycotoxicosis⁷ because of the clinical and pathologic similarities in calves affected by the three *Aspergillus* toxins and also to differentiate this toxicosis generically from stachybotryotoxicosis which in many ways is similar.

Although the *A. chevalieri* and *A. clavatus* had been isolated from feedstuffs which did not contain chlorinated naphthalene compounds, other feedstuffs and fodders suspected to be related to the incidence of bovine hyperkeratosis⁶⁴⁻⁶⁵ were not subjected to definitive mycologic and mycotoxic study. Therefore, the possible role of mycotoxins in the production of hyperkeratosis, when it occurs apart from feeds contaminated with highly-chlorinated naphthalene derivatives, still remains indefinite. Nevertheless, the literature does indicate that various fungi, including many aspergilli, have been isolated from various feedstuffs which have been shown to induce toxicoses in animals.

Aspergillustoxicosis may occur sporadically and outbreaks vary in degree and severity. Under natural conditions, this toxicosis appears to be most prevalent from the Fall until late Spring, although it can be produced experimentally during any season of the year. This toxicosis primarily affects cattle although horses, rabbits and mice also are affected.

As with the other mycotoxicoses, aspergillustoxicosis also exists in both an acute and a chronic form.

The acute form develops following ingestion of large amounts of toxic substratum and is characterized initially by lachrymation, intermittent salivation, depression, and an increase in body temperature, pulse and respiration. As the toxicosis progresses, the plasma vitamin A decreases and the animal develops intense malaise, characterized by congested
conjunctival mucous membranes, profuse fetid diarrhea, dehydration, anorexia, Cheyne-Stokes respiration, and prostration. Death may follow within five to 20 days from the onset of the symptoms. The most prominent necropsy findings are hemorrhage and congestion in many tissues, including the trachea, superficial muscles of the thorax, lungs, heart, abomasum, small intestines, caecum, kidneys, adrenals, pancreas and in the tracheal, mediastinal, hepatic and mesenteric lymph nodes. On histopathologic examination, in addition to the above findings, the principle changes are observed in the liver and kidneys. The primary changes in the liver are thrombosis of the blood vessels, necrosis and necrobiosis and vacuolation of the liver cells, and subendothelial edema. Dilation of the collecting tubules and hemorrhages occur in the cortex of the kidneys, and in some renal tissues the epithelial cells are separated from the basement membrane and from casts in the lumina.

The chronic form develops in calves that ingest small quantities of toxic fungal substrata over extended periods of time. The chief clinical observations initially are slight to moderate depression, slight anorexia, lachrymation, and intermittent salivation. As the toxicosis progresses these conditions intensify. The skin along both sides of the neck and cheek may become thickened, the plasma vitamin A drops and an agranulocytosis occurs. Pathologic findings include, in addition to most of those observed in the acute form, frequent overproduction of the keratin layer of the corneum, numerous eosinophiles in the corium, polymorphonuclear infiltration of the corneum and corium, and hypertrophic reaction of the hepatic reticuloendothelial tissues.

**Moldy Corn Toxicosis in Swine.** In 1952, Sippel and co-workers studied this toxicosis intensively and in 1953 reported on a widespread outbreak in swine that foraged moldy corn in the field. Some cattle also were affected. In examining previous laboratory reports, Sippel concluded that this malady apparently first occurred as early as 1949 though not diagnosed as such at that time. Although toxicoses in livestock associated with ingestion of moldy corn and other mold-infected grain and feedstuffs have been indicated prior and subsequent to the report of Sippel et al, none, however, was so widespread and involved such economic loss. In 1957, Burnside and co-workers found that two fungi, a strain of *Aspergillus flavus*, Link and one of *Penicillium rubrum*, Stoll, when grown in pure culture on sterilized corn and fed to swine, produced a similar toxicosis.

Although swine of all ages are affected by this toxicosis, young pigs are particularly susceptible. Cattle that had foraged moldy corn in the field also developed this syndrome, and under laboratory conditions this toxicosis has also been reproduced with both the *A. flavus* and *P. rubrum*. Using the *P. rubrum* which was the only one of the two toxic fungi tested the toxicosis was also produced in the horse, goat and mice.

Moldy field corn is the primary source of the typical field cases; however, instances have occurred where moldy peanuts (groundnuts, *Arachis hypogaea*) have been incriminated. In fact, the author examined one lot of such toxic peanuts submitted to him in October 1952 by Dr. Joseph Burnside, then stationed at the Georgia Coastal Plain Station, Tifton, Georgia and isolated a toxic strain of *Aspergillus flavus*, Link
Figure 1. Toxic moldy peanuts (groundnuts—*Arachis hypogaea*). The predominating fungus, a toxic strain of *Aspergillus flavus*. Link was isolated from these peanuts in October 1952.
Morphologically and taxonomically this fungus was identical with the toxic strain of *A. flavus*, Link isolated from the field cases of moldy corn toxicosis. The latter fungus has been designated, *A. flavus* CD#5 and is commonly referred to as such among various mycologists and other investigators familiar with this strain. This strain of *A. flavus* appears to be morphologically similar to that found by the English workers in certain lots of Brazilian groundnut meal peanut meal. (Burnside *et al.* and Sippel stated that there is evidence that formulated feedstuffs containing corn grown in enzootic areas may also be toxic to animals, and commercial feeds have also been alluded to as possible sources of this toxin in which low quality corn or peanuts have been used as ingredients.

Members of the *A. flavus* group of fungi are distributed world-wide and can proliferate on any substance capable of supporting any type of fungal growth. Some strains produce aspergilllic acid, a heat-stable, toxic antibiotic also resistant to acid or alkaline conditions. Sippel *et al.* observed that carcasses of animals dead from the moldy corn toxicosis decomposed slowly. This circumstance suggests that the presence of an antibacterial substance could have inhibited decomposition. In addition, Burnside *et al.* reported that their toxic strain of *A. flavus* produced an antibacterial substance when cultured in broth. Perhaps the aspergilllic acid produced by the strains of *A. flavus* used by White, White *et al.*, and Jones also may have been produced by the strain used by Burnside and co-workers.

Both toxic and nontoxic strains of *A. flavus* have been isolated from corn in the field cases of moldy corn toxicosis. Of nine strains of *A. flavus* isolated Burnside *et al.* found only one toxic. No data have been reported to date indicating presence of toxic and nontoxic strains of *P. rubrum*, although it is reasonable to assume that both do exist.

Moldy corn toxicosis in general appears to be seasonal, the first cases appearing during late Summer and early Fall as the animals are allowed to forage corn (or peanuts) in the field and the toxicosis terminates with availability of the feed supply.

The toxicosis occurs naturally and also has been reproduced experimentally in an acute and a chronic form. The acute form develops after swine consume lethal quantities of toxic substratum. Affected swine develop anorexia, depression, staggering in the hind quarters, and paleness of the mucous membranes. Death usually ensues within two to five days. Body temperature, hematocrit, hemoglobin, prothrombin time, and blood counts remain normal. A drop in plasma vitamin A level frequently occurs, values ranging from 4.2 to 7.2 gamma per 100 ml. of plasma being reported by Sippel and co-workers. The paramount lesion at necropsy is profuse hemorrhage in many tissues, in many cases death being caused by hemorrhages.

The chronic form develops in swine that consume sublethal amounts of toxic moldy substratum for extended periods. If physiologic damage to the host is severe, the animal neither responds to dietary change nor makes profitable gains. If death does not occur from cachexia, it is considered economically expedient to destroy the animal. Swine affected by the chronic form are depressed, anorexic and cachecic; they stand with
heads lowered, backs arched and flanks tucked in, and walk with a stiff gait. Affected swine usually have a low erythrocyte count, prolonged prothrombin time, and appear yellow and icteric, the icteric index being high. The principal gross necropsy findings include a general icterus of the body, a yellow to orange fibrous liver, presence of a straw-colored fluid in the thoracic and peritoneal cavities, paleness and edema of the kidneys, gelatinous infiltration of the peritoneal covering of the colon, hemorrhages and a watery consistency of the lymph glands, and ecchymotic hemorrhages on the superficial muscles of the anterior of the thighs, in the subcapsular region and in other tissues. According to Sippep a rather constant lesion is subendocardial hemorrhage in the ventricles.

Histopathologic examination of swine affected with the acute or chronic toxicosis indicates an essentially similar process, the difference being only in degree. Hemorrhages in many tissues appear to be the primary lesion. Changes characteristic of an acute or subacute toxic hepatitis are observed in the liver. In the acute form a fatty degeneration and necrosis of the liver cells occur. In the chronic form, lesions of subacute toxic hepatitis are observed characterized by intralobular cirrhosis, bile duct proliferation and marked centrolobular necrosis, often times extending to the periphery of the lobule. Glomerular atrophy, tubular dilation, edema, and necrosis of the convoluted tubules, together with fatty degeneration of the epithelial cells are observed in the kidneys. The spleen shows evidence of lymphoid exhaustion. Areas of hemorrhage, focal necrosis and fibrosis occur in the pancreas.

Recently, Loosemore and Harding73 observed a similar chronic toxicosis in swine that were fed a diet containing Brazilian groundnut meal. The toxin present in the meal, as indicated earlier, apparently is of mycologic origin. At least one, if not the principal fungus, that may be responsible for the mycotoxin in the groundnut meal is a strain of A. flavus, Link morphologically similar67 to that isolated by Forgacs from moldy toxic peanuts in Georgia in 1953 and by Burnside et al2 from toxic field corn in 1957.22 The clinical, gross and histopathologic findings in swine that consumed the toxic Brazilian groundnut meal as reported by Loosemore and Harding73 in 1962 appear identical with those reported nine years earlier by Sipple et al21 for the chronic form of moldy corn toxicosis in swine, although the former investigators would like to characterize their syndrome into an acute, subacute, and a chronic toxicosis based on hepatic changes in affected swine and would appear to parallel those described in Cunningham et al74 and others for liver lesions in ruminants affected with the facial eczema toxin.

Moldy Feed Toxicosis in Poultry. Poultry hemorrhagic syndrome as it has been described in the field75-78 closely resembles some of the mycotoxicoses. It has been shown that various fungi isolated particularly from feed spilled in litter collected from the major broiler areas in the United States where poultry hemorrhagic syndrome was enzootic produced a toxicosis23-28,75 strikingly similar to that observed in field cases of the syndrome.78 This mycotoxicosis has been produced in an acute and a chronic form in battery birds experimentally, and in those maintained under field conditions. The toxicosis associated with ingestion of
fungus-infected substrata by poultry, accordingly, has been named tenta-
vively mold feed toxicosis in poultry.7,26

Among the numerous fungi isolated from feed and litter where the
poultry hemorrhagic syndrome was enzootic, the following fungi have been
found toxic to chickens: Aspergillus chevalieri, A. clavatus, A. flavus, A.
flavus-oryzae, A. fumigatus, Paecilomyces variotii, Penicillium citrinum,
P. purpurogenum, P. rubrum, several unidentified species of Penicillium
and an Alternaria. Undoubtedly there exist other fungi yet to be isolated.

Moldy feed toxicosis also exists in an acute and in a chronic form. This
toxicosis can be produced during any season in batteries using toxic
fungal substrata. Under field conditions where chickens are maintained on
wood shavings litter, it is most readily produced from late Fall through
Spring when conditions are ideal for fungal growth and toxin formation.

The acute form develops during the early stages of the toxicosis. This
may occur within 24 hours or may extend from 17 to approximately 21
days, depending on amount and potency of mycotoxin consumed. There-
after the chronic form develops.

The acute form is characterized clinically by depression, diarrhea
(frequently blood-tinged) and paleness of the combs and wattles, followed
by death. Necropsy findings include for the most part subcutaneous and
intramuscular hemorrhages, hemorrhages and congestion in the thymus,
proventriculus, gizzard (which also becomes eroded, discolored, and fre-
quently edematous), small intestines, caecum, liver, and heart, together
with paleness and petechial hemorrhages in the liver. In addition on histo-
pathologic examination, the liver shows areas of necrosis, vacuolations
containing pleomorphic nondescript bodies that stain red with Giemsa stain
and enlargement of intracellular spaces due to edema or atrophy of the
liver cells, and in the hepatic veins, thrombi containing bacteria. In addi-
tion to hemorrhages, the kidneys show tubular necrosis and interstitial
round-cell infiltration. Free blood is occasionally seen in the lumen of the
intestinal tract with or without any alteration of the epithelial lining or
evidence of focal hemorrhagic lesions. Accumulations of hemosiderin are
observed in the muscle tissues, liver, spleen, and bone marrow.

If the host does not succumb to the acute form of the mycotoxicosis,
symptoms of the chronic form develop. Depression, diarrhea, paleness of
combs and wattles, anorexia and cachexia which are observed in the acute
form intensify in the chronic form. Morbidity and mortality vary. The
chief necropsy findings include, in addition to those observed in the acute
form, paleness of the bone marrow and focal hemorrhagic lesions in the
small intestines readily visible through the serosa. The major histo-
pathologic changes consist of blood pigments in the liver, proteinaceous
and hematogenous casts in the distal convoluted and collecting tubules of
the kidneys and, in the bone marrow, depletion of the erythropoietic centers
and hypoplasia of the myelopoietic centers with development of lymphocytic
foci and many fat cells.

Under field conditions, the acute form prevails during the early stages
of the toxicosis. Later, both the acute and chronic forms usually appear
concurrently. In the field, the toxic fungi which are omnipresent in broiler
mash proliferate in feed spilled by chicks in damp litter and from toxic
substances as early as 10 days.\textsuperscript{25,26,7} Thus, chicks that ingest such feed develop the acute form of the toxicosis during the early stages. Thereafter, both the acute and the chronic forms appear within the same flock which is dependent upon time and amount of toxic feed consumed. The overall intensity of the toxicosis may reach a peak within about two weeks and then gradually subsides. Mycologic examination of feed spilled in litter indicated that development of a strain of \textit{Scopulariopsis brevicaule} coincided with the decline in severity of the clinical signs of the toxicosis.\textsuperscript{26,27,7}

\textbf{Facial Eczema in Ruminants.} This mycotoxicosis which occurs primarily in New Zealand\textsuperscript{74} has been classified by Clare\textsuperscript{29,30} as one of the hepatogenous photosensitivity diseases in which the sensitizing agent, pyelloerythrin, a breakdown product of ruminant digestion, reaches the peripheral blood circulation due to pathologic lesions in the liver. Early studies supported the hypothesis that the toxicosis was of microbiologic origin.\textsuperscript{80,33} In recent years, it has been demonstrated that the fungus known during earlier stages of the study as \textit{Sporodesmium bakeri}, Syd., but recently reclassified as \textit{Phytomyces chartarum}\textsuperscript{46} growing as a saprophyte on dead perennial rye grass is the source of the hepatotoxin.\textsuperscript{31-35} Sheep and cattle are the large domestic livestock affected.\textsuperscript{74,29,30}

Facial eczema in ruminants, so far, has been described apparently as a chronic disease. Affected sheep develop lachrymation, salivation, a nasal discharge, hyperirritability, ichiness and seek shade for relief. Subsequently, the afflicted animals may develop a leucocytosis, bilirubinemia, icterus, cachexia, and may perish. Affected cattle first show a drop in milk flow, followed by local manifestations of photosensitization usually confined to the teats, udder, escutcheon, perineal region, muzzle, around the eyes, under the tongue, and generally, to lightly-pigmented areas of the skin. Necropsy findings include primarily gross changes in the liver, with presence of a bile-stained fluid in the dermis and in the subepidermal layer of the skin. Histopathologically, the primary changes indicate varying degrees of an obliterative cholangitis with subsequent bile ductile proliferation and fibrosis and atrophy of the liver cells.

A strikingly similar disease has been reported in cattle in the United States that grazed moldy Bermuda grass,\textsuperscript{37-40,41-43} and moldy legume pasture.\textsuperscript{41-43} The predominating fungus on toxic forage is a strain of \textit{Periconia minulissima}.\textsuperscript{39,40} Although cattle that had been fed grass contaminated with this fungus developed hepatogenous photosensitization, feeding pure culture media on which this fungus has been grown has not been successful so far.

\textbf{HUMAN MYCOTOXICOSES}

Alimentary toxic aleukia (ATA) develops in human beings who have ingested overwintered moldy grain, its by-products, or cooked foodstuffs such as bread or gruel prepared from infected grains. The causal fungus, \textit{Fusarium sporotrichioides}, grows saprophytically on various cereal grains, particularly proso millet that have been allowed to overwinter under the snow. This fungus, unlike many others, when dry is usually not colored and therefore, grain infected with this fungus is not readily detected visually.
Surprisingly, ATA appears to be confined to certain urban areas of the USSR, although *F. sporotrichioides* is distributed world-wide. Mayer\(^5\)\(^4\) critically reviewed over 200 research publications on ATA, chiefly in Russian, and the interested reader is referred to his comprehensive review for a detailed description of various aspects of this toxicosis. Gajdusek\(^5\)\(^4\) also published an English summary of selected Russian works on this and other mycotoxicoses and other diseases endemic in the Soviet Union. Recently, Forgacs and Carl\(^7\) reviewed the work of Mayer and Gajdusek as well as additional Soviet works and have compiled a historical, epidemiologic, clinical, pathologic, biologic and ecologic study of ATA.

Like other mycotoxicoses, ATA does not have a well-defined epidemiology. It has been estimated that approximately three lbs. of proso millet or its equivalent in by-products eaten over six weeks will induce pathologic changes in the hematopoetic system.

*F. sporotrichioides* is extremely resistant to cold weather and will grow slowly at temperatures as low as $-10^\circ C$, although its optimum like most saprophytic fungi is approximately $24^\circ C$. In the past, the optimal temperature for toxin production has been regarded as 1.5 to $4^\circ C$. More recent information, however, would indicate that the fungus is capable of forming its toxin at higher temperatures.

The toxin is resistant to heat, acids or alkalis. Although most of the data indicate that man has been the most affected by the toxin, other publications indicate that most farm animals are also susceptible to the toxin.\(^3\)\(^4\)

ATA is now recognized to exist in four stages. The first stage is characterized chiefly by changes in the buccal cavity and gastrointestinal tract. These include, in mild cases, a stomatitis, diarrhea, nausea and vomiting, and excessive prespiration. In more severe cases, convulsions and cardiac failure may occur. The nonlethal manifestations may last for five to nine days and then subside, even though ingestion of toxic food is not interrupted. Thereafter the second stage develops.

The second stage of this toxicosis, as in the second stage of the typical form of equine stachybotryotoxicosis, is quiescent clinically but damage to the hematopoetic centers occurs. Examination of the peripheral blood reveals a progressive leucopenia, granuleucopenia, and a relative lymphocytosis. This stage usually lasts for three to four weeks, but in some cases it may extend over a period of two to eight weeks.

The clinically-quiescent second stage is rapidly followed by symptoms of the third stage which can be violent, especially under the influence of stress. Minute hemorrhages occur over various parts of the body necrotic areas in the buccal cavity, and a severe gangrenous pharyngitis. These manifestations are followed by hemorrhages from the nose, mouth, gastrointestinal tract, and kidneys. Blood dyscrasias observed in the second stage become more pronounced. The leucocyte count may drop to 100 per cubic millimeter, erythrocytes to one million, thrombocytes to 8,000, lymphocytes may increase to 90 percent, and prothrombin-time and clot-retraction time increase significantly. Thus, with the depletion of the hematopoetic centers, secondary infections may occur. Duration of this stage may extend over a period of five to 20 days.
The fourth stage is designated as the period of convalescence and varies from two weeks to over two months depending on damage to the patient.

**ISOLATING FUNGI**

The first prerequisite for the isolation of fungi from a suspected substratum is a thorough knowledge of the morphology and taxonomy of fungi. Since most research workers interested in moldy feed or fodder poisoning may not be trained in mycology, the assistance of a mycologist, or perhaps a qualified phytopathologist proves invaluable. A mycologist trained in mycotoxicoses research, of course, would be the ideal; however, an individual with this background is indeed scarce.

Suspected material, freshly collected, should be examined with a dissecting microscope. This procedure must be carried out carefully to retain the structure of fungal fructifications necessary for tentatively identifying the predominating fungi as they occur on the natural substratum. Using this information, the mycologist usually selects the proper media to be used for isolating the fungi and knows what fungi to expect during subsequent cultural studies.

When fungi thriving on a natural substratum fail to grow successfully on a conventional medium, it is recommended that isolation be then attempted on a variety of media, including some which incorporate components found in the natural substratum.

Various media and methods have been employed for isolating fungi. Those described here, for the most part, are based upon our own experience and do not necessarily imply that they may be suitable under all circumstances. Indeed, the interested worker may find it profitable to modify these procedures or even perhaps devise his own techniques. Details of the methods used for isolating fungi described in the selections to follow have been presented elsewhere.7

**Fungi From Hay, Straw, and Other Fodder.** Freshly-collected samples of suspected hay or fodder should be examined for fungal growth and fructifications. Hollow-stemmed roughage should be examined not only from the exterior but from the interior as well since in many cases some fungi, although not easily detected on the outside, frequently flourish within the stem.

Most hays and fodders may be contaminated with mucors and other fungi that proliferate rapidly and overgrow the culture plates, thus observing growth of the most fastidious and less rapid growing fungi. Therefore, it is expedient to use methods that effectively suppress growth of all fungi, particularly the luxuriant and, frequently, undesirable molds. We have found the following procedure satisfactory: A sample Petri dish is prepared in the laboratory by placing a small layer of absorbent cotton on the bottom, overlaid by two pieces of filter paper the same diameter as the Petri dish. The plate is then sterilized by dry heat at 150 to 160°C for one hour, or alternately, autoclaved by 15 lbs. pressure for 50 minutes. The suspected material is aseptically cut into 1/4-inch pieces, and several are placed on the filter paper in the sterile Petri dish. For nutriment,
about eight ml. of Czapek's solution broth (Difco) are aseptically pipetted onto the cotton under the filter paper. The inoculated plate is incubated upright for several days at approximately 25°C and examined for fungal proliferation by means of a dissecting microscope. When fungal growth has advanced to the stage of formation of aerial fructifications, a small portion of the aerial mycelium is carefully removed aseptically with a dissecting needle and transferred onto a more nutritious medium, such as Mycological agar (Difco), Littman's oxgall medium (Difco), Czapek's solution agar (Difco) or any other suitable medium. A medium consisting of two percent milled wheat and two percent plain agar in distilled water has been found highly satisfactory for culturing *Stachybotrys* and other cellulose-decomposing fungi.

**Fungi From Grain.** Suspected grain can be examined in situ by means of a dissecting microscope to determine relative predominance of types of fungi. Then by means of forceps which can readily be sterilized in the field using an alcohol lamp, seeds of grain showing typical fungi can be placed on suitable mycologic agar in Petri plates. Several media have been found satisfactory. Littman's oxgall agar is particularly useful since it partly inhibits growth of contaminating bacteria and restricts growth of all fungi, thus reducing excessive bacterial growth and preventing overgrowth by the troublesome mucors and other luxuriant growing fungi. Czapek's solution agar and Mycological agar to which have been added a wide-spectrum antibiotic for inhibiting contaminating bacteria are also useful. The antibiotic is added to the sterilized agar after it has been cooled to approximately 50°C and just prior to pouring into plates.

Another method used for isolating fungi from grain consists of aseptically grinding a composite sample of suspected grain and making serial dilutions of a one gram sample in distilled water containing a nontoxic surfactant and inoculating an aliquot, either on the surface of a mycologic agar, or by means of pour plates.

Litchwardt and co-workers found Christensen's malt-salt agar satisfactory for isolating fungi and stored shelled corn. Bonner and Fergus used several agars for successfully isolating a wide variety of fungi from grain, fodders, and silage that were suspect in toxicoses in farm animals.

**Fungi From Broiler Mash.** Suspected broiler mash or feeds of a similar textural nature can be diluted and plated using the same technique as that described for isolating fungi from ground corn. The feed also can be sprinkled very lightly onto the surface of several mycologic agars, including Littman's oxgall medium. The inoculated plates are observed after four to five days for fungal growth, using the dissecting microscope. Transfers are made of isolated fungal colonies onto suitable mycologic agars.

Broiler mash, when mixed with litter, should first be examined with the dissecting microscope for active fungal growth and, if possible, for prevailing fungal types. The proliferating fungi can be transferred onto solid media and routinely subcultured for purity. A composite sample of the suspected feed and litter can be subjected to fungal isolation, using the methods described for isolating fungi from broiler mash.
Christensen, in 1946 devised a malt-salt agar for successfully isolating fungi from flour and later modified the medium slightly. We found both media satisfactory for isolating many fungi from broiler mash.

Fungi From Litter. The fungal types present in a litter depend on the nature of a litter. For example, bagasse litter because of its hypertonic nature contains a predominance of xerophytic fungi, such as members of the *Aspergillus glaucus* group that flourish on media having a high osmotic tension. Wood shavings litter is an excellent source of species of *A. fumigatus* and cellulose-decomposing fungi. Litter which contains spilled feed contains a wide array of fungi common to both the litter and the feed.

Microscopic examination of litter, which in most cases in the field is damp, reveals the predominating fungal types. Litter samples should be examined for fungi, using both the procedures recommended for isolating fungi from hay and coarse fodder and those for isolating molds from broiler mash.

TESTING FUNGAL ISOLATES FOR TOXICITY

Screening and testing fungal isolates for toxicity is both tedious and exacting. Since toxic fungal species may be morphologically identical with nontoxic species, all fungal isolates must be individually screened for toxicity. The techniques recommended here are not described in detail; however, the interested reader may refer to another source for detailed descriptions of the techniques. In addition, since these procedures can be regarded only as general, modifications may be necessary to meet the particular problem at hand.

The initial type of substratum for growing a fungus for toxin production should closely approximate the composition of that on which the fungus originally grew. The optimum conditions for toxin production can be determined then by experimentation with a variety of media, temperatures, time of incubation, and other factors.

Cellulose-Decomposing Fungi. Cellulose-decomposing fungi such as species of *Stachybotrys* or *Mennoniella* can be cultured successfully on wheat straw, hay, or other roughage. The substratum is packed into two-liter, wide-mouth, Fernback flasks; 50 ml. of distilled water are added; the openings are plugged with cotton, and the contents autoclaved for 30 minutes at 15 lbs. pressure. After cooling, the contents of the flask is aseptically inoculated with the culture to be tested and incubated at room temperatures for approximately three weeks, removed, dried, and ground to a powder in a Wiley mill or other suitable apparatus.

For dermal toxicity testing, an extraction thimble is filled with the dried, powdered substratum and is reflux-extracted for three hours in a Soxhlet apparatus, using anhydrous ethyl ether as the solvent. The other is partly evaporated under reduced pressure from the extract, then 10 ml. of olive oil are added to the extract, followed by a complete removal of the ether. A volume of 0.125 ml. of the oily suspension is applied topically for three consecutive days on a shaved area of the back of a rabbit and the treated area is observed for a positive reaction, characterized by a hyperemia 24 hrs. after the first dose and a pronounced hyperemia and serum
exudation 24 hrs. after the third dose. Nontoxic extracts produce no dermal inflammatory reaction.

Fungal substrata of isolates yielding a positive dermal reaction are then tested for oral toxicity in various animals. The final animal species selected for oral toxicity testing should include the particular species originally affected by the suspected substratum from which the fungus or fungi had been isolated. The dosage selected for testing should be determined by titration of the toxin fed and should be calibrated for each isolate and for each test animal species employed for both acute and chronic toxicity tests.

Testing Other Fungi. Species of *Alternaria*, *Aspergillus*, *Paecilomyces*, *Penicillium* and similar fungi isolated from grain and processed feeds may be inoculated on sterile, moist, whole-grain corn alone, or mixed with other grains. Prior to sterilization, the grain should be steeped in warm water to soften the kernels and thus facilitate proper steam sterilization. After draining the excess moisture, approximately one lb. of grain is placed into wide-mouth, two-liter, Fernbach flasks which are plugged with cotton and the contents then autoclaved for one hour at 15 lbs. pressure. After cooling, the autoclaved grain in the flasks is inoculated aseptically with the test fungus and then incubated at room temperatures for approximately three weeks. The fungus-infected grain is then removed from the flasks, dried and ground to a meal. The dried meal is tested for dermal and oral toxicity as described in the previous section.

Other Methods of Testing. Other methods have been used for testing fungi for toxicity. Two which show promise are briefly described here.

Burnside and co-workers\textsuperscript{22} macerated with water in a Tenbroeck tissue grinder, Mycological agar slants on which had been cultured fungal isolates, and stomach-fed the homogenates to mice using a hypodermic syringe fitted with a blunted 18-gauge needle. Mice weighing 20 to 25 gms. were force-fed daily 0.5 to one ml. of the homogenate. These workers did not use other animals in this test procedure, although there is no reason why this cannot be accomplished.

Burnside \textit{et al}\textsuperscript{22} also successfully tested culture filtrates of 13 fungi for toxicity in embryonated chicken eggs. Approximately 0.2 ml. of cell-free filtrates of selected fungi was injected and embryo viability was observed for three days.

Both the mouse force-feeding test and the embryonated chicken egg procedure were in agreement with the dermal and oral toxicity tests that Burnside and co-workers used in their moldy corn studies. Of 13 fungi isolated only two, a strain of *A. flavus*, Link and one of *P. rubrum*, Stoll were found to be toxic.

PRECAUTIONARY MEASURES

A word of caution is given to the investigator who selects to work with the toxigenic fungi. The possibility exists that some of the toxin-producing fungi also may be pathogenic to man. Strains of *A. fumigatus*, *A. flavus* and, possibly *S. atra*, have been demonstrated pathogenic not only to animals, but to man as well. Aerosols from toxic fungal substrata may affect
man. Drobotko and Drobotko et al. indicated that workers exposed to aerosols from *Stachybotrys*-infected substrata may develop a dermatitis and also absorb sufficient toxin via the respiratory route to evidence blood dyscrasias characteristic of the second stage of the mycotoxicosis in animals. Toxic manifestations also may occur in human beings exposed to other toxigenic fungal substrata and extracts. Furthermore, many fungi because of their xerophytic nature are readily dispersed in air currents into adjacent areas and thus may be a source of infection or toxin to the individual who inhales fungal spores, as well as a source of contamination in the laboratory.

It is strongly recommended that isolation of fungi be performed under an exhaust-equipped hood. Similarly, the drying and grinding of fungal substrata should be conducted in a separate room equipped with an exhaust hood and exhaust filter. All exposed individuals should wear a suitable respirator (preferably a gas mask), protective clothing, and should change clothing and shower before re-entering a noncontaminated area.

GENERAL COMMENTS

The mycotoxicoses as they affect animal and human health have not been considered economically important in the past for the most part in the western world. Actually, the economic significance of this group of diseases cannot be evaluated simply because of a lack of diagnosticians familiar with the clinical and pathologic manifestations of this group of elusive diseases, as well as a dearth of interested scientists trained in methods of mycotoxicoses research. A study of the scientific literature, however, reveals that in the past there have been described various toxicoses of unknown etiology, many of which in retrospect resembled various mycotoxicoses. Although, in most of the reported cases of these toxicoses of unknown etiology, the role of bacteria, viruses and nutritional factors have been studied as possible factors, in the absence of positive findings, the cause had been alluded to be chemical in nature. Yet the possible role that toxigenic fungi could have played in these toxicoses had not been determined, even though it has been established that the fungi because of their highly complex biochemical characteristics are capable of synthesizing a vast array of chemical compounds under highly variable natural conditions, frequently highly complex chemically, structurally unrelated, and having diverse physiologic properties. A good example are the fungi used to synthesize the wonder-drugs or antibiotics.

The fact that some fungi may be toxigenic should not be surprising, for among the many thousands of fungi which are tested annually for antibiotic-producing ability, only a very few indeed are retained for further testing. Most are rejected because they either produce no antibiotic substances or because they produce toxic products, in essence mycotoxins. Nevertheless, the possibility that the fungi can synthesize toxic compounds (under natural conditions) appears to astound many individuals. Even recently, it has been stated that, "one should not become overly concerned about this mycotoxicosis as it is being called, because all of these molds have been with us for a long time and this has never become a serious
problem—."

This erroneous, scientifically unfounded conclusion is indeed hazardous as would be the case if one should apply such reasoning, for instance, to the omnipresence of bacteria and viruses in nature and their relationship to animal and human health.

The author of this presentation does not by any means wish to de-emphasize a continued need for the intensive study of the role of bacteria, viruses, nutrition, and chemical agents in maladies of animal and human beings. Obviously, this study must continue. Rather, it is the sole purpose to call attention to a group of diseases, the mycotoxicoses, which although in the past have been neglected somewhat, deserve their rightful place in the scientific endeavors of scientists, both in the veterinary and human fields. The voluminous research conducted by Soviet scientists, and the limited but fruitful investigations of scientists in the western world clearly indicate the importance of these diseases and the need for further investigations not only from the standpoint of their direct influence on health, but also in terms of their influence upon infections by other agents. For it has been established that when the defense mechanisms become depleted, the host is subject to secondary infections. In the chronic forms of those mycotoxicoses studied, one of the primary changes includes depression of the hematopoetic centers of the host and thus secondary infection indeed occur.

The mycotoxicoses described here do not encompass all described in the literature. There exist others, some of which have been described, and others yet to be studied. The mycotoxicoses described here are relatively severe in their manifestations. Others exist which are mild in their manifestations but nevertheless exert their toxic effects on the host.

Although the mycotoxicoses in animal health have been neglected to some extent, their influence upon human health has been ignored almost entirely in the western world. The Soviet scientists have shown that a toxin produced by a saprophytic fungus *F. sporotrichioides*, produces severe toxic manifestations in man, frequently terminating fatally. Molds, some of which are toxigenic, are universally distributed in nature and many fungi are utilized in industrial processes. Yet the possible influence that these fungi might have on animal and human health remains yet to be determined. For example, high grade baking flours have been found highly contaminated with fungi. Some of these fungi may be toxigenic. Although baking destroys virtually all molds and their spores, the mycotoxins in the fungal mycelia and spores can be heat-stable, and thus not be affected by the baking process. One may therefore justly wonder what influence these mycotoxins may have on those of us who consume products prepared from substrata contaminated with mold fragments and spores.

Molds, particularly members of the *A. flavus-oryzae* group are used in the Orient for fermentation of soy products, yet the toxigenic properties of these fungi or their natural mutants has not been entirely studied. Evidence presented here indicates that a toxic strain of *A. flavus*, Link has been isolated from peanuts in the United States as early as 1952 and subsequently from corn and other natural substrata. This fungus is capable of growing on any substratum capable of supporting any type of fungal growth. More recently the English workers have confirmed these
findings and indicate that the preformed toxin in groundnuts (peanuts) is not destroyed by heat generated during extraction of the oil but remains in the peanut meal and has caused a mycotoxicosis in small animals, poultry and large domestic livestock. The implications of this and similar mycotoxins in human health remain to be determined.

Various fungi are used to ferment malt grains, and in the past this process also has been given little attention from the standpoint of toxigenic potentialities of the associated fungi. Yamamoto\textsuperscript{86} isolated a toxic strain of \textit{Penicillium urticae} from malt grain that caused a high mortality among cows fed the grain. A strain of \textit{Penicillium islandicum} has been isolated from yellowed rice\textsuperscript{87} and has been found to produce toxic substances.\textsuperscript{88}

Aging of meat is an old practice and although it has been demonstrated that many fungi, some of which actually proliferate at low temperatures, may be found on such meat, the possibility that some of the fungi may form mycotoxins also has not been determined.\textsuperscript{85}

\textit{Byssochlamys fulva}, considered by Raper and Thom to be very similar if not identical with \textit{Paecilomyces variotii} is important in the canning industry because of its ability to withstand high temperatures and its ability to grow in fruits and fruit syrups. In our studies in moldy feed toxicosis in poultry, we frequently isolated toxic strains of \textit{P. variotii} from feed samples.

Various fungi have been isolated from many food products,\textsuperscript{85} yet the toxigenic potentialities of the fungi remain to be studied. Unfortunately space does not permit here to list the numerous food products examined and the wide array of fungi isolated.

It is the sincere hope that this brief presentation of a broad subject will stimulate much-needed research in the fruitful field of mycotoxicoses research both from the standpoint of animal and human health.

REFERENCES

(The references included in the manuscript are by no means exhaustive, but rather selective. For purposes of brevity and convenience, the titles of Russian publications have not been transliterated from the Cyrillic to the Arabic, but have been presented only as the English translation.—author.)


A REPORT OF THE USE OF A GEL DIFFUSION TEST FOR THE DIAGNOSIS OF VIBRIO FETUS INFECTION IN CATTLE

Edwin I. Pilchard, D.V.M., M.S., and Miodrag Ristic, D.V.M., Ph.D.

Vibrionic abortion of cattle, caused by *Vibrio fetus* remains an important uncontrolled cause of losses to cattle breeders throughout the United States. A major difficulty in devising an effective control program for this disease has been the lack of a reliable and simple diagnostic test. A rapid gel diffusion test, using heat stable (HS) soluble antigens was recently introduced by Ristic and Murty (1961)** as a diagnostic test for bovine vibriosis.** This paper is a preliminary report of the use of this test at the Diagnostic and Research Laboratory of the University of Illinois College of Veterinary Medicine during a period of 21 months ending on June 31, 1962.***

Ideally, a test for vibriosis must detect the presence of all strains of *V. fetus* pathogenic to cattle, be devoid of cross-reactions with factors not specifically related to vibriosis, and be sufficiently sensitive to allow diagnosis in a high percentage of infected individuals and herds. In addition, test reagents must be stable upon storage and of uniform reactivity.

*Vibrio (Spirilla)* was first associated with abortion and infertility in cattle and sheep in 1909 (McFadyean and Stockman, 1909 and 1913). To this day a definitive diagnosis of vibriosis requires the isolation of the causative organism. Unfortunately isolation of *V. fetus* is an impractical procedure.

The widely used serum agglutination (Smith *et al.*, 1920)** and vaginal mucus (Szabo, 1951)** tests for vibriosis employ antigens which are difficult to standardize and which have been shown to give false positive reactions (Plastridge *et al.*, 1951;** McEntee, Hughes and Gilman, 1954).**

McEntee, Hughes and Gilman (1954)** reported that the vaginal mucus test was positive during a period from 60 days to seven months after inoculation of heifers with *V. fetus*. Although the presence of traces of blood in the mucus resulted in false positive test results, these workers found that the vaginal mucus test was more satisfactory than the serum agglutination test.

For the serum agglutination test Plastridge *et al.*,** recommended the collection of samples within a period of two weeks following abortion. McEntee *et al.*,** found positive serum agglutination tests appeared in experimentally infected heifers during several months after positive mucus tests were obtained.

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**The term "vibriosis" in this paper refers only to the infertility and abortion caused by *Vibrio fetus* infection of cattle.

***The Diagnostic and Research Laboratory is operated by the College of Veterinary Medicine, University of Illinois, in cooperation with the Illinois Division of Livestock Industry.

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The purpose of the current study is to correlate with certain clinical and bacteriologic evidence of vibriosis the results of the rapid gel diffusion tests of bovine sera collected under farm conditions.

MATERIALS AND METHODS

The test used in this study is that of Ristic and Murty (1961). Test antigen was composed of approximately equal parts of a heat stable (HS) soluble fraction of each of two bovine strains of *V. fetus*, B-10 and Illinois (Ristic and Brandly, 1959; Ristic and Murty, 1961). Commercial formaldehyde at a final concentration of 0.2 percent was added as a preservative. Each of the strains used has shown broad overlapping spectra of reactivity with sera of rabbits inoculated with different isolates (Ristic and Brandly, 1959; Ristic and Walker, 1960; Ristic and Murty, 1961; Ristic, 1962).

Reactivity of the antigen remained undiminished during 12 months storage at -45 F, and during two months at 32 F to 40 F. Blood sera tested were received principally from Illinois veterinary practitioners. Although histories were received with only 57 percent of the samples, it is probable that the majority of sera were from herds in which there existed a reproductive problem.

Isolations of *V. fetus* were made from fetal stomach content and liver, amnionic fluid and placenta. Modified tryptose crystal violet agar and blood agar was heavily inoculated and incubated for from three up to seven days at 37 C in an atmosphere of 10 percent carbon dioxide (Rhoades, 1962).

RESULTS AND DISCUSSION

One thousand nine hundred sixty (1,960) sera from 410 herds were tested (Table I). Approximately one-half of these sera were accompanied by a request that the gel diffusion test for vibriosis be applied. The remainder of sera were tested for vibriosis following negative routine brucellosis and leptospirosis testing of herds in which infertility or abortion was reported. Sera negative for vibriosis were routinely tested for leptospirosis and brucellosis.

Two hundred eighteen (218) sera from 41 herds representing 10 percent of the herds tested gave positive gel diffusion tests for vibriosis. Three herds positive for vibriosis were also positive for leptospirosis at agglutination-lysis titers in excess of 1:1000. Positive rapid plate agglutination tests for brucellosis were obtained in three vibriosis-positive herds. In contrast to these figures, 17 herds in which there were histories of infertility or abortion, and which were negative for vibriosis, contained brucellosis reactors. In 25 herds in which there were histories of infertility or abortion and which were vibriosis negative, leptospirosis was diagnosed.

The large proportion of serum samples tested which gave no evidence of the presence of precipitating antibodies against *V. fetus* HS antigen favors the probability that cross reactions with other common pathogens were absent.
TABLE I

Summary of Results of Rapid Gel Diffusion Tests of Bovine Sera for V. fetus Antibodies, October 1, 1960 through June 31, 1962

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herds tested</td>
<td>410</td>
</tr>
<tr>
<td>Herds positive</td>
<td>41</td>
</tr>
<tr>
<td>Herds positive, history of abortion</td>
<td>16</td>
</tr>
<tr>
<td>Herds positive, history of infertility</td>
<td>8</td>
</tr>
<tr>
<td>Herds positive, history not given</td>
<td>17</td>
</tr>
<tr>
<td>Herds negative</td>
<td>369</td>
</tr>
<tr>
<td>Herds negative, history of abortion</td>
<td>113</td>
</tr>
<tr>
<td>Herds negative, history of infertility</td>
<td>34</td>
</tr>
<tr>
<td>Herds negative, history not given</td>
<td>222</td>
</tr>
<tr>
<td>Herds brucellosis positive, gel test negative</td>
<td>17</td>
</tr>
<tr>
<td>Herds brucellosis positive, gel test positive</td>
<td>3</td>
</tr>
<tr>
<td>Herds leptospirosis positive, gel test negative</td>
<td>25</td>
</tr>
<tr>
<td>Herds leptospirosis positive, gel test positive</td>
<td>3</td>
</tr>
<tr>
<td>Samples tested</td>
<td>1,960</td>
</tr>
<tr>
<td>Samples positive</td>
<td>281</td>
</tr>
<tr>
<td>Samples negative</td>
<td>1,679</td>
</tr>
</tbody>
</table>

The question of relative sensitivity of the gel-diffusion test in detecting vibriosis remains unanswered. For the purposes of this study, it was decided initially that the sole criterion for a diagnosis of vibriosis is the isolation of V. fetus from the typically infected bovine or from the fomites.

A large number of bull semen samples were cultured and examined microscopically during the course of this study but no vibrio organisms were found.

Two serum samples provided during September, 1961, by Dr. W. N. Plastridge from V. fetus-infected cows gave a positive gel diffusion test result.

During the 21 month period of this study V. fetus was isolated from four aborted feti (Table II). Blood samples were collected from the aborting dams immediately and at from three weeks to two months following abortion. Sera were tested for vibriosis, leptospirosis and brucellosis. A positive gel diffusion test was obtained with serum from one cow from which V. fetus was isolated at our laboratory.

The gel diffusion test was positive in one herd in which the aborting cow produced both V. fetus organisms and a negative gel diffusion test.

Blood samples were obtained from only two cows in each of the two remaining herds mentioned. Test results were negative.

The relative sensitivity of the gel diffusion test has not been determined because of the small number of culturally confirmed cases available for this study. Additional data is needed. Sera from experimentally infected animals is of limited value in evaluating a test which is to be applied to cattle which are naturally exposed to many different field strains of the organism, as well as to widely varied ecological factors. Therefore, it is not possible to estimate the period which will be required to complete the current investigation.
TABLE II
Summary of Data on *V. fetus* Isolations

<table>
<thead>
<tr>
<th><em>V. fetus</em> Isolated from</th>
<th>History</th>
<th>Result of &quot;Herd&quot; test</th>
<th>Result of test of serum from cow from which <em>V. fetus</em> was isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aborted fetus #B5406, 1962</td>
<td>One abortion in herd; no additional abortion or sterility reported</td>
<td>Herd not tested</td>
<td>Positive</td>
</tr>
<tr>
<td>Aborted fetus #B3689, 1962</td>
<td>3 cows aborted within 7 days; all were 5 to 7 months pregnant</td>
<td>2 samples; both negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Aborted fetus #A4319, 1961</td>
<td>3 cows aborted in 21 days, all were 5 to 6 months pregnant</td>
<td>4 samples, 1 positive, A104, 876–881</td>
<td>Negative</td>
</tr>
<tr>
<td>Aborted fetus #A5666, 1961</td>
<td>1 abortion at 4th month of pregnancy; herd history of sterility and &quot;early abortions&quot;</td>
<td>2 samples; both negative, A116, 091–092</td>
<td>Negative</td>
</tr>
<tr>
<td>2 aborted feti</td>
<td>Received serum samples from 2 infected dams. Plastridge, September, 1961.</td>
<td>Both samples</td>
<td>Positive</td>
</tr>
</tbody>
</table>

As reported in the papers cited and in this report, the gel diffusion test using a heat-stable (HS) antigen gives little indication of cross reactivity with antibodies other than those produced following *V. fetus* infection.

**SUMMARY**

One thousand nine hundred sixty bovine sera from 410 herds were tested for the presence of *V. fetus* precipitins using a heat-stable (HS) soluble antigen derived from two strains of *V. fetus*. The antigen was incorporated in a rapid gel diffusion test for vibriosis (Ristic and Murty, 1961). The antigen is stable indefinitely when stored at -45 F. It may be stored for two months in a refrigerator (32 to 40 F).

The period during which blood serum samples should be collected for the rapid gel diffusion test for vibriosis was not determined. However, it is suggested that samples be collected immediately following abortion and about 60 days thereafter. Where there is infertility only, blood samples should be drawn at several two-week intervals.

Preliminary results of rapid gel diffusion tests for vibriosis conducted on sera collected under farm conditions suggest freedom from false positive reactions and confirm the ability of the test to detect vibriosis in individual dams in naturally infected herds. Additional sera from naturally infected cows must be examined before the relative sensitivity of this test can be determined.
REFERENCES

The title of this presentation should have been "An Evaluation and a Correct Diagnosis of the Problems Confronting the Mutual Insurance Associations and the Veterinary Profession in regard to Lightning Losses to Livestock." In Iowa the livestock insurance companies have clearly indicated that the payment of claims of livestock losses, ostensibly caused by lightning, is increasing each year to the point that it is becoming a serious financial problem for them. Complacency toward the so-called losses of livestock caused by lightning is a luxury that the insurance companies cannot afford to continue in the future. The payment of legitimate claims is a responsibility that every insurance company eagerly accepts and desires to do. These livestock mutual insurance associations are aware that they need competent diagnostic service to determine legitimate claims and have turned to the veterinary profession that is qualified to help them. Are we willing to accept the challenge?

Diagnosis and prevention of diseases in animals have always been primary objectives of our profession. Therefore, it is apparent that we must accept our professional responsibilities of rendering diagnostic services to these livestock insurance companies.

Before we can effectively help these insurance companies we must have a clear concept of their problems and a knowledge of the type of service that they expect from the veterinary profession. They believe that we should definitely state that the animal was or was not killed by lightning. Some personnel of these insurance associations do not understand the necessary procedures and the factors involved in making a diagnosis. Many accusations are hurled at the veterinary profession stating the lack of courage and inability to make a specific diagnosis as to the cause of death of an animal.

From the above remarks it is apparent that the above parties must frankly discuss all aspects of the above situation. Each must have a mutual understanding, concern and respect for these mutual problems and a desire to formulate the plans necessary for proper evaluation of animal losses caused by lightning.

Through the years the veterinary profession has been confronted with calls to determine whether or not lightning was the cause of livestock losses. It is hard to realize the paucity of information in standard veterinary literature concerning the effects of lightning on livestock.

It was interesting to note in reviewing the available veterinary literature in the United States for the past 50 years that very little new specific information has been added to the subject concerning symptoms and lesions produced by the effects of lightning in our domesticated animals. It is known that lesions will vary from animal to animal and most certainly are not very specific for lightning. Information regarding this subject is
sparse and extremely fragmentary. It is obvious that scientific research in this area has been very meager. Very few records of complete post mortem examinations with detailed histological findings of animals killed by lightning are available for study.

The procedure used in evaluating reported cases of lightning by nearly all insurance adjusters and unfortunately by some veterinarians is comprised of some degree of superficial examination of the carcass and the consideration of circumstantial evidence. In examining the carcass the presence of any one of the following conditions may be enough to warrant a positive diagnosis by some people. Singed or burned hair (occasionally this is produced by matches, soldering iron, or a blow torch), dilated pupils, relaxed anus, presence of froth from the nostrils, absence of froth from the nostrils, leathery patches of skin, clean feet, grass in the mouth, lying with the back down hill and the flow of dark blood from incisions made through the skin of the fetlocks. That lightning can kill an animal and not leave any external marks is recognized by most of these people. In instances where no lesions are found the investigator considers circumstantial evidence such as: the recent occurrence of an electrical storm, the fact that the animal died near a tree, fence or building that may or may not show signs of being hit by lightning, that it did not die near a building or a fence, that there was no sign of struggling, or that no evidence of struggling could be found, and if any one of these conditions prevail some feel justified in ascribing the cause of death to lightning.

At the Midwest Midyear Meeting of the National Association of Mutual Insurance Companies on May 18, 1961 in Des Moines, Iowa, "The Responsibility in Lightning Losses—Whose? What? When?" was discussed. This made me very cognizant of the fact that the Insurance Associations and the general public are not properly informed about the methods, procedures and details involved in arriving at a correct diagnosis.

The insurance companies were asked the following questions concerning the recognition of problems now facing them in regard to losses reported as caused by lightning:

1. Have you insurance adjusters listed the specific causes for the present predicament in which you find yourself in regard to so called "lightning losses?"
2. Since World War II, have the insurance companies that sell livestock insurance worked hard to increase their business?
3. Have these insurance companies been quite liberal in paying claims in order to promote more sales?
4. Is it possible that this liberal attitude of the insurance companies has encouraged clients to present some fraudulent claims?
5. Is there a growing tendency for all of us to attempt to collect on our insurance on the slightest pretense? Have you heard people say this is the reason why we have insurance?
6. Why have the automobile insurance premiums increased so much in the last 15 years?
7. Have you any idea how many unjust claims that you are paying each year?
8. Have you made any attempts scientifically to evaluate the exact causes of death in animals supposedly killed by lightning?
9. Can you increase your insurance premiums for livestock insurance and continue to sell the same amount of insurance.

10. Can you from the standpoint of business radically change or rewrite your insurance policies now with specific stipulations in reporting, examining, and evaluating an animal supposedly killed by lightning?

11. Isn't it in reality true that you are actually paying most claims that are presented to you primarily because the "Proof is the responsibility of the insured?"

12. Isn't it true that the livestock owner is always going to get the benefit of the doubt when there is a question in regard to the specific cause of death?

13. In evaluating the present problem it would appear that the insurance company exerts no specific initiative in attempting to determine the specific cause of death and are completely at the mercy of the insured. Am I incorrect in regard to this matter?

14. Would it be worthwhile for all livestock insurance companies to publish an authentic treatise on lightning covering the various aspects as significance of meteorologist's report, history of the health of the animal, environmental circumstances surrounding the case, autopsy procedure, and the gross and microscopic lesions?

15. Has the insurance company asked itself any of these questions?

SUGGESTIONS

1. The insurance companies should have a specific form with a logical sequence of questions to be answered by the owner and the veterinarian in regard to a claim submitted for lightning loss.

2. The insurance company should establish a more realistic time limit for the reporting of the lightning losses. Unfortunately in many states the insurance companies have no control over the time limitations as it is determined by state laws.

3. The insurance adjuster should get acquainted with veterinarians in his territory and discuss mutual problems with them.

4. Insurance adjusters and veterinarians should make an extended effort to be present at the time on the premise and evaluate the claim together. Taking of photographs should be encouraged.

5. Insurance companies should have a complete meteorologist's weather report for the insurance adjuster and veterinarian before one goes to the premise.

6. The insurance company must insist on animals not being moved until the insurance adjuster and veterinarian have the opportunity to evaluate environmental circumstances and apparent evidence of external gross lesions.

7. A detailed history should be obtained.

8. It is emphasized that every suspected case of lightning should be autopsied before the insurance company will pay any claim. Furthermore, veterinarians should be encouraged to submit specimens to Veterinary Diagnostic Laboratories if necessary for
bacteriological and other laboratory findings in order to establish a diagnosis. Who should assume the responsibility of paying for these services? I feel that this is a question that must be resolved by the insurance company.

9. A thorough and complete examination of each carcass should be made in order to accumulate specific information for future action by your insurance company. A careful necropsy on each animal should be performed even if circumstantial evidence is completely conclusive that the animal was killed by lightning. Why should it be done? First, it would be informative for the insurance adjuster, the client, other parties, and the veterinarian to know exactly the history, circumstances, and gross lesions of lightning.

10. It is suggested that probably the insurance companies could well afford to employ veterinarians with special training in pathology to help in evaluating the losses of insured livestock.

11. In closing, the insurance adjuster and the veterinary profession must realize and cause the public to be aware of the health hazard aspects. The animal or animals may have died of some highly infectious diseases as rabies, anthrax and clostridial diseases whose etiologic agents are readily transmissible to man and other livestock. The owners of the livestock may have been exposed. In this instance a correct diagnosis of rabies or anthrax in which there has been human exposure is much more valuable to the farmer than paying for a dead animal.

As a result of the paper presented at the Midwestern Regional Mid-year Meeting of the Farm Conference Sections of the National Association of Mutual Insurance Companies in Des Moines, Iowa on May 18, 1961 the Mutual Insurance Associations asked the Department of Veterinary Pathology to accumulate accurate information and evaluate these facts concerning the losses of livestock caused by lightning and electricity within a radius of some 50-75 miles around Ames. The Mutual Insurance Associations are contributing $65,000.00 for this study for a duration of three years.

During the lightning season and summer we will be conducting field studies of livestock that have been killed or injured by lightning or electricity. Investigative studies will be designed and done during the winter to increase our knowledge of the above research. It is believed that the information derived from the field studies combined with the basic research in the laboratory will help in making a more accurate diagnosis of losses caused by lightning and electricity. Our objectives are summarized in the suggestions that we have made.

In our initial study of seven definitely known cases of lightning we are focusing our attention on those areas in body that may prove to be target tissues and organs revealing rather consistent gross and microscopic changes. Our best leads to date indicate that the skin, subcutaneous tissues, lymph nodes and the heart are the most promising leads for gross changes. We trust that this group will invite us back next year so that we can present our evaluation of the gross and microscopic lesions of lightning.
CRITERIA FOR THE DIAGNOSIS OF VPP AND ATROPHIC RHINITIS

Oliver D. Grace, D.V.M., M.S., James W. Dunn, D.V.M., and George A. Young, D.V.M.
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The positive diagnosis of virus pneumonia of pigs (VPP) or atrophic rhinitis (AR) must be based on an acquaintance with the clinical and pathological changes associated with these diseases. Whittlestone¹ stated "In diagnosis of respiratory infections in pigs, it is relatively easy to recognize the existence of broad pathological and clinical conditions, particularly as a number of respiratory infections often occur simultaneously. Even with laboratory facilities it is not simple to make a diagnosis, because of the paucity of knowledge of some of the common conditions." Our knowledge of all methods of transmission of VPP and the specific etiology and transmission of AR are not complete. The same can be said regarding prophylaxis and treatment of these two diseases.

The Specific Pathogen Free (SPF) swine repopulation program was established to aid in elimination of AR and VPP from the swine population. Both diseases can be spread from infected to susceptible animals by direct contact, inhalation of air from quarters shared by infected animals or entry to quarters immediately after they have been vacated by infected animals. In the SPF program this cycle of transmission is broken by preventing direct contact of infected and susceptible individuals. Pigs are taken from the dam by hysterectomy and are raised to four weeks in the laboratory. Meanwhile, the hogman can clean his premises and equipment so that the new SPF stock will not contact previously infected areas.

The acceptance of the SPF program has resulted in the establishment in Nebraska of an organization composed mainly of farmers who raise breeding stock for swine replacement purposes. This organization has created certain standards for certification as an SPF herd. Both pure-bred and commercial producers cooperate in this program. It is known as the Nebraska SPF Swine Certification Program. Certification under the program permits the member to sell qualifying hogs as SPF breeding stock. The following items are included among others:

A. Requirements for enrollment - Primary or SPF stock
B. Health Requirements
C. Performance Requirements

Vaccination for hog cholera by a veterinarian using modified hog cholera virus vaccine of rabbit origin is recommended. Herds which experience outbreaks of erysipelas, leptospirosis and enteric infections may be certified 30 days after the disease is brought under control providing all these requirements are met. Other infectious diseases such as swine influenza, pseudorabies, listeriosis, clostridial infections, NUD and edema disease are handled in a like manner. The presence of lice or
mange mites is interpreted as a break-down in management or contact with non-SPF swine since no certified SPF pigs among the 20,000 raised in Nebraska have had these present.

Validation under the Brucellosis Free Swine Herd Program is part of the plan. Blood samples collected for Validation of 75 Nebraska herds have also been tested for leptospirosis.

Certification of animals as being free of virus pneumonia and infectious atrophic rhinitis is done by examination of tissues. Extreme cases can be easily diagnosed and the herd denied certification. However, in the Nebraska program herds experiencing severe outbreaks of either disease have been rare. This is not intended to mean that no cases have occurred. To accurately diagnose an early case of either disease requires that an individual making such examination be well trained, exceedingly observant and capable of sound professional judgement. It also requires some standards of measurement for diagnosis of the disease or diseases.

Virus pneumonia of pigs may be suspected clinically by the presence of a dry unproductive cough. If the animals combine this with a slow and inefficient rate of gain virus pneumonia should be given serious consideration. In infected herds virus pneumonia may make its presence known in pigs three to 10 weeks of age. This may develop in seven to 30 days after exposure but some pigs develop pneumonia without obvious signs. The cough may persist for weeks or months. Some animals may make a gradual recovery, some apparently recover completely but may still harbor the infectious agent. Mortality in VPP is usually low but is usually more severe under crowded conditions, when it is newly introduced into a herd, or complicated with secondary bacterial infections.

Macroscopic lesions of VPP are confined to the lungs and immediately associated lymph nodes. The lesions are most commonly confined to the apical and cardiac lobes of the lungs. In most cases they are well demarcated and are plum-colored or greyish. The rest of the lung may be normal in appearance and be collapsed. It has been suggested that localization of the disease in the anterior lobes may be associated with the more vertical bronchi in this area. A combination of visual observation and palpation will be of value in the diagnosing of VPP. The affected lobes have a meaty appearance and firm consistency creating greater resistance to the knife than normal lung tissue.

Recognition by Pattison (1956) of certain histopathologic changes in lungs from infected pigs has provided information which permits more exact diagnosis by use of these procedures. The viral agent of this disease causes extensive lymphoid hyperplasia in the lung. This lymphoid hyperplasia is primarily peribronchial, peribronchiolar and perivascular in distribution. Use of this lymphocytic infiltration will aid in differentiating VPP from other pneumonias of swine.

Failure of pigs to develop measurable antibody titer to VPP eliminates the use of serological means as a method of confirming the presence of VPP. However, serum neutralization can be of value in determining whether antibodies to swine influenza are present and by use of this technique one can receive help in differentiating lesions of influenza and VPP. Failure to develop antibodies to VPP also rules out the possibility of immunizing animals for prevention of the disease.
Diagnosis of atrophic rhinitis is based primarily on examination of the nasal passages by cutting the snouts in cross-section at about the level of the first premolar teeth. Switzer stated that at least three different organisms *Pasteurella multocida*, *Hemophilus suis* and *Bordetella bronchisepticus* are capable of causing inflammation and turbinate atrophy. There are probably other organisms capable of producing similar changes in the structures of the nasal passages. Careful examination of the sectioned snouts and comparison with normal is essential in making a diagnosis of AR. If one animal in the sample being examined is found to have some change which might be considered atrophy, closer examination of other subjects is of course indicated.

Certification of herds under the Nebraska SPF program is based on a regular check at the packing house on each group of pigs farrowed. This examination is the last step before certification of the herd and from the disease standpoint is probably the most important step. It is made after the pigs reach an age of 140 days and when pigs reach market weights. The animals examined are barrows and cull gilts the breeder would be selling at that time.

The accompanying graph (Figure 1) shows the number of pigs which must be examined to give a 99 percent probability of detecting the disease. If 40 percent of the pigs in the herd have VPP, a sample of seven would be required from a herd of 20 pigs, a sample of eight from a herd of 40 pigs, and in herds of over 40 a sample of nine would be necessary. As a general rule, we ask for a minimum of 10 animals for these examinations. When the examination is not conclusive, another sample is requested.

Hogs are tattooed when they arrive at the yards to positively identify each producer's animals. After the animals are "put on the rail" the SPF animals are located by these tattoo numbers and each pig in turn is given

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Theoretic curve showing the sample size required to detect a virus pig pneumonia-infection in a specific pathogen-free swine herd at a 99 percent probability level.
an individual number. At this time a mark is made on the hard palate with an indelible pencil for future identification as a test pig. A numbered plastic coated tag corresponding to the leg number is placed about one inch deep in the nostril to identify the individual animal. These identities are established as the pigs leave the polisher (dehaiser). Gross inspection of the lungs for evidence of VPP is made on the viscera trays of the packing plant. If VPP lesions are found or if any other abnormality is observed, a sample of tissue is collected for histopathologic examination. The livers are also checked for scars made by migration of ascaris larvae. The results of these inspections are recorded.

While the carcass moves down the processing line, the skin, meat and mandible have been removed from the head. Plant personnel locate the SPF heads by the mark on the hard palate and place them in a gondola for detailed examination. The snout is then cut transversely with a meat saw to expose the structure of the nasal cavity.

Examinations made in the packing plant for VPP and AR have some difficulties which are not present in routine postmortem examinations. Certain conditions are observed which would not be encountered under routine methods. Blood aspirated into the lung or scalding water which finds its way to the lungs are not uncommon in such lungs. The constant movement of the carcass does not permit a great deal of time to ponder each animals case.

Histologic examination of the lung tissue discloses many conditions. Included are parasitic pneumonia, bacterial pneumonia, blood and/or water, simple atelectasis, a relatively small percent of lesions suggestive of VPP and lungs that are normal.

From 1958 through the fall of 1961, 193 farrowings out of 198 passed the VPP examination or 97.5 percent. During the same period 195 farrowings out of 198 passed the AR examination, or 98.5 percent. On a herd basis those that passed both VPP and AR at the market examination were 92 percent of the herds enrolled in the program.

SUMMARY

Diagnosis of virus pneumonia of pigs (VPP) and atrophic rhinitis (AR) must be based on a knowledge of the pathology of these diseases as well as the pathology of other respiratory diseases. Criteria for such a diagnosis of VPP includes lesions on the anterior lobes of the lung, the plumb color and meaty consistency, the gross examination and the observation of peribronchial, peribroncholar and perivascular lymphoid hyperplasia by histopathologic techniques. AR can only be diagnosed by determination of gross changes in the turbinates and septum of the nasal cavity.

REFERENCES

THE ETIOLOGY AND DIAGNOSIS OF ALEUTIAN DISEASE IN MINK

James D. Russell, Jack M. Bennett and Edward W. Marty

Madison, Wisconsin*

Aleutian disease is a widespread chronic debilitating disease of mink characterized by loss of weight, polydipsia, tarry droppings, and hypergammaglobulinemia progressing to eventual death of affected animals. The disease insidiously causes a marked reduction in the number of viable kits produced and is often responsible for early mortality in kits from affected dams. The syndrome was first described by Hartsough and Gorham\(^1\) as a malady affecting mink double recessive for the Aleutian gene. Until recently, it was believed that the disease was an hereditary condition since it was seen primarily in mink which carried the Aleutian gene. The disease is manifested as an autoimmune response and is similar histopathologically to certain diseases of man, such as periarteritis nodosa, lupus erythematosus and other associated conditions.

The gross and microscopic lesions of Aleutian diseases have been described in detail by Hartsough and Gorham\(^1\) and by Helmboldt and Jungherr.\(^2\) A similar disease has been described in Sweden by Obel.\(^3\) Characteristic gross lesions of Aleutian disease are pale, yellow, mottled and enlarged kidneys which may become pitted and shrunken late in the course of the disease; spleen enlarged two to four times normal size; mottled, slightly enlarged liver occasionally occurring with fatty infiltration. Hemorrhage from the mouth and gums are sometimes seen as a terminal manifestation of the disease. Microscopic lesions which are pathognomonic are marked plasma cell infiltration of the periportal areas of the liver with bile duct proliferation, plasma cell and lymphocyte infiltration of the renal cortex, hyalinized glomeruli, tubular casts and sclerosis. The kidney changes are primarily those of an early acute interstitial nephritis with chronic nephritis late in the course of the disease. The microscopic lesions represent those of a chronic inflammatory response since necrosis and fibrosis are always absent. Plasma cell and lymphocyte infiltration also occurs in the brain, heart, lungs and uterus. The disease must be differentiated from distemper, mycobacteriosis, and lipidosis. Although the disease resembles leptospirosis in other animals, the latter is extremely rare in mink. A large percentage of animals with AD will develop a terminal bacterial pneumonia which can at times complicate the diagnostic picture unless histopathology is conducted. Death is usually attributable to the nephritis which is present and the resulting uremia. Henson \(et\ al.\)\(^4\) described the hypergammaglobulinemia which is associated with the disease and Henson \(et\ al.\)\(^5\) reported the development of a rapid iodine agglutination serological test for the detection of affected mink.

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Concurrent with the completion of data for this report, Karstad and Pridham and Trautwein and Helmboldt reported on the experimental reproduction of the disease in both Aleutian and non-Aleutian type mink with cell-free filtrates and tissue suspensions prepared from affected mink. This paper describes experimental results which further demonstrates the infectious viral etiology of Aleutian disease. Field observations on the extent and spread of the disease obtained through use of the iodine agglutination test are also reported.

**Studies on Infectious Etiology**

One hundred and forth-five mink obtained from an Aleutian disease free ranch were used in the initial study. Seventy-two of these mink were homozygous recessive (aa) for the Aleutian gene and the remaining 73 were homozygous dominant (AA) for the Aleutian gene. The latter are known by mink ranchers as non-Aleutian type mink whereas the former, which are double recessive for the Aleutian gene, are called Aleutian mink or Aleutian carriers. The mink were divided into seven separate groups, each of which contained Aleutian and non-Aleutian mink, and the groups were separately inoculated with the following:

- **Group A**: One ml inoculated intraperitoneally; sterile saline solution.
- **Group B**: One ml inoculated intraperitoneally; 15 percent tissue suspension consisting of tissues taken from typically affected Aleutian diseased mink.
- **Group C**: One ml inoculated intraperitoneally; Berkefeld N bacteriologically sterile filtrate from the above (B).
- **Group D**: One ml inoculated intraperitoneally; Berkefeld N filtrate from the above (B) treated for 24 hours at 37°C with 0.2 percent betapropiolactone (97 percent - Wilmot Castle Company, Rochester, New York).
- **Group E**: One ml inoculated subcutaneously; 15 percent tissue suspension, (B) above, treated for 24 hours at 37°C with 0.5 percent commercial formalin.
- **Group F**: One ml inoculated subcutaneously; 15 percent tissue suspension, (B) above, treated for 24 hours at 37°C with one percent commercial formalin.
- **Group G**: One ml inoculated intraperitoneally; 15 percent tissue suspension as in (B) above. Mink in this group were also given two mg of prednisone per mink daily in the feed beginning with the day of inoculation and continuing until all animals in the group were dead.

All inocula were checked for the absence of bacterial and mycotic contamination in thioglycollate broth, tryptose agar slants, and Sabouraud’s agar prior to inoculation into mink. Each preparation was also checked for general toxicity and safety by the inoculation of 0.2 ml intraperitoneally into five mice. In all instances bacteriological and toxicological tests were negative.

Mink were observed for four months. All animals which died were necropsied and sections of liver, spleen, and kidney fixed in 10 percent
TABLE I
The Artificial Transmission of Aleutian Disease to Aleutian and Non-Aleutian Type Mink

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inoculum</th>
<th>Summary of Results*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aleutian (aa)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Affected/inoculated)</td>
</tr>
<tr>
<td>A</td>
<td>Controls</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>(1 ml Sterile Saline Inoc. Intraperitoneally)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Raw Affected Tissue Suspension</td>
<td>12/12</td>
</tr>
<tr>
<td></td>
<td>(1 ml Intraperitoneal Inoc.)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Berkefeld N Filtrate</td>
<td>7/10</td>
</tr>
<tr>
<td></td>
<td>(1 ml Intraperitoneal Inoc.)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Berkefeld N Filtrate Treated with 0.2% Betapropiolactone</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>(1 ml Intraperitoneal Inoc.)</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Formalized Tissue Suspension 0.5% formalin Treated</td>
<td>6/10</td>
</tr>
<tr>
<td></td>
<td>(1 ml Subcutaneous Inoc.)</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Formalized Tissue Suspension 1.0% Formalin treated</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>(1 ml Subcutaneous Inoc.)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Raw Affected Tissue Suspension 2 mg Prednisone/mink/day</td>
<td>9/10</td>
</tr>
</tbody>
</table>

*Based on histopathological lesions of Aleutian disease.
**Three mink died from heat exhaustion early in the experiment and were not included in the final count.

formalin or absolute denatured alcohol,* imbedded in paraffin, sectioned, and stained with either hematoxylin-eosin or methyl-green-pyronin stain for plasma cells. All sections were examined for the presence of typical histopathological lesions of Aleutian disease. All animals remaining four months after inoculation were sacrificed and examined as described above for gross and histopathological lesions characteristic of Aleutian disease. Results are given in Table I.

Aleutian disease was artificially transmitted to both Aleutian and non-Aleutian type mink with a single intraperitoneal inoculation of a 15 percent tissue suspension prepared from Aleutian diseased mink. The typical syndrome also developed in 70 percent of both Aleutian and non-Aleutian mink inoculated with a Berkefeld N filtrate prepared from the same affected tissue suspension. Only 10 percent of the mink inoculated with the betapropiolactone-treated ultrafiltrate showed lesions of Aleutian disease.

*Solox, U. S. Industrial Chemicals Company.
In this instance, both of these mink were of the non-Aleutian type. Of the mink inoculated with the formalin treated tissue suspensions, 45 percent of those receiving the 0.5 percent formalin treated material developed the disease whereas only 10 percent of those receiving the one percent formalin treated material showed typical signs. Mink given prednisone in the feed developed Aleutian disease much sooner and with greater severity than those mink which received affected tissue suspension only. Control mink remained normal throughout.

These results demonstrate quite conclusively the infectious nature of Aleutian disease. Contrary to the formerly-believed hereditary theory of the disease, it has now been shown that non-Aleutian type mink can be as easily infected as can the Aleutian type. The data presented also strongly suggest that the agent causing Aleutian disease is smaller than ordinary bacteria and is probably viral in nature. The agent is apparently quite resistant to the action of classical inactivating agents; however, formalin and betapropiolactone markedly reduce its infective ability. In this experiment, prednisone (a corticosteroid) definitely lowered the animals resistance to the disease. All of the animals within the group receiving prednisone succumbed to Aleutian disease within six weeks following challenge whereas only six of the animals which received only infective material died of Aleutian disease over the entire four month post-challenge period. Since it is known that in certain instances corticosteroids may reduce an animals natural ability to resist infection, this data further supports the hypothesis that Aleutian disease is caused by an infectious agent.

**Rapid Passage Studies**

An Aleutian disease virus challenge pool was prepared from tissues taken from typically affected mink obtained from several separate naturally occurring outbreaks of Aleutian disease. A finely ground, 40 percent tissue suspension containing 200 units of penicillin and 10 mg of dihydrostreptomycin per ml was prepared.

Five fawn male mink (non-Aleutian type) negative to the iodine agglutination test were used in this experiment. One mink was inoculated intraperitoneally with five ml of the pooled AD challenge suspension. Fourteen days following inoculation, selected internal organs were removed and a 40 percent tissue suspension prepared as described previously. A second mink was inoculated intraperitoneally with two ml of the freshly prepared infective tissue suspension from mink #1. The second mink was sacrificed after seven days and a tissue suspension prepared in the manner described above. This procedure was repeated until all five mink had been inoculated. The fifth mink was negative to the IAT reaction when tested at seven and 14 days following inoculation, and was found dead in his cage on the 15 day postinoculation.

The virulence of the AD virus was apparently increased with each succeeding passage as evidenced by gross pathologic changes characteristic of AD. The first passage mink had pronounced lesions of AD 14 days following a massive intraperitoneal inoculation. By the third and fourth passage, the spleens were increased three to four times over normal
size, and kidneys appeared acutely affected. The latter were enlarged, soft in consistency with petechiations, whitish foci, and pitted roughened surfaces. Livers appeared mottled, but not otherwise noticeably affected at this level.

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The mink inoculated with fourth passage material died from acute AD within 15 days following challenge. This was unusual since death cannot consistently be produced within a short time after challenge even with high titer AD virus. The increased severity of AD with each succeeding passage may be due to an increase in virus titer.

Fawn mink which do not have the recessive Aleutian gene are very susceptible to Aleutian disease and can be readily infected with an intraperitoneal inoculation of AD-virus-containing tissue suspension. All five mink used for rapid passage studies developed gross lesions of Aleutian disease, but remained negative to the IAT test.

Titration of AD-Virus-Containing Tissue Suspensions

Fifty pearl (non-Aleutian type) male mink free of Aleutian disease as tested by the IAT test were used to titrate a second passage AD challenge pool. Ten ml of second passage AD virus (40 percent tissue suspension—wt/vol) were diluted with 30 ml of half strength tryptose broth containing antibiotics to give 40 ml of a 10 percent tissue suspension. This represented the $10^{-1}$ dilution. Serial ten-fold dilutions through $10^{-5}$ were then prepared in tryptose broth. The dilutions were then inoculated separately into groups of 10 mink each using one ml intraperitoneal injections.

Four weeks following inoculation all mink were tested by the IAT method, sacrificed, and examined for gross lesions of AD. Results are given in Table II.

<table>
<thead>
<tr>
<th>Virus Dilution (Per gram tissue)</th>
<th>IAT Reaction (affected/inoculated)</th>
<th>Gross AD Lesions (affected/inoculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-1}$</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>6/10</td>
<td>5/10</td>
</tr>
</tbody>
</table>
Although there appears to be excellent correlation between the IAT reaction and pathology, this was true only in those dilutions in which all animals were grossly infected; i.e. through the $10^{-4}$ dilution. Where lesser amounts of the virus were present for initial infection, there were discrepancies not evident in Table II. Two mink showing gross lesions of AD showed negative IAT reactions and three showing positive IAT reactions were negative on gross exam. Since the IAT test is non-specific, it cannot serve as a precise technical method of evaluating AD infection. This can only be done pathologically, and hence end points were determined accordingly.

The endpoint of the virus titer was not reached at the $10^{-5}$ dilution; however, as assessed by gross pathology the titer was approximately $10^{5.0} \text{ID}_{50}/\text{gram}$ of tissue. It has not been determined whether or not the type of mink used would have an affect on the titer. Mink of all genetic types, including darks and half-breds, have been infected with the second passage AD virus challenge pool. However, it has taken the darks longer to develop visible AD lesions and the lesions are seldom as severe as those seen in mutation mink. On the other hand, a positive IAT reaction occurs at approximately the same time following challenge of the darks as in mutation mink. This suggests a difference in secondary resistance, but not in primary susceptibility which may create unrecognized carrier animals.

Eighteen pastel (non-Aleutian) mink were used for titration of a third passage tissue suspension. All titrations were conducted on a per gram tissue basis; i.e. $10^{-1}$ dilution equals a 10 percent tissue suspension, $10^{-2}$ equals a one percent tissue suspension, etc. Serial ten-fold dilutions through $10^{-7}$ were made in half strength tryptose broth containing antibiotics and the prepared dilutions were titrated in groups of three mink each using a one ml intraperitoneal inoculation and separate inoculating needles for each animal. Mink were separated by at least one empty pen and there was no direct contact from one mink to another. These mink were blood tested at four and six weeks postinoculation, sacrificed at six weeks and examined for gross and histopathological lesions of AD. The splenic index which is the weight of spleen divided by the body weight times 100 was calculated for each mink. The splenic index demonstrates the increase in size of this organ in AD infected mink over normal animals. Results are given in Table III.

The AD virus titer of the third passage pool was $\geq 10^{7.5} \text{ID}_{50}/\text{gram}$ of tissue as evaluated six weeks following inoculation. A distinct endpoint was again not obtained although two of three mink inoculated with $10^{-7}$ dilution did not develop a positive IAT reaction until two weeks after mink given the lower dilutions. The results were evaluated primarily on the basis of gross and histopathologic lesions of AD. It is unlikely that all three mink at the $10^{-7}$ dilution would have developed the disease simultaneously from an incidental exposure to the virus. The data further suggest that as the virus titer decreases, it takes longer for the disease to develop. This observation appears even more valid since originally we could not produce the disease in less than two months without concurrent use of a corticosteroid.
<table>
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<tr>
<th>Mink Number</th>
<th>Virus Dilution (Per Gram Basis)</th>
<th>Initial</th>
<th>4 Week PI*</th>
<th>6 Week PI</th>
<th>Gross Microscopic (** - explanation)</th>
<th>Spleen/Body Weight X 100 (Normal range is 0.35 to 0.55)</th>
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</table>

*Post Inoculation.
**Capillary Tube containing serum broken in centrifuge.
***Degree of observed AD lesions of spleen, kidney, liver, lymph node and thymus; numbers indicate degree of microscopic lesions found in kidney tissue slides; 1 = minor round cell and plasma cell infiltration; 2 = moderate round cell and plasma cell infiltration; and tubular casts; 3 = extensive infiltration and tubular casts.
The iodine Agglutination Test

The iodine agglutination test (IAT) has been used by us to test approximately 30,000 mink in the field. The test can be a valuable diagnostic tool in the detection of affected mink before the animal demonstrates visible signs of illness. The test as described by Henson et al. is about 95 percent accurate in detecting affected mink. However, it is a non-specific test and other conditions which lead to an increased gamma globulin or lowered albumin will give false positive reactions. A positive reaction depends upon an approximate one to one or greater serum globulin:albumin ratio. As the gammaglobulin level approaches 22 percent of the total serum protein, agglutination of serum protein occurs when combined with the reagent. The higher the level of gamma globulin, the more pronounced the agglutination becomes. A positive IAT reaction does not normally occur until about three to five weeks following virus infection and therefore the IAT test cannot be relied upon to detect early infection in mink. This observation also suggests that the hypergammaglobulinemia which develops as disease advances is not a reaction of the body against the virus per se. but rather, it is a reaction stimulated by remodeled, virus-infected cells or by cellular debris, thus appearing as an auto-immune phenomenon. Gross and microscopic pathology appear to be the only reliable final criterion for determining whether an animal is affected with AD.

The IAT test is conducted by obtaining a drop of blood from a clipped toenail in a plain non-heparinized capillary tube. The blood is allowed to clot and centrifuged for three to five minutes in a microhematocrit centrifuge. The capillary tube is broken at the serum-clot interphase and the serum tapped onto a clean glass plate. A drop of iodine reagent (one part iodine crystals, two parts potassium iodide dissolved in 20 parts distilled water) is added and mixed with the serum. The reaction is read immediately. Negative samples remain absolutely clear and transparent; positive samples become cloudy, are more viscous and contain clumped, precipitated, granular material. The reaction is rated as one, two, three, or four plus, depending upon the size of the agglutinated particles and the rapidity with which the reaction occurs.

It is important that mink be tested before rather than immediately after feeding since large amounts of circulating fat in the serum may cause cloudy reaction and interfere with the reading of the test. Furthermore, it is important that nonhemolyzed samples be used since this will also lead to false positive reactions. We have found that the iodine solution is rather unstable, especially at higher temperatures, and it cannot be relied upon to give accurate results after two to three weeks even when kept at refrigeration temperatures in amber colored bottles. Solutions held at room temperature or higher have given false positive reactions within 48 hours after preparation. This apparently results from the precipitation of iodine crystals. The reagent should not be kept in bottles with metal caps. Known positive and negative serums should be used to test the reagent before use.
The TAT test has been used to survey the incidence of Aleutian disease on 15 mink ranches in the midwest. Only one of these ranches showed a complete absence of positive reactors and from this survey it is evident that Aleutian disease is much more widely distributed than was formerly thought (Table IV). The results of this survey also show that AD occurs in all types of mink regardless of color, genetic types, or age (Table V). Mink double recessive for the Aleutian gene are more
susceptible and have a higher mortality rate than non-Aleutian mink. The more resistant color types probably serve as inapparent reservoirs of infection as indicated by the large percent of reactors found in the dark color phase.

The ranch incidence usually approaches 20-25 percent before the rancher is cognizant of the problem. More important than direct mortalities is the interference with reproduction and the early kit losses that occur with this disease, thus presenting serious economic hazard. Studies are continuing to determine the means of spread and routes of infection which occur under ranch conditions. Stringent testing, sanitation, and quarantine procedures should be used to limit the spread of the disease on infected premises and throughout the industry by infected breeding stock. The use of vaccines is now under study.

**SUMMARY**

1. Data has been presented to demonstrate the infectious etiology of Aleutian disease.
2. The IAT test can be used as a valuable field diagnostic tool to detect infected animals in apparently normal herds; however, since the test is nonspecific, the final criterion for diagnosis must be based on gross and microscopic pathology.
3. The disease does not present a difficult diagnostic problem since the gross lesions present are very characteristic and they are not easily confused with other conditions of mink.
4. A survey using the IAT test on 15 mink ranches in the midwest has indicated a higher incidence of this disease than was formerly recognized. In the field, the disease has been observed in virtually all color types and phases of mink.

**REFERENCES**

FREQUENTLY ENCOUNTERED DISEASES AFFECTING THE CENTRAL NERVOUS SYSTEM WITH SOME USEFUL TECHNIQUES FOR DIAGNOSIS

L. W. Turner, D.V.M., M.S., and G. H. Barney, D.V.M., M.S.*

Nashville, Tennessee

In routine diagnostic work, it is common to observe numerous cases where one or more of the animals submitted reveal symptoms of central nervous system disturbances. The diagnostician is confronted with the problem of deciding if the symptoms are caused by a specific disease entity or are secondary to some other systemic disease. To make the correct choice of tissues for laboratory examination, considerable physical effort and time is required to remove the brain from the cranial cavity of adult bovine, equine, ovine and porcine, preserve the entire brain in 10 percent formalin and later select the desired sections for histopathologic examination. This procedure, which delays the final diagnosis, requires approximately ten days to two weeks. Therefore, many times sections of the central nervous system are not saved along with other tissues because of this inconvenience.

The diagnosis of a disease should be made with accuracy in the shortest period of time by finding lesions that are pathognomonic. The lesions described below are considered by the writer to be diagnostic if found with a history suggesting the disease. One cannot over-emphasize a study of the history since the selection of tissues will depend upon this information.

It is the purpose of this paper to describe the lesions found and techniques used in the Tennessee Animal Disease Diagnostic Laboratory to diagnose seven disease entities.

Collecting and Processing Tissues

After removing the head from the carcass, the skull is opened longitudinally so that the brain is divided equally. One hemisphere is removed. Using a brain knife, desired portions are selected. The sections of the brain are fixed in 10 percent formalin and placed in the autotechnicon with the other tissues collected that day. The processing of the tissues through mounting and staining requires two days. Since routine culturing is performed on every case, no effort is made to shorten this time. Three days is the average time required for bacteriology. Hematoxylin and Eosin stain is used routinely and extra tissue sections are saved, if needed, for differential staining. The sections are cut at a thickness of seven microns.

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Salt Poisoning in Swine

A history of convulsions in all affected animals will suggest salt poisoning (sodium salt poisoning). The unavailability of drinking water for the animals should be determined because this factor is a common cause of the disease. If live animals are presented, the diagnostician should attempt to excite one or more animals. The typical symptoms of salt poisoning will be observed in the form of epileptiform seizures. The pathognomonic lesions for diagnosis are found in the cerebral cortex. These consist of meningoencephalitis characterized by edema and infiltration of eosinophiles into the meninges and around the blood vessels. Hematoxylin and Eosin stain is ideal for demonstrating these lesions.

Bacterial Encephalitis (Streptococcosis)

In baby pigs convulsions may be due to microabscesses in the brain caused by suppurative bacteria. Streptococcus infection is one of the most common causes. Portions of brain tissue should be collected from several sites when there is a history of convulsions and post mortem lesions of a septicemia in baby pigs. In this condition, no specific portions of the brain are affected. Examination of Hematoxylin and Eosin sections will reveal minute abscesses composed of foci of polymorphonuclear neutrophiles. A meningitis may also be present. One or more slides that show typical lesions should be stained with Brown and Bren stain for bacteria in tissue. In these slides coccoid forms in single, paired and chained forms will be seen within the areas of neutrophile infiltration. Some of the organisms will be found within phagocytes. These findings, along with a history of an encephalitis, are sufficient proof of a bacterial encephalitis caused by streptococcus organisms.

Hog Cholera

The history and gross lesions will in many cases be sufficient to make a tentative diagnosis of hog cholera. A leukopenia is also significant. A few pigs may exhibit encephalitis symptoms in the form of convulsions and paralysis. When none of the above conditions are pronounced, brain sections from the midbrain and brain stem will supply the needed information for a positive diagnosis. Perivascular cuffing with mononuclear cells and areas of gliosis will be found using Hematoxylin and Eosin stain. When any gross lesions of hog cholera are present and perivascular cuffing is found, a positive diagnosis is in order.

Listeriosis

In the spring months listeriosis is encountered. Although it is often associated with sheep, the writer has seen several cases in cattle. Examination of the history shows that the animal walks aimlessly, and may or may not circle. An affected animal will frequently push against objects. The animal lives approximately five to seven days and becomes ataxic before death. Usually only sporatic cases are seen, yet several animals in one herd are known to have succumbed to this disease.
Sections are removed from the brain stem and spinal cord. The remainder of the brain can be refrigerated if culturing is desired. When listeriosis is suspected, Hematoxylin and Eosin stained tissue sections should be examined. Microabscesses are found throughout the tissue and are composed of clumps of neutrophiles. Another important lesion is extensive perivascular cuffing with neutrophiles and mononuclear cells. If these lesions are found, Brown and Bren stain is used on several slides that represent the best lesions. In the area of neutrophil clumping, small gram positive rods will be found. A confirmed diagnosis of listeriosis can be made by the demonstration of microscopic lesions and gram positive organisms in tissue sections of the brain stem.

Avian Encephalomyelitis

Symptoms are usually seen in baby chicks under seven weeks of age. They are observed in the form of leg weakness and tremors of the head or entire body. Vibrations caused by the disease can be felt by holding one of the birds loosely in one's head and lightly shaking it. The mortality is usually about 10 percent. Hematoxylin and Eosin staining of slides taken from the cerebellum, brain stem and midbrain will reveal lesions of perivascular cuffing with mononuclear cells and areas of gliosis. In the spinal cord, the motor nerve cells will be undergoing degenerative changes. The most pronounced change will be the loss of the tigroid elements of the cytoplasm. These findings are considered diagnostic in the presence of a history suggesting the disease.

Scrapie

Restlessness, tremors, grinding of the teeth, pruritus, and the loss of wool are some of the early symptoms of scrapie. Later the animal is unable to stand; death soon follows. Sections of the brain should be taken from the medulla and brain stem. The diagnostician will find vacuolization of the cytoplasm of the neurons. The vacuoles may be single or multiple in the single cell. This lesion is considered pathognomonic.

Rabies in Cattle

Some diagnostic laboratories do not perform rabies examinations. Most state health departments are adequately equipped to make this test. Nevertheless, in cases where the clinical symptoms are not typical, specimens will be sent to the laboratory for pathologic examination. In routine examination of nerve tissue Negri bodies may be found. Recently, a suspected case of rabies in our laboratory was diagnosed as listeriosis. The cerebrum was sent to the State Health Department and the rabies examination was negative. Listeriosis was found in the remainder of the brain that was processed in the Diagnostic Laboratory. This practice is recommended because other encephalitic diseases in the bovine will be detected.

The two most important symptoms that rabid cattle exhibit are continuous bellowing and tenesmus. One or both of these symptoms may be observed. With this history and the demonstration of Negri bodies in the Purkinje cells of the cerebellum, a diagnosis of rabies can be made.
Hematoxylin and Eosin stain can be used to demonstrate Negri bodies, however, it is not the best technique. When intracytoplasmic bodies are seen with Hematoxylin and Eosin, one of the more specific stains should be used to confirm this finding. Schleifstein's rapid method for the demonstration of Negri bodies is recommended.6

DISCUSSION

The above descriptions of symptoms are only a summary of the more significant encephalitic diseases encountered in the Diagnostic Laboratory. In many cases the symptoms will be misleading. To make a thorough examination, culturing from animals that represent the typical disease pattern is recommended. Culture material can be taken from the liver, spleen and heart blood. These recommendations are for the elimination of a secondary disease.

SUMMARY

The need for rapid and accurate tests for diseases that affect the central nervous system was shown. Suggestions were given for collection of tissue, methods of processing and recognition of lesions. Important symptoms and diagnostic lesions of seven diseases were described.

A positive diagnosis can be made when the history suggests one of these diseases and the characteristic lesions are present.

REFERENCES

A NEW HEMAGGLUTINATION TEST FOR HOG CHOLERA*

E. I. Pilchard, D.V.M., M.S.** and D. Segre, D.V.M., Ph.D.***

A simple, economical hemagglutination (HA) test for hog cholera virus recently described by Segre (1962) has been used experimentally at the University of Illinois College of Veterinary Medicine since October, 1961. Hog cholera virus was detected in blood and spleen of affected hogs and in tissue cultures. The purpose of this paper is to discuss the optimal conditions for the preparation of the HA reagent and for testing, and to report the results obtained on a number of naturally and experimentally infected swine.

MATERIALS AND METHODS

Hog cholera virus was received from commercial sources, from the National Animal Disease Laboratory, Ames, Iowa and from Dr. D. P. Gustafson, Purdue University.

The HA test reagent consists of an aqueous suspension of formalin-fixed erythrocytes which have been coated with anti-hog cholera antibody by means of diazo linkages (Segre, 1962). All steps of preparation were done in a cold room or in a salt-ice bath in order to maintain temperatures below 7°C.

Erythrocytes were formalinized according to the method of Csiszas (1960) and were stored as a 50 percent suspension in 0.85 percent sodium chloride solution at 4°C. Formalinized rabbit erythrocytes were also received as a 10 percent suspension in 0.85 percent sodium chloride solution.

Phenol-free anti-hog cholera serum was obtained from hogs that had been repeatedly inoculated with hog cholera virus. Rabbits were

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***Professor of Veterinary Research, University of Illinois College of Veterinary Medicine Department of Veterinary Pathology and Hygiene, Urbana, Illinois.


*****Formalinized rabbit erythrocytes were kindly supplied by Mr. A. L. Lane of Difco Laboratories, Detroit, Michigan.

******Hog cholera serum was obtained through the courtesy of Dr. R. M. Scott, Sioux Falls Labs., Sioux Falls, S. D. and from a specific-pathogen-free boar kept at the University of Illinois.
also repeatedly inoculated with virus of swine origin, rabbit origin, or with virus propagated in swine cell tissue culture. Serums were stored at -25 C. Gamma globulin was lyophilized and stored at 4 C.

Bis-diazotized benzidine (BDB) was prepared by the method of Kabat and Mayer (1961). It was placed in glass vials in 10 ml quantities and stored at -45 C.

Spleen extracts and heparinized blood samples were collected from individual swine. In most cases, whole spleen, heparinized blood or tissue culture was stored at -45 C before being tested. Spleen samples were first ground in glass tissue grinders with nine parts of distilled water. The spleen extracts were clarified by centrifugation and the clear supernates were diluted in two-fold steps in 0.5 ml volumes of distilled water contained in 12 by 75 mm tubes. Blood samples were diluted in the same way. In both cases, the lowest dilution tested was 1:20. In the case of tissue cultures, samples were dialyzed 24 hours against three changes of 20 volumes each of distilled water at 4 C before testing. Tissue culture samples were then centrifuged for 10 minutes at 9000 x g at 0 C. Portions of the supernate were tested at 25 C, both undiluted and at dilutions of 1:5 and two-fold higher in 0.5 ml volumes of distilled water. One drop (approximately 0.05 ml) of reagent was then added to each tube. The tubes were shaken and allowed to stand undisturbed for 90 minutes. The pattern of sedimentation of the erythrocytes was then observed with the aid of a mirror placed at a 45° angle under the rack holding the tubes. Agglutination was shown by a uniform layer of cells covering the entire bottom of the tube, or by a wide ring of erythrocytes with serrated margin. Absence of agglutination was shown by a small dense button of erythrocytes or by a small ring with smooth margin.

Attempts were made to define the best conditions for the preparation of the HA reagent, the selection of specimens which most consistently permit a diagnosis of hog cholera, and the physical chemical parameters required for maximum specificity and sensitivity of test reagent. Attempts to increase the sensitivity of HA reagent included the removal of non-globulin portions of the immune serums by ethanol fractionation (Deutsch, 1952) before linking antibody to the formalinized erythrocytes. The reactivity of HA reagent was tested repeatedly during periods of storage at 4 C. Reagent was also lyophilized, sealed under vacuum and stored at 4 C. After various periods of time, lyophilized reagent was resuspended in distilled water at a concentration of two percent and was tested for reactivity.

RESULTS AND DISCUSSION

Erythrocytes from rabbit, sheep, swine, guinea pig, dog, horse, opossum or skunk were used successfully as a constituent of the HA reagent. One batch of bovine erythrocytes and one of chicken erythrocytes was found unsatisfactory. None of the species which have been tried have appreciable advantages in comparison with the rabbit as donor of erythrocytes.
HA reagent prepared with anti-hog cholera gamma globulin was able to detect hog cholera virus in dilutions two-fold greater than the end-point obtained with the reagent prepared as originally described (Segre, 1962). HA reagents prepared with the serums of rabbits repeatedly inoculated with rabbit-adapted virus were not active. Reagents prepared with the serums of rabbits repeatedly inoculated with virus of swine origin or with virus propagated in swine cell tissue culture were agglutinated by normal swine specimens, as would be expected. HA reagents prepared with the same serums following absorption by thrice-washed normal swine erythrocytes were not agglutinated by hog cholera virus.

The temperature during BDB linkage of erythrocytes to antibody was maintained just above the freezing point of the liquid as well as at 7°C. Temperatures above 7°C result in rapid decomposition of the BDB and failure of the diazo linkage to develop. Extension of the time for coupling to as long as three hours resulted in no apparent increase in the sensitivity of the HA reagent. This was expected, since coupling is known to occur rapidly.

Identical titers were obtained by reacting hog cholera virus with the HA reagent for one hour at room temperature and at 37°C. When the test was carried out at 4°C, settling of the HA reagent required more than six hours and normal swine specimens were agglutinated at dilutions of 1:20 and 1:40. The titers of hog cholera specimens tested at 4°C exceeded the titers obtained at room temperature by as much as four-fold. However, upon placing the test tubes at room temperature, the titers rapidly decreased to approximately the level of those obtained in tests carried out entirely at room temperature.

Spontaneous agglutination of HA reagent occurred in solutions of sodium chloride at concentrations of 0.085 percent or higher as well as in 0.02 molar ammonium sulphate solution, 0.02 percent ammonium oxalate, 0.02 percent potassium oxalate, and 0.02 percent sodium citrate. Normal blood at dilutions of 1:20 in distilled water agglutinated the HA reagent incompletely in most cases. The HA test requires the use of distilled water as diluent. Attempts to prevent the agglutination caused by electrolyte solutions by the use of surface active agents were unsuccessful.

The HA reagent retained reactivity upon storage at 4°C for as long as 10 days. Lyophilized HA reagent gave positive tests upon rehydration after 30 days. Not all batches of lyophilized reagent retained reactivity. These tests are continuing.

Based upon the results of tests with specimens from hogs experimentally infected with hog cholera, heparinized blood samples may be collected at any stage of the disease (Table I). Fresh spleen samples should be obtained immediately upon death or at the time of euthanasia of moribund animals. A few urine samples which were collected at necropsy and dialyzed against distilled water gave negative HA tests.

Modified live hog cholera virus of tissue culture origin containing $10^5$ PD50* per ml gave an HA titer of 1:80. Fluid from uninfected tissue

*One PD50 (50 percent protective dose) will protect 50 percent of susceptible swine against challenge 21 days following inoculation.
HEMAGGLUTINATION TEST FOR HOG CHOLERA

TABLE I
Hemagglutination Tests of Blood and Spleen of Pigs Inoculated with Hog Cholera Virus: Total Positive Tests*/Total Swine Tested

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<th>Blood, day following inoculation with virulent hog cholera virus</th>
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<tr>
<td>16 7 7 7 15 16 6 8 6 8 4 5 2 1 0 1 0 1 0 1 1 9</td>
<td></td>
</tr>
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</table>

Percent positive

| 0 0 43 71 80 63 33 50 66 100 100 100 0 0 100 100 100 66       |

*HA test of blood was considered positive for hog cholera when agglutination occurred in dilutions of 1:40 higher. Spleen suspensions were considered positive when agglutination occurred at dilutions of 1:20 or higher.

cultures was negative to the HA test. Hog cholera virus of swine origin of known infectivity titer was not tested. However, titers of 1:40 to 1:80 were obtained with blood or spleen of experimentally infected hogs. Specimens from swine experimentally infected with agents other than hog cholera virus have not been tested. It was found preferable to test fresh heparinized blood samples owing to the development after freezing of a precipitate which interferes with the detection of positive test results at titers of less than 1:80.

More than 400 field specimens obtained at necropsy were tested for hog cholera. HA titers of cases diagnosed as hog cholera ranged from 1:320 to negative. Negative results obtained with specimens from hog cholera cases may be due to low concentration of virus in the tissue tested. A small number of field cases diagnosed as hog cholera and giving negative HA tests may have been misdiagnosed and must be tested by inoculation of susceptible swine.

Both the HA test for hog cholera virus and the pancreatic amylase test proposed by Taylor (1961) for the diagnosis of hog cholera were applied to 45 swine examined at necropsy at the diagnostic laboratory* (Table II). Laboratory diagnoses were based upon history, gross and microscopic lesions, and the results of hematologic and bacteriologic examinations. Diagnoses of hog cholera or of possible hog cholera were made when respectively all or some of the findings were those associated with hog cholera. Cases considered to be possible hog cholera but in which characteristic lesions were absent included acute enteritis-pneumonia, encephalitis, possible enterotoxemia, and acute pneumonia. Considered not to be hog cholera were cases of mycotic enteritis, chronic pneumonia, salmonellosis, atrophic rhinitis, verminous pneumonia, chronic enterocolitis, ascariasis, balantidiasis, otitis media, eperythrozoonosis and edema disease. Of 11 swine with a diagnosis of hog cholera,

*Diagnostic and Research Laboratory, University of Illinois College of Veterinary Medicine operated in cooperation with the Illinois Division of Livestock Industry.
Necropsy laboratory diagnosis | Positive | Negative | Pancreatic amylase test
--- | --- | --- | ---
Hog cholera | 10 | 1 | 7 | 4
Possible hog cholera | 6*** | 11 | 5*** | 12
No hog cholera lesions but possible hog cholera**** | 4***** | 3 | 3***** | 4
Not hog cholera****** | 0 | 10 | 2 | 8

*Segre (1962).
**Taylor (1961).
***Three of the pigs with possible hog cholera reacted to both the HA and the amylase test.
****Includes acute enteritis-pneumonia, encephalitis, possible enterotoxemia, and acute pneumonia.
*****None of the pigs reacted to both the HA test and the amylase test.
******Includes mycotic enteritis, chronic pneumonia, salmonellosis, atrophic rhinitis, verminous pneumonia, chronic enterocolitis, ascariasis, balantidiasis, otitis media, eperythrozoonosis, and edema disease.

10 were positive to the HA test and seven were positive to the amylase test. All swine in this comparative study which were considered not hog cholera were negative to the HA test. Two of these were positive to the amylase test. Of 24 swine diagnosed as possible hog cholera, 10 were positive to the HA test and eight were positive to the amylase test. Specimens from three pigs with a diagnosis of possible hog cholera were positive with both the HA test and the amylase test.

Tests to determine the optimum time during which to collect blood samples from infected swine are summarized in Table I. HA tests were considered positive when hemagglutination occurred in dilutions of heparinized blood of 1:40 or greater and in dilutions of spleen suspension of 1:20 or greater.

Positive HA tests were obtained with blood collected from 48 hours after inoculation up to the time of death, excepting on days 12 and 13 post-exposure. Additional experiments are planned which will include daily testing of all individuals. However, based upon the data shown, the HA test for hog cholera virus should be applied to two or more pigs from a naturally infected group at any time during apparent disease or immediately following death.

SUMMARY

A simple, economical hemagglutination (HA) test for hog cholera (Segre, 1962) has been used experimentally at the University of Illinois College of Veterinary Medicine since October, 1961. Hog cholera virus was detected in blood and spleen of affected hogs and in tissue cultures.
Blood samples should be collected in heparin from apparently diseased swine. Spleen samples should be obtained immediately after death. Two or more apparently diseased swine from a group suspected of having hog cholera should be tested. Work is in progress toward improving the sensitivity and specificity of the HA test and characterizing the reliability of the test for the diagnosis of naturally occurring hog cholera.

REFERENCES

PERSONNEL CLASSIFICATIONS AND APPROPRIATE QUALIFICATIONS FOR VETERINARY MEDICAL LABORATORIES

Virgil B. Robinson, D.V.M., Ph.D.*
Indianapolis, Indiana

Some of the factors relating to the organization and function of a Veterinary Medical Laboratory were discussed at this Conference at its Fourth Annual Meeting.** The presently assigned topic seems a logical sequel, and I am pleased that we can discuss it here today. Some of the general personnel and management principles projected here are much more important than details, such as specific job titles, terminology and the like, but all of these must be considered. In order for a laboratory to excel in service to its constituents, it is essential that the people making up the staff be well qualified for their respective responsibilities, and it is just as important that specific positions are not overstaffed. The duties and responsibilities of each position must be a challenge to the incumbent. It is essential that each position be clearly described and accurately evaluated so that the individual can be properly matched to it. This actually amounts to definitive evaluations of both people and positions.

It seems convenient to begin by naming some of the positions necessary to the proper function of a veterinary medical laboratory. After each position is evaluated, personnel qualifications for the various job classifications can be more intelligently approached. In setting up a complete laboratory, the following positions should be considered:

- Laboratory Director
- Medical Technologist
- Veterinary Pathologist
- Laboratory Technician
- Laboratory Veterinarian
- Secretary
- Microbiologist
- Laboratory Assistant
- Toxicologist
- Animal Technician, and possibly others

Some of the larger laboratories may require all of these classifications and possibly more than one person in each job. The smaller laboratories can probably operate effectively with fewer classifications by combining responsibilities.

PROFESSIONAL STAFF

Laboratory Director

This position requires a veterinarian with several years experience in a veterinary medical laboratory or a similar organization. He should

*From the Department of Pathology, Research Laboratories, Pitman-Moore Company, A Division of the Dow Chemical Company, Indianapolis, Indiana.

have a demonstrated competence in administration and broad technical knowledge in animal diseases. Familiarity with the disciplines represented in his laboratory such as microbiology, pathology, and toxicology, is necessary and he may be a specialist in one of these.

**Veterinary Pathologist**

This position is most adequately filled by a Diplomate of The American College of Veterinary Pathology. The next in order of preference is a veterinarian with several years training and experience in applied veterinary pathology and who is an advanced trainee for certification. In this connection, proper consideration must be given to opportunities for a continuing training program for persons in the latter category. For instance, a properly staffed laboratory may qualify as an approved training institution for veterinary pathologists. A classification of pathologist and senior pathologist might be used for purposes of designating training, experience, and salary scale.

**Laboratory Veterinarian**

This designation should apply to veterinarians active in any of the several disciplines utilized in a veterinary medical laboratory. A classification such as Laboratory Veterinarian I, II, and III or a similar designation may be used to delineate training, experience, and salary scale. Examples of professional personnel conveniently falling in the classification might be some of the following:

1. A veterinarian early in a training program leading to Diplomate, American College of Veterinary Pathology.
2. A veterinarian with special training and experience in microbiology. A veterinarian should not be expected to assume the responsibility of a microbiology section unless he has had advanced training and experience and has developed competence in the discipline.
3. A veterinarian with special training in biological and analytical chemistry, and performing the duties of a clinical toxicologist.
4. A veterinarian with advanced training and experience in parasitology and functioning on the staff as a veterinary parasitologist.

**TECHNICAL STAFF**

**Pathogenic Microbiologist**

It is logical from the standpoint of technical knowledge and function to combine the interrelated sciences of bacteriology, mycology, virology, and serology into a single section of microbiology. The supervisor should have a Master's Degree and some experience or a Bachelor's Degree and several years experience in pathogenic microbiology. Some proficiency in administration will be necessary in a section having staff members. Other staff members may be of the Bachelor's level in microbiology and on-the-job training may be provided in the various subdivisions.
A laboratory veterinarian may serve as supervisor if he has had specialized training and experience, but this is not considered necessary or even desirable in most situations. Scientific talent of the D.V.M. or Ph.D. level will be fully utilized and challenged here only when an active research program is maintained in addition to a general consultation service.

**Clinical Toxicologist**

A full time position of this kind will be necessary only where there is an exceptional demand for service in toxicology. Minimal qualifications should be a Master's Degree in chemistry with specific training in the biochemical and analytical area. Some on-the-job training and experience in clinical toxicology is desirable. A veterinarian or a chemist of the Ph.D. level qualified in toxicology should be employed for this position only when opportunities for an active research program are provided along with the consultation service.

**Veterinary Parasitologist**

The need for this position will depend somewhat on the geographic location of the area served and the objectives of the laboratory. In warm, moist climates where parasites are a serious problem, a full time qualified veterinary parasitologist would probably be desirable. In areas less favorable to parasites and/or in small laboratories the clinical parasitology may be done in the clinical pathology section under the direction of the veterinary pathologist. When a veterinary parasitologist is employed, adequate opportunities for research should be provided.

**Medical Technologist**

Persons trained in medical technology and Certified by the American College of Clinical Pathologists (ASCP) should supervise the Clinical Pathology and Histology sections of the laboratory under the direction of a veterinary pathologist. Laboratory technicians with the Bachelor's Degree in one of the biological sciences may be given on-the-job training for specific services and assist in these areas.

**Laboratory Technician**

The technical personnel of the Bachelor's or Master's level serving in the various sections will fit into this category. Some may be needed in the microbiology, clinical pathology, histology, and toxicology laboratories. A high degree of specialization and proficiency may be attained by these individuals in on-the-job training and they may well form the bulwark of the laboratory force.

**SECRETARIAL STAFF**

In order to function most satisfactorily in this position, secretaries must be endowed with a generous share of inherent capabilities. In addition, their training and experience should qualify them to take case histories, keep medical records, execute simple data processing systems,
use calculators, and do medical correspondence. Good secretarial help can relieve the professional staff of many time-consuming details and play a major role in the operation of an efficient, smooth-running organization.

SEMI-TECHNICAL STAFF

*Laboratory Assistant*

A high school graduate is desirable for this classification. These are people who may be trained to do many of the routine duties of the laboratory that will conserve the time of those with more formal technical training and capabilities. Laboratory assistants may clean and sterilize glassware, use analytical balances, prepare chemical solutions and media, do filtrations, and perform many other similar procedures that are so numerous in veterinary medical laboratories. These people are usually interested in and dedicated to their work and make stable valuable employees.

*Animal Technician*

Persons well trained as animal technicians are essential to the successful operation of a laboratory. They should be endowed with considerable innate ability and be of the high school graduate level. In addition to caring for animals, their routine duties will include making simple clinical observations for behavioral changes, taking temperatures, keeping records, and the like. Simple treatments can be administered orally and parenterally under the supervision of the scientific staff and specimens of blood, urine, and other material may be collected. Animal technicians may be trained to a high degree of skill in routine post mortem dissection and collection of tissues and other specimens under the supervision of the veterinary pathologists. This will conserve much time for the pathologist who may often restrict his activities at the post mortem table to examining exposed organs, dictating notes, and giving instructions for further study of the case.

**DISCUSSION**

It was mentioned earlier that all the possibilities discussed here will not be universally applicable. The administrative staff must evaluate present conditions in their own laboratory and calculate probable growth of both the consultation service and research programs. A projection of needs in personnel and physical facilities to meet this growth should be made for as many years into the future as possible. Major personnel policies must be well thought out and projected over a long period of time. Frequent major changes may adversely affect staff morale and decrease efficiency.

This kind of long-range planning will permit a continuous up-grading of the entire organization and hence the quality of the consultation and research work performed. For instance, the program of veterinarians already employed in the basic science areas may be enriched and the overall productivity of the laboratory improved.
The scarcity of individuals certified in the desired specialties is especially acute in some areas. When individuals with the most desirable qualifications are not locally available, other interim arrangements must be made until the supply is more favorable. In the case of the pathologists, however, it is urged that the qualifications spelled out by The American College of Veterinary Pathology not be compromised if there are any possible means of avoiding it. Human individuals and political and business groups being what they are, everyone will agree that ideal conditions in every area of a Veterinary Medical Laboratory will likely never be a reality. However, insistence on high standards for individuals and the laboratory as a whole will do more to elevate morale and the quality of work than any other single procedure.

SUMMARY

1. The objectives of the veterinary medical laboratory should be thoroughly determined and clearly stated.
2. The positions necessary to carrying out these objectives should be set up and each job accurately described and evaluated.
3. Overall qualifications of individuals should be as carefully matched with each position as possible.
4. The upgrading of each position should be a continuous process so it will always challenge the incumbent.
The occurrence of equine piroplasmosis in the United States has been previously described.\textsuperscript{1,2} It is the purpose of this paper to discuss available diagnostic techniques and problems associated with control and eradication of the disease.

**Diagnosis**

**Symptoms:** The symptoms are characteristic\textsuperscript{1,2} and should suggest the possibility of piroplasmosis, infectious anemia, leptospirosis, severe parasitism or possibly certain plant poisonings. The first two are very difficult to differentiate clinically or with presently available laboratory tests. Anemia and icterus are the cardinal symptoms noted in these diseases. Briefly, in piroplasmosis, fever, depression, weakness, with frequent lying down, jaundice, anemia, edematous swelling of the eyelids and supraorbital fossa, edema in lower limbs, prepuce or posterior sternal area, rapid loss of weight and lacrimation may be seen. All of the above symptoms are seldom seen in the same animal.

**Treatment:** Treatment provides a valuable diagnostic tool as piroplasmosis responds dramatically, whereas the other conditions mentioned above do not. Drugs in use include 15 ml. of a 40 percent solution of phenamidine isethionate given subcutaneously.* Other drugs reported to be effective in South Africa include Aspirin (Bayer) and Berenil (Squibb).

Trypan blue, one percent aqueous sol., one gram per 500 lbs. of body weight, has been used. Severe reactions are sometimes encountered and the dosage may be divided.

**Necropsy Lesions:** Icterus, anemia and an enlarged spleen are the prominent lesions noted at necropsy. The degree of anemia varies, but can be expected to be noticeable in a case severe enough to be fatal. Mortality has been about 20 percent in Florida cases.

The spleen is enlarged, but the pulp is not as fluid as in anthrax.

Subcutaneous edema in the ventral portions of the body is usually noted, which may not have been obvious prior to death. Serous fluid is often noted in the abdominal, thoracic and pericardial spaces.

**Laboratory Tests:** In the acute stages of the disease, the piroplasma can be seen in the erythrocytes in Giemsa or Wright's stained blood smears. The percentage of infected cells has not exceeded 10 percent in our experience and usually is one percent or two percent. The typical bilobed pear-shaped organisms connected at the narrow end are seen. They may occur singly or extracellularly. The organisms divide by binary fission, in contrast to *Piroplasma equi* which divides by budding and develops four organisms within an erythrocyte.\textsuperscript{3} There is some difficulty in distinguishing between these organisms, especially in mixed infections.

These parasites stain well by the acridine orange-ultraviolet light fluorescence stain. Examination of blood fixed in formol-saline has been adopted as a routine diagnostic procedure in our laboratory. (Add one part of formalin to nine parts of physiological saline solution. To eight to ten ml. of this solution add not more than one or two ml. of blood taken from the acutely ill horse.) The technique is not suitable for detecting carrier horses as the organisms are not sufficiently numerous.

Due to the low percentage of infected erythrocytes in acute cases, several techniques for concentrating the infected cells have been described. One of these involved making touch preparations of the edge of the tip of the ear after the epidermis had been removed with a sharp instrument. Our experience has not indicated this technique to be a consistent improvement or equal to jugular blood.

Salyaev has described a concentration technique based on the fact that infected erythrocytes are lighter than normal ones. This technique is as follows: Mix equal parts of two percent sodium citrate and suspect blood. Centrifuge at 500 to 700 r.p.m. for three to five minutes. Decant the supernate and recentrifuge at 1500 - 2000 r.p.m. for 15 - 20 minutes. Discard the supernate and prepare a smear from the sediment. Infected erythrocytes are lighter than normal ones and are concentrated by this technique. Stain with one of the above methods.

Watkins described a modification of this procedure at the 99th meeting of the American Veterinary Medical Association: Allow blood, to which an anticoagulant has been added, to settle for 20, 30 or 60 minutes. (We have found 15 minutes to be best.) Remove the plasma and centrifuge at 2500 to 3000 r.p.m. for five to ten minutes. Decant the plasma, prepare smears of the sedimented cells, stain as above and examine.

Bilirubin content of the serum is elevated in this disease to levels of four milligrams percent or above. This, of course, is not diagnostic, but indicative of piroplasmosis. There is a leucocytosis with a shift to the left and sometimes a monocytosis and/or eosinopenia. Affected animals have a fever, are lethargic and off feed, in contrast to those infected with infectious anemia that continue to eat.

Specific serologic tests are not yet available, however, techniques in use for other blood parasitic diseases such as one of the tests based on labeled antibody fluorescence, precipitin reactions or complement fixation should provide one or more acceptable tests in the near future.

**Regulatory Aspects of Piroplasmosis Control**

Several facts make piroplasmosis difficult to control. Among these are:

1. The inapparent carrier state which develops in recovered animals and may last four years or longer;
2. The lack, at present, of a specific serologic or other simple test to determine which horses are carriers of the disease;
3. Biological transmission by certain ticks (Dermacentor and Rhipicephalus) that have been reported to be able to transmit the disease after five generations, without feeding on horses in the intervening generations; and
4. The relatively low mortality rate (in Florida about 20 percent) which influences the owner to gamble and not report suspected cases for treatment and subsequent quarantine and applied control measures, since recovered horses exhibit no apparent ill effects which would differentiate them from perfectly healthy animals.

In the Florida outbreak, quarantine measures were not adopted at first because of the long and indefinite length of the infection in recovered horses and due to the expected failure of owners to report new cases that invariably follows application of a quarantine. When it became known that owners were shipping animals to areas not known to be previously infected, both within and out of the affected area of the lower east coast of Florida, a quarantine was reluctantly applied on all animals previously known to be infected. As expected, the reporting of new cases dropped off abruptly.

Current steps toward control include:

1. A careful study of the epidemiology of the disease, including follow-up on all cases confirmed by laboratory tests.

2. Careful examination for ticks of all horses in stables where the disease has appeared. Infected horses are freed of ticks and are sprayed at 21-day intervals with emulsifiable concentrates or wettable powders of Delnav (0.15 percent solution), or Toxaphene (0.5 percent solution). Ears are treated by cleaning and applying a solution of one percent Lindane in cottonseed oil by means of a plastic squeeze bottle.

3. All stables, stalls, pens, and corral areas used by horses on infected premises are sprayed at 21-day intervals with Delnav or Toxaphene in the foregoing stated strengths.

4. Education of horse owners in the endemic area and throughout the state on facts about the disease and especially on the importance of tick control and abstention of the promiscuous use of needles and vaccination equipment to prevent mechanical spread of the disease.

5. Appropriation by the State of Florida Cabinet of $100,000 from the Contingency Fund for control (and limited research) of piroplasmosis.

6. Continued re-evaluation periodically by the Florida Equine Health Research Advisory Committee (an advisory group of equine industry representatives and veterinarians appointed by the Governor to study piroplasmosis and infectious anemia) to recommend revised action on this disease as conditions warrant.

All animals which have been determined by acceptable laboratory procedure, or other diagnostic methods acceptable to the Florida Department of Agriculture, to be infected with equine piroplasmosis are permanently identified by a lip tattoo or visible body brand. By such identification the promiscuous movement of infected animals will be curtailed.

A "crash" program is underway to develop a suitable diagnostic test for carrier animals and some success appears to be imminent through the complement fixation and other tests.
Additional "crash" measures are being instituted in treating the carrier state, and it seems reasonable to expect that a successful treatment will be developed. Such therapy is mandatory inasmuch as eradication of the suspected vector (*Dermacentor nitens*), a multiple-host tick, appears to also be an insurmountable task.

The successful control and eradication of piroplasmosis in the United States, therefore, awaits the result of experimentation now in progress.

REFERENCES

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RHODE ISLAND

Official member  
T. J. Grennan  

SOUTH CAROLINA

Official member  
R. W. Carter
<table>
<thead>
<tr>
<th>State</th>
<th>Official member</th>
<th>Individual members</th>
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<tbody>
<tr>
<td>SOUTH DAKOTA</td>
<td>M. D. Mitchell</td>
<td>B. W. Bierer, J. B. Guess, Jr., C. L. Vickers, S. D. Stockyards Ass'n, W. F. Waddell</td>
</tr>
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<td>TENNESSEE</td>
<td>C. E. Kord</td>
<td>J. B. Champlin, L. E. Fredrickson, H. L. Fry, O. E. Harrison, L. W. Lawrence</td>
</tr>
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<td>VERMONT</td>
<td>A. E. Janawicz</td>
<td>J. W. Armstrong, Holstein-Friesian Ass'n</td>
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<td>VIRGINIA</td>
<td>W. L. Bendix</td>
<td>R. J. Anderson, R. A. Barton, M. Bay, Jr., W. L. Bendix</td>
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I. Erickson
G. B. Estes
O. F. Foley
R. F. Gentry
F. B. Gluckstein
J. M. Hejl
D. W. Johnson
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D. Miller
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Wyoming Stock Growers Ass'n

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L. A. Fahland
<table>
<thead>
<tr>
<th>Country</th>
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<tr>
<td>Argentina</td>
<td>B. D. Blood, C. Rosenbusch, F. Rosenbusch, B. Szyfres, D. C. Blood</td>
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<tr>
<td>Australia</td>
<td>A. Webster, G. W. Ward</td>
</tr>
<tr>
<td>Brazil</td>
<td>W. M. Henderson, A. Killinger</td>
</tr>
<tr>
<td>England</td>
<td>Royal Society of Medicine, A. O. Betts, Commonwealth Bureau of Animal Health Veterinary Laboratory, G. N. Gould</td>
</tr>
<tr>
<td>Germany</td>
<td>D. Strauch</td>
</tr>
<tr>
<td>Holland</td>
<td>R. C. Fish</td>
</tr>
<tr>
<td>Israel</td>
<td>Y. S. Gorr</td>
</tr>
<tr>
<td>Italy</td>
<td>P. R. Ellis, M. Petek</td>
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<tr>
<td>Mexico</td>
<td>F. Camargo N., K. Schaaf</td>
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<td>New Zealand</td>
<td>S. Jamieson</td>
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<tr>
<td>Norway</td>
<td>R. Vollan</td>
</tr>
<tr>
<td>Peru</td>
<td>M. Moro, Universidad Nacional</td>
</tr>
<tr>
<td>Switzerland</td>
<td>H. Fey, E. Hess, M. M. Kaplan, H. Keller, C. Kilchspberger, W. Leeman</td>
</tr>
<tr>
<td>Uruguay</td>
<td>H. Trenchi</td>
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<tr>
<td>Venezuela</td>
<td>M. Villegas D.</td>
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Proceedings
of the
Third Annual Meeting
of the
Interstate Association
of
Live Stock Sanitary Boards.

Chicago, Illinois, October 11-12, 1899.

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MORTIMER LEVERING, - - - - Lafayette, Indiana

Treasurer,
W. B. TULLIS, - - - - Quanah, Texas

The next Annual Meeting will be held at Louisville, Kentucky, October 2, 1900.
The meeting was called to order by C. P. Johnson, President of the Association, at 11 o'clock A. M., October 11th.

By direction of the President, the Secretary called the roll of States and the following responded:

- Arizona—Colin Cameron.
- Colorado—Dr. Sol. Bock.
- Illinois—J. H. Paddock, J. P. Lott, J. M. Darnell, C. P. Johnson and Dr. C. P. Lovejoy.
- Indiana—Mortimer Levering.
- Kansas—F. H. Chamberlain and Taylor Riddle.
- Kentucky—G. A. Birch, John M. Letterle, G. W. Embry and Dr. F. T. Eisenman.
- Massachusetts—Dr. Austin Peters.
- Minnesota—Dr. M. H. Reynolds.
- Pennsylvania—Dr. Leonard Pearson.
- Texas—W. B. Tullis and R. J. Kleberg.
- Virginia—Dr. E. P. Niles.
- Wisconsin—Dr. H. P. Clute.
The Bureau of Animal Industry was represented by Dr. D. E. Salmon and Col. Albert Dean.

Mr. Letterle moved that the foregoing be accepted as the membership of this convention, subject to the addition of such delegates as may subsequently report. Carried.

The minutes of the last meeting of the Association were read by the Secretary, and on motion of Mr. Letterle, were ordered to stand approved.

THE CHAIR—In connection with the action of the Ways and Means Committee, which was appointed on a resolution adopted at the last meeting of the Association, I would request the Secretary to read to the convention a statement of the returns received in pursuance of that resolution. I wish to say at this point that because sufficient returns have not been received, the minutes of the last convention were not published.

The Secretary thereupon read the following report of receipts for per capita assessments:

<table>
<thead>
<tr>
<th>State</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illinois</td>
<td>$5.00</td>
</tr>
<tr>
<td>Indiana</td>
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</tr>
<tr>
<td>Tennessee</td>
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<td>Arizona</td>
<td>5.00</td>
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<tr>
<td>Kansas</td>
<td>5.00</td>
</tr>
<tr>
<td>Colorado</td>
<td>5.00</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>5.00</td>
</tr>
<tr>
<td>Missouri</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Twelve States, total ... $60.00

THE CHAIR—I would state that the Secretary is open to receive contributions to this fund for printing the minutes of our proceedings. All the expense of correspondence and printing circulars has so far been borne by the Illinois Board.

MR. RIDDLE—For information, I would like to ask if there will not be two assessments due at this meeting.

THE CHAIR—When this Association was organized at Fort Worth, no provision was made for ways and means. The Illinois Board published the minutes of that meeting in its Annual Report and copies were sent to all members; but at the Omaha meeting it was thought wise to create a fund so the minutes might be printed independently for distribution to all the boards interested.

I will state, while this matter of business is being attended to, that the next order of business on the programme I have prepared is the introduction of Resolutions, and following that the appointment of the necessary committees for the transaction of our business. If there are any resolutions to be offered, this is the proper time to introduce them.
MR. RIDDLE—I would suggest that we are expecting delegates from two states that represent quite an important part of our Association, and if it would not delay matters too much, we might adjourn, at least until after the dinner hour, and they might then be present. We will want them on some of the committees.

THE CHAIR—I thought perhaps it might be wise to make up the committees before the noon recess. There were two regular committees created: the Committee on Line and Open Season, and the Committee on Resolutions. The former committee, by action of the convention, was constituted of one delegate from each state; therefore, each state represented is entitled to representation on that committee, and any state coming in after the appointment of the committee will be represented by the addition of their member.

MR. TILLMAN—I think it would be well to appoint the committees before the resolutions are introduced. The State of Tennessee has her state delegates here—Capt. Paine, Commissioner Dunn, and Mr. Hamill. The others of us are here simply representing county and city boards of health, and I would suggest that, in appointing the committees, one of the State delegates be appointed.

THE CHAIR—Col. Skinner, formerly General Manager of the Fort Worth Stock Yards, informs me that the Union Stock Yard Co. extends a cordial invitation to all the delegates to lunch at the Transit House, at one o'clock, in the ordinary. What is the will of the convention?

MR. LETTERLE—I move that the invitation be accepted with thanks. Carried, by a rising vote.

MR. LETTERLE—I move that the Chair appoint a committee of five on resolutions, and that each state appoint, by its Chairman, a representative of the state on the Committee on Line and Open Season.

MR. RIDDLE—I believe that the question of open season should be given to another committee, because it affects only the states bordering on the quarantine line. The states far north can have an open season and the line states can allow cattle to pass through them for those northern states. I think the line states are practically a unit against what has been known as the open season. I therefore move to amend the resolution by striking out that part of the resolution allowing the Line Committee to determine the question of the open season.

The amendment was lost.

The question then recurring on the original motion, it was carried.

The Secretary then called the roll of states for the appointment of the Committee on Line and Open Season, and the following were named by the Chairman of each delegation to represent their respective states on said committee:
Arizona.......................... Colin Cameron.
Illinois.......................... Dr. C. P. Lovejoy.
Indiana.......................... Mortimer Levering.
Kansas............................ Taylor Riddle.
Kentucky.......................... Dr. F. T. Eisenman.
Massachusetts..................... Dr. Austin Peters.
Pennsylvania....................... Dr. Leonard Pearson.
Tennessee......................... W. H. Dunn.
Texas.............................. W. B. Tullis.
Virginia........................... Dr. E. P. Niles.
Bureau of Animal Industry........ Dr. D. E. Salmon.
Colorado.......................... Dr. Sol Bock.
Minnesota......................... Dr. M. H. Reynolds.
Wisconsin.......................... Dr. H. P. Clute.

The Chair announced the following Committee on Resolutions:

R. J. Kleberg Thomas H. Paine Dr. F. R. Eisenman
Taylor Riddle J. H. Paddock

THE CHAIR—Gentlemen: We have with us to-day, by invitation, which he has very kindly accepted and acted upon, Dr. D. E. Salmon, Chief of the Bureau of Animal Industry since its creation. I now take pleasure in introducing Dr. Salmon to the convention and he will address us on questions that he deems of interest.

DR. SALMON.

MR. PRESIDENT AND GENTLEMEN: I have no intention of making a formal address. In the first place, I have not had time to prepare one, and in the second place, if I had, on account of the severe cold from which I am suffering, it would be impossible for me to deliver it. I do wish to say a few words, however, and in the first place to impress upon the members of this Association, so far as I can, the importance and value of the organization to which they belong. There was great need for cooperation between the sanitary authorities of the different states, in order to secure uniform action, and to enable the owners and shippers of live stock to get their animals to market without unnecessary or burdensome restrictions, and this is one of the most important steps towards securing such results.

The federal government has done something to make the regulations uniform, but in order to satisfy those in the various parts of the country, it was desirable to have something more; it was desirable that those who are interested should have some voice in the conclusions that were reached. The Department of Agriculture has endeavored to follow, so far as possible, the recommendations which have been made from time to time by your organization at its past sessions. It has done so, partly because it recognized the fact that such recommendations should be given great weight, and partly because it desired to do as much as it possibly could to raise the prestige and standing of your organization. It was believed that there was need of such an organization in order that
representatives from all parts of the country might come together once a year to deliberate upon these questions.

Now, there will be questions coming before this meeting which you have had in mind, most of you, which will have considerable influence on the live stock industry during the year. It is not necessary that I should review these questions and discuss them at this time, because they will come up and be discussed by you during the progress of this meeting.

It is unfortunate that one expectation we had a year ago has not been realized. We thought, when the regulations for the current year were made, that we had a dip by which we could free southern cattle from infection and allow them to go anywhere without danger to northern native cattle, and we made arrangements in the regulations by which such dipping should be recognized; and we also left out of consideration the open season, which heretofore we have allowed. Unfortunately the dipping of last year injured the cattle to such an extent that the department did not feel that it could recommend it for the current season, and we concluded to make no such recommendations. The oil used last year, which proved so unsatisfactory, was purchased from the Standard Oil Co. I presented the matter to them and explained the effect it had upon the cattle, and they told me the oil was probably refined with sulphuric acid and it was impossible to get all the acid out of it. I arranged to get some oil which had not been treated in that way and was free from acid. I got such an oil and dipped some cattle in Washington without injury to them, and we concluded the oil was satisfactory. A little later in the season, when we got some ticky cattle, we dipped them in the oil and found it did not kill all the ticks. I went back to the Standard Oil Co. and asked them if they knew exactly the kind of oil used last year. They told me they furnished the oil, that it came from their refinery at Whiting, near Chicago, and that they could furnish an identical oil at any time. I arranged with them to send me some of the oil from Whiting to Norfolk, Va., where we have no trouble in obtaining suitable cattle for experimentation. When that oil came we dipped ticky cattle in it, but we failed to kill all of the ticks. You can see that I was somewhat embarrassed by these experiments and was unable to go ahead with the dipping. Since then we have been making further experiments but they are not concluded, and I can only say that we have some encouraging results which lead us to hope for success in time for the next dipping season.

The question of the open season is one that must soon be settled. There are many cattle in the South seeking a market; and the department must decide in a very short time to what extent they can be let out with safety to the cattle of the North. I have come here to listen to your views in regard to the suppression of the infection, and hope to see my way clear to make some recommendation to the department. The Secretary of Agriculture is desirous of doing anything in his power that shall be for the best interests of the greatest number interested in the cattle industry.

I thank you for your attention, and shall be glad to give you any further information possible upon the questions that come before this
convention. I shall also be glad to hear from you in reference to these
questions, and to receive any information that we have not yet been able
to secure.

THE CHAIR—Is there any one who desires to ask any questions of
Dr. Salmon?

MR. RIDDLE—In regard to the question of an open season, to which
Dr. Salmon has alluded, I can hardly conceive how our Association here
can do anything more than recommended, as each state can pass on that
question for itself. The state of Illinois may want an open season and can
have it. The state of Kansas might not want an open season, without in-
pection, and the state of Kansas would not have it if she didn't want it.
It is a question to be decided by each state for itself, and it should be left
in the hands of the live stock authorities of each state. Any resolution
that we might adopt would have very little effect. I would like to ask Dr.
Salmon if the Secretary of Agriculture allowed an open season, what would
be the effect upon those states that do not desire it?

DR. SALMON—There is always this to be considered. When you al-
low cattle to pass the line of any state you do not know where they will go
to from that state. Cattle are shipped from Texas, south of the quarantine
line, to Minnesota. That is all right for that state, but who knows whether
they are going to stay there? They are liable to be re-loaded and shipped
elsewhere. The question of an open season does affect more than the par-
ticular state to which cattle go direct, and this is a part of the question
that must be considered. I do not know to what extent you feel like acting
upon it, but I have always been somewhat embarrassed when the question
came before the department, because I have no way of deciding where
cattle that are allowed to pass the line are destined. Dealers ship cattle
to one place, unload them, and then ship them to some other point. That
must be considered in discussing and deciding this question.

MR. RIDDLE—Of course this question of line and open season is not
before the convention—simply an informal discussion—but my object in
offering my amendment a while ago, to take from the line committee the
power to recommend as to the open season, had in effect the very ques-
tion that is raised by Dr. Salmon. The fact that I believe that Minnesota
can take cattle safely from south of the quarantine line, is not altogether
a good reason why they should be permitted to do so. We have had cases
of fever this year from ticks that were no doubt brought in in the month
of December. In my judgment, the most important question to be decided
by this body is the question of an open season. After cattle cross the
quarantine line, that being the only obstacle to the traffic, so far as Texas
fever is concerned, they are at liberty to go anywhere and can go into any
state without hindrance.

A DELEGATE—Could not the state of Kansas make its own line
around the state—not only the fever line, but a line all around it?

MR. RIDDLE—That would make it necessary for us to have inspectors
all around the state, and that would work a great hardship on shippers and
would cause a great many innocent people unnecessary annoyance.
MR. DUNN—We have seven inspectors employed by our state to keep cattle from south of the line from coming in.

MR. RIDDLE—Allow me to ask if you have any inspectors on the north to keep infected cattle out?

MR. DUNN—We require all cattle from north of the quarantine line to have a certificate from the county from which they come.

MR. RIDDLE—In case of an open season they might come into your state from Kentucky, Missouri, Arkansas, or any other state.

THE CHAIR—I wish to announce that there will be a slaughter of twelve cattle that have been tested with tuberculin and have reacted, at 10 o'clock tomorrow morning, under the supervision of our Board at B. Wolf's slaughter house, corner of Emerald Avenue and Forty-first Street. These cattle were not tested under the supervision of our Board, but the Board will take charge of the post-mortems, and extends a cordial invitation to all the delegates attending this convention to be present and witness the post mortem examinations.

On motion of Mr. Riddle, a recess was taken until 2:30 o'clock P. M.

AFTERNOON SESSION—2:30 P. M. OCTOBER 11

The convention was called to order by the President.

The Committee on Line and Open Season not being ready to report, a recess was taken until 4 o'clock, P. M.

The convention reconvened at 4 o'clock P. M.

THE CHAIR—Gentlemen of the Convention: In view of the live question that is before the people and the sanitary boards of many of the states of the Union, of the infectiousness of tuberculosis, and the practical efforts that have been made for its eradication, I have, as your President, arranged for a series of papers on this subject. The State Board of Live Stock Commissioners of Illinois, some time ago made arrangements with the Columbus Medical Laboratory of the College of Physicians and Surgeons of Chicago, to conduct some original investigations for the purpose of demonstrating to what extent, if any, milk from tuberculous animals whose udders are not affected, is infectious and contains the bacillus tuberculosis. Prof. Adolph Gehrmann, Bacteriologist, and Prof. W. A. Evans, Pathologist, who took this work in charge, are present with us today, and have kindly consented to present papers on this subject. I take pleasure in introducing Prof. Gehrmann, who will address you.

THE INFECTIVE POSSIBILITIES FROM TUBERCULAR MILK

Mr. President and Gentlemen: The study of experimental tuberculosis as related to cows has been treated by all investigators in the direction, first, of demonstrating the infectiousness of the secretions and
of tubercular cows to other domestic animals; and secondly, in an attempt to demonstrate the real relations between the disease in the cow and in man. Many series of experiments have been conducted to show that cattle tuberculosis can be transmitted to other animals. These experiments have been by feeding and injecting material derived from cows in which the evidence of tuberculosis was more or less positive.

From a view of the experiments presented one is obliged to recognize that there is a regular increase in the certainty of infecting an animal proportionate to the evidence of the tubercular nature of the material used in the experiments.

The results reported vary to a considerable degree. These variations are probably owing to the numerous difficulties always present in a study of tuberculosis. These are: the chronic nature of tuberculosis, the slow multiplication of the bacilli, their slow removal from the infected individual and the careful technique required to demonstrate them in a given specimen.

Examination of Milk for Tuberculosis.
(From Rabinowitsch and Kempner)

<table>
<thead>
<tr>
<th></th>
<th>NO. OF COWS</th>
<th>TIMES T.B. FOUND</th>
<th>PER CENT.</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>6</td>
<td>1</td>
<td>16.6</td>
</tr>
<tr>
<td>Stein</td>
<td>14</td>
<td>4</td>
<td>28.5</td>
</tr>
<tr>
<td>Bang</td>
<td>63</td>
<td>9</td>
<td>14.0</td>
</tr>
<tr>
<td>Hirschberger</td>
<td>20</td>
<td>11</td>
<td>55.0</td>
</tr>
<tr>
<td>Ernst</td>
<td>36</td>
<td>10</td>
<td>28.5</td>
</tr>
<tr>
<td>Smith &amp; Schroeder</td>
<td>6</td>
<td>2</td>
<td>33.2</td>
</tr>
<tr>
<td>Schroeder</td>
<td>31</td>
<td>2</td>
<td>6.5</td>
</tr>
<tr>
<td>Delepine</td>
<td>37</td>
<td>9</td>
<td>24.3</td>
</tr>
<tr>
<td>Nocard</td>
<td>54</td>
<td>3</td>
<td>5.5</td>
</tr>
<tr>
<td>Rabinowitsch u. Kempner</td>
<td>15</td>
<td>10</td>
<td>66.6</td>
</tr>
</tbody>
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These figures of results show a variation in infectiousness of 5.5 per cent to 66.6 per cent.

Briefly stated, the following is a summary of some reported experiments.

Van Gersten of Brussels was the first to call attention to frequency of tuberculosis in the mammary gland of cows and the consequent danger of infection from the use of milk from such animals.

Gerlach, Klegs and Bollinger caused tuberculosis in animals by giving them milk from tubercular cows.

Schlieber (Inaugural Dissertation, Koenigsberg, 1875), fed 18 rabbits and three guinea pigs on milk from tubercular cows. None became tubercular.
Bollinger, 1879, at German Congress at Baden, announced that milk from tubercular cows, the mammary gland being healthy, could cause tuberculosis when injected into the peritoneum of a healthy pig.

In 1883, May, under direction of Bollinger, conducted some experiments at the slaughter house in Munich. He took milk from tubercular cows and injected two to three c.c. into the peritoneum of guinea pigs. All were negative except one. This was in a case of general military tuberculosis with involvements of the mammary gland. Ordinary cooking was sufficient to remove all virulence even when virulent tubercle bacilli were purposely placed in milk. Archiv. i. Hygiene, '83.

Stein (Inaugural Dissertation, 1884, Berlin), under Bollinger's direction injected eighteen rabbits intraperitoneally with milk from advanced tubercular cows. Four showed tuberculosis.

Hip Martin (Revue de Medicine, 1884, page 156), bought milk as sold at the door in Paris. This he injected into guinea pig's abdomen. He injected nine pigs, three contracted tuberculosis. He concluded that 33 per cent of the milk of Paris was tubercular. Commenting on this, Straud (Tuberculose et bacille), says, experiments made both before and after this are unanimous in showing that in cases where the milk is from an animal certainly and profoundly tubercular the inoculation gives proportionately very much less.

At International Congress held in Paris in 1884, Bang said that mammary tuberculosis, far from being common, still is not very rare.

At the Tuberculosis Congress in 1888, Bang wrote of milk from tubercular cows but with healthy udders.

In twenty-one cases of this nature, where 2 c. c. was inoculated into peritoneal cavity of rabbits, two showed tuberculosis. The cows had advanced tuberculosis. Says Bang, these results are very reassuring. He injected milk from eight tuberculosis women (advanced) with negative results.

At the International Congress of Hygiene in London in 1891, Bang reported experiments on fifty-eight cows; milk inoculated in rabbits and guinea pigs; infected—15.5 per cent.

In these cases nearly all the cows were in advanced tuberculosis. Some succumbed to the acute miliary form. In three cases there was mammary tuberculosis, too slight to be recognized with the naked eye. In one, nothing was found in the mamma, but in the supermammary glands tuberculosis was found.

Galtier (Tuberculosis Congress in 1888) said tuberculosis was only found when the mammary gland is affected. Nevertheless, always refuse milk from cows with tubercular mammary gland, boil all milk from suspected or proven tubercular cows; never drink any unboiled milk unless you know its source.
Hirschberger (Deut. Archive. fur Klin. Med., '89), took twenty-one cows—all tubercular; cut off mammary gland, incised and milk taken out by pressure and a syringe and examined it microscopically. Then 1 to 2 c. c. milk was injected in peritoneum of guinea pigs. Eleven times pigs became tubercular—58 per cent—of cows five had general tuberculosis. Their milk gave four positive results. Six others had moderate tuberculosis; milk examination four positive, two negative; nine had localized tuberculosis; three positive, six negative.

Gebhart (Virchow's Archiv. 1890) bought milk on streets of Munich, injected 2 c. c. into peritoneum of guinea pigs. Ten experiments all negative. On the other hand, milk from tubercular cows with sound mammae gave positive results. If this same milk was diluted with 40 to 500 parts of water, no tuberculosis was produced. A certain degree of dilution causes milk to lose its tubercular virulence. The case in which the danger is prevalent is where the milk supply is from a cow and that cow is tubercular.

Gaffky (Deut. Med. Wochenensch, 1892), calls attention to the fact that tubercle bacilli can get in milk from faeces, hair, dirt on teats even when that gland is healthy.

Strauss (Tuberculose et Bacille) summarizes as follows: Milk which comes from tubercular cows is nearly certain to be infected if the mammary gland is tubercular. When the gland is healthy the milk is generally harmless, even if the tuberculosis is advanced and general. He also calls attention to the fact that in these experiments milk was injected subcutaneously or intraperitoneally, and this gives a much greater insurance of infection than when it is taken by mouth.

Bang has taken milk rich in tubercle bacilli, centrifuged (60,000 revolutions) for one hour. Separated three layers—1st, cream; 2nd, milk; 3rd, sediment. Layer No. 3 was rich in tubercle bacilli layers 1 and 2 about equal to each other, but much less number than in No. 3. Inoculation with 1, 2 and 3 gave positive results.

Scheurlen arrived at the same conclusions as a result of similar work.


Four Devon cows were tested. Tuberculin shows clean cut reaction. No mammary involvement demonstrable. Eight calves from these and other mothers (healthy) were fed on milk from these cows—none developed tuberculosis while being fed on milk. Tests extended from November, 1896, to February, 1899.

Conclusions: In the experiments here reported, eight calves have been fed. But it is certain that we strangely exaggerated the importance of the intestinal mode of infection. (Strauss ibid.) Calves were fed upon the milk of tubercular cows for periods varying from three months to sixteen months without developing the disease. The results of these experiments coincide with the general results of European observations, and
advocate that the danger from the spread of tuberculosis through the milk of cows to man or to other animals, is not as great as has been supposed. In the earlier stages of the disease, and at all times when the udder is not affected, the danger from the use of the milk is quite limited. Great stress, however, should be laid on the danger of using milk from cows which show any symptoms of udder infection.

Dr. Lydia Rabinowitsch and Walter Kempner. Zeitschrift fur Hygiene, 31 Band, 1st heft.

The important questions to be decided are tubercle bacilli in milk, in general cases of general tuberculosis or in cases of tuberculosis of the udder.

Nocard's experiments seem to show that microscopic tuberculosis of udder must be present to have the milk infectious.

Rabinowitsch and Kempner proposed to answer these questions:

First—Are tubercle bacilli in milk from cows with beginning tuberculosis apparent by physical signs and no udder infection?

Second—Are tubercle bacilli in milk from cows with latent tuberculosis shown by the tuberculin test? Fifteen cows tested with tuberculin were used.

These questions are answered in the affirmative. Three of the results in which tuberculosis was found are positive answers for the first question, as no udder tuberculosis was present while there were physical signs of pulmonary tuberculosis.

Two of the results in which tuberculosis was found are positive answers to the second question, as in these two cows absolutely no clinical evidence of tuberculosis was present.

The general conclusion that is reached is—that the milk of all cows reacting to tuberculin is infectious. The tuberculin test is therefore of much wider significance than the physical examination of the cattle.

Test with Milk for the Illinois State Board of Live Stock Commissioners

As to the way in which these samples were examined, we may present the following condensed summary:

Bottles of a capacity of 200 cubic centimeters were sterilized by steam and well corked. These were sent to the slaughtering house and were filled with the milk, one sample being taken from each of the four teats or as many of the teats, as would give milk. It was also attempted to obtain a mixture of the first portion of the milk and the strippings from each test. The bottles were tightly closed, marked and sealed. They were packed in ice and were sent at once to the College of Physicians and Surgeons, where the examination was conducted. The samples were received
there three or four hours after their collection. The examination was begun at once.

The first step in the preparation of the samples was to shake them thoroughly and swing the milk in the Purdy centrifuge. The tubes of the centrifuge were filled with milk and swung at a speed of about two thousand revolutions a minute for five minutes. A portion of the cream layer was then removed with a sterilized platinum spatula and placed in a sterile test tube. The milk down to the sediment was then emptied and the tube was refilled from the bottle and swung. This procedure was continued a number of times, each time removing a small portion of the cream layer. The sediment was then removed from the bottom of the centrifuge tube and a direct microscopical examination was made without any other preparation. This was for the purpose of showing the presence of pus cells, blood cells and epithelium.

Three slides were then spread from the cream that had been collected from the tubes, and three similar slides were spread from the sediment. These were stained for tubercle bacilli, using the usual method of Ziel Nielson. The cream that was obtained and the sediment were then mixed and about five cubic centimeters was injected subcutaneously in a guinea pig. The guinea pigs were marked and remained under supervision until they died, or were killed at the end of five or six weeks and a post mortem examination made.

The tables which we present indicate the number of the sample; the special test from which the sample was removed; the physical character of the milk as received; the amount of milk; the results of the direct microscopical examination; the results as to the presence of the bacillus tuberculosis, or other bacteria from the stained slides prepared from the cream and the sediment; also the weight of the guinea pigs, the amount of milk injected and the length of the period that they survived; and finally the results of the post-mortem examination and the details of the examination of the tissues taken from the animals.

It has been our intention throughout to conduct the experiments as rapidly after receiving the samples as possible, and to use the greatest possible precaution in every step to avoid any possible contamination or interference with an exact result.

RESULTS.

The milk from thirty cows was examined and there were 120 samples of milk. The milk of eight cows was shown to be tubercular, or 26 per cent of the number examined.

Upon microscopical examination of the milk samples, tubercle bacilli were found in all samples, or 9.1 per cent of the entire number. Tubercle bacilli were found in the cream alone four times and in the sediment alone three times. In the other samples, where tubercle bacilli were found they were both in the cream and in the sediment.
One guinea pig was inoculated with the mixed specimens from each cow. Macroscopically and microscopically, eight guinea pigs showed tuberculosis. These were all from the cows in the milk of which tubercle bacilli were found.

In this series of cows physical examination of the udders showed an absence of tuberculosis.

Reviewing the data of the experiments, it is apparent that the milk of two cows, Nos. F 95 and C 94, are of a positively dangerous character. In the other cows but few tubercle bacilli were found and the guinea pig infection was not extensive.

The result speaks strongly for the possibility of having a dangerously tubercular milk from cows apparently free from udder tuberculosis.

THE CHAIR—Gentlemen: I take pleasure in introducing to you Prof. Evans, Pathologist of the Columbus Medical Laboratory, a colleague of Prof. Gehrmann.

DISCUSSION BY PROF. EVANS.

Mr. Chairman and Gentlemen of the Convention: This discussion forms a part of the work which is going on in every country that claims to be civilized.

The American Medical Association at its last session took official cognizance of the movement, to the extent that it appointed a committee with large powers. In the state of Illinois there is a society for the suppression of tuberculosis. There are similar societies in Pennsylvania, in Massachusetts, and in other states. Cook county has recently erected a hospital for consumptives. There is effort, somewhat unorganized and somewhat inconsistent, but certainly opportune, that is spreading all over America. But America is not a pioneer in this work. There is the British Association for the Prevention of Tuberculosis. Last year the Berlin Congreee was held. France has been investigating the subject for several years. The King of Sweden gave his Jubilee fund to the suppression of the disease. The State Board of Illinois is to be commended for adding its efforts to those of other communities. In every work of this character the coefficient of error must of necessity be large. The massing of material then, is the proper method of obtaining opinions that are correct.

The importance of the subject is beyond compare. It is commonly held that of all the people, one in seven dies from tuberculosis. Of those who die between fifteen and sixty years of age, one-third die from tuberculosis. Before fifteen, the child is dependent upon its parents; after sixty, the adult becomes dependent upon either the state, his family, or the accumulation of his years of greater capacity. The division then, is in reality a division into burdens and burden carriers, and one-third of those who die while carrying the load of the world, die from this disease. Bertillon's statistics for Paris show that, of 1,000 people dying from tuberculosis, 76 were between 20 and 60 years of age. The census of
1890 showed that 109,000 people died that year from this disease. Allowing for the disposition of any statistics to understate the number of deaths from tuberculosis, it is safe to say that 150,000 people die each year from the disease in the United States. Vaughan estimates that at any given moment there are 1,500,000 people in the United States who have this disease. As regards the disease in animals, there is the greatest variance in opinion. It is universally accepted that it is at once of greatest importance and of greatest frequency in milch cows. The work of the Illinois Board would indicate that 20 per cent of the milch cows are affected. There are $36,000,000 worth of milch cows in the state. This would indicate that $7,000,000 represents the value of diseased animals in this state. The fact that the cows of institutions and cows open to suspicion have been examined with greater frequency than any other, would indicate that this percentage is higher than the facts would warrant. Let us say that it is 10 per cent or 2 per cent, the figure given by one of our neighbors, as proper for this state; the matter is not materially changed. There is more tuberculosis this year than there was last, and there will be more next year than there is this. If it would be expensive this year, it would be more so next year. In a recent address by Dr. Adami to the Association of Canada, Dr. Adami argues strongly that Canada should handle the question before it becomes so expensive and so troublesome.

The State Board of Illinois is to be commended for adding its efforts to the world wide movement looking toward the control of this disease. Even if no new fact is uncovered, or even sought, the massing of information is desirable, for the adjudication of a question of such stupendous moment is not to be entered upon until there is sufficient data to guard against serious error.

When we come to consider the question of the infectiousness of milk two avenues of inquiry branch off. The one relates to the spread of the disease to other domestic animals; the other relates to its relation to the disease in the human subject. The communicability of the disease through the milk—first, to sucking calves and animals fed on milk—second, to animals associated with the diseased animals.

A piece of investigation eminently proper in its bent, was that of Phelps. He raised calves on milk from cows that had responded to tuberculin. While his method was proper, study of the period of reaction of the mothers, and the date of feeding of the calves, forces us to believe that conclusions cannot be drawn from the work. The very rapid spread of the disease in stock, the virulence of the germ in bovine tuberculosis and the virulence of the germ in milk, are good evidence that the disease is spread by the milk.

Theobold Smith has recently shown that the tubercle bacillus that has passed through the cow has gained in virulence. The British Royal Commission for 1890 said: The milk of cows with tuberculosis of the udder possesses a virulence which can only be described as extraordinary.

The statistics from Copenhagen show the great prevalence of tuberculosis in pigs fed on skim milk from creameries.
Its relation to the disease in the human subject. This is in reality the greater question of the two, even to the stockman. The life of a man and of his family must ever be superior to any question of property. People with tuberculosis are not fit companions for stock any more than stock with tuberculosis are fit companions for people. If the annual loss from tuberculosis in the United States is $750,000,000, the stock owner must suffer in common with every one else. The work of Bang at Copenhagen and the experiments at Thurbyville, England, show the disease is spread by association. The Bang experiments are familiar to most of you. An exposition of them can be found in the valuable report of Prof. Conn at Thurbyville in 1892. Sixty-three per cent of the herd tuberculous—segregation has applied. In 1897 this percentage has fallen to 27.

What evidence have we that tuberculosis can be transmitted from cows to man?

To begin with, we must pose the general proposition that every animal that has tuberculosis serves as a culture bed for the bacilli of that disease. It is true that the disease may be more or less modified as to its virulence by the animal, in which it has found a home. This does not destroy the force of the original statement. In birds the bacillus has been so modified as that its virulence towards other animals has been in a great measure lost. This represents the extreme of divergence. Yet it would hardly be denied that the presence of avian tuberculosis contributes in some measure to the possibility of the spread of the disease.

In autopsies of calves it has been found that 68 per cent showed intestinal tuberculosis as the primary lesion. This tends to show that milk was the medium of infection. Tatham's statistics for England show that whereas pulmonary tuberculosis had decreased 45.4 per cent, when we compare 1851 to 1860 with the present time, intestinal tuberculosis has only decreased 8.5 per cent. If we study intestinal tuberculosis as shown in children of different ages, we find that under one year of age it has increased 27.7 per cent, and under five years of age it has decreased only 3 per cent. This tends to show that improved hygienic conditions, better light, better air, better drainage, cleaner homes and work places, have lowered the amount of tuberculosis enormously. But even these bettered conditions—a betterment assuredly as helpful to babies as to adults—have not been sufficient to compensate for a food supply become more dangerous. And what is the food supply of children under five, and especially of babies under one year of age? Tatham's statistics show that for each 100,000 people in England, 206 die each year from pulmonary tuberculosis, 225 from all forms of tuberculosis, and 44 from intestinal tuberculosis. That of 100,000 children under four years of age, 151 will die each year from intestinal tuberculosis. Of 100,000 babies under one year of age, 404 will die from intestinal tuberculosis.

Dr. Gehrmann has spoken at some length on this phase of the question. It is scarcely denied in reputable quarters that milk from cows with tubercular udders is infectious to animals and to man. The Jena statistics for 1897 show that 1 per cent of the animals having tuberculosis have
tuberculosis of the mammary gland. Nocard has emphasized the fact that it is not always possible to demonstrate tubercular nodules in the mammary gland when there is indication of such involvement. We are willing to accept the statement of Conn, who says—after careful study of the problem for many years there appears to be at the present time in the minds of scientists an undoubted tendency to regard the danger as very much less than has been supposed. In the series of examinations that we have made, whilst 26 per cent of the milks of cows with sound udders showed tubercle bacilli in the milk, yet in only two of these cases was there a number of tubercle bacilli that would have constituted a considerable element of danger in a person with a sound intestinal tract. The single organism found after prolonged searching of several slides in each of the other specimens would probably have been easily handled by the resisting or protecting forces.

In spite of all this, the milk tuberculosis question presents itself in about this fashion:

First, we are trying to eradicate a disease that kills one-seventh of the people and costs $700,000,000 annually.

Second. Milk constitutes one of the avenues of infection.

Third. Milk can sometimes contain larger numbers of the bacilli when the mammary gland is not infected.

Fourth. It is not always possible to demonstrate tuberculosis in the gland, either before or after death, when other evidence conclusively demonstrates its presence.

Fifth. No man can say when the walls of his intestines are capable of resisting even a small number of bacilli.

The conclusion of the British Commission is correct, to-wit: Any person who takes tubercular matter into the body as food, incurs risk of acquiring tubercular disease.

Says Dr. F. W. Smith, of the New York Tuberculosis Commission: The first great step toward the prophylaxis of tuberculosis in man is to stamp out the disease in cattle.

The question is one worthy of our most judicious thought, from whatever standpoint we view it; and by no means the least important point of view is that of the man who, clear headed and far sighted, tried to save the stock interests of the country, not only from the impending ills, but from those that, farther removed, are still well above the horizon.

In Northrup's series of seventy-seven post-mortems in children: forty-two appeared to have a primary thoracic tuberculosis; nineteen appeared to have primary abdominal tuberculosis. He calls attention to the fact that in apparently primary pulmonary tuberculosis, the bacillus might have entered the intestines, traversed the wall, passed to the lymphatics, thence to the thoracic duct and the lungs.

At the conclusion of Prof. Evans' paper the convention took a recess until 10 o'clock A. M. the following day.
The convention was called to order by the President.

Dr. Pearson offered the following resolution, which was read, and, on motion of Mr. Tillman, was referred to the Committee on Resolutions.

WHEREAS, Tuberculosis of cattle is now attracting much attention in all parts of the country and has been the subject of much legislation in the eastern states and in some of the central states; and,

WHEREAS, In some of the discussions on this subject certain fundamental facts have been lost sight of, or are misstated, and there is need of a definite statement as to some of the points in regard to tuberculosis that are so fully established as to be placed beyond reasonable doubt and have been accepted and endorsed by the conventions that have taken action on this subject during recent years, be it

RESOLVED. That the following points in regard to tuberculosis may be considered as established and should enter into all plans for controlling this disease:

1. Tuberculosis is a contagious disease.

2. Tuberculosis prevails extensively in many parts of the United States and is spreading, except in unstabled beef producing herds and in states that are combating it actively.

3. While the losses caused among cattle by this disease are large and sufficient to justify energetic efforts to eradicate it, tuberculosis is also of much importance on account of the intimate relation of this disease of cattle to the public health.

4. Tuberculin furnishes by far the most accurate means that is at present available for recognizing tuberculosis in living animals, and the mistakes made where it is carefully used are so few as to be of no practical importance.

5. It is necessary that states shall authorize measures planned to suppress this disease if it is not to be permitted to continue to spread.

6. This convention recommends that all states that have not already done so shall authorize and empower the proper authorities to adopt well planned and conservative measures to repel the encroachments of this disease and to eradicate it where it is already established.

Mr. Charles W. Baker, Secretary of the Chicago Live Stock Exchange, in behalf of the Exchange, extended an invitation to the delegates to visit some of the large packing houses at the Stock Yards as its guests, and stated that carriages would be in waiting at Wolf's slaughter house at the conclusion of the post-mortems for the use of those delegates who desired to make the trip.
Dr. Leonard Pearson, State Veterinarian of Pennsylvania, read the following paper on

THE CONTROL OF TUBERCULOSIS.

Although the general subject of tuberculosis of cattle has been discussed in so many papers and at so many meetings during the past few years, I feel that the magnitude and importance of the question justify its frequent consideration until there is greater crystallization of opinion and union of effort in regard to the main points of the situation. And perhaps a brief review of an actual endeavor to check the progress of this disease may not be entirely without value.

If no exact observations were recorded in regard to tuberculosis, if no careful scientific inquiries and investigations as to the multitudinous bearings of this disease were being made and reported and if no effort to repress tuberculosis were actually under way, it would be possible for the theorizers and disputants to wrangle endlessly over these questions. But when the established facts in relation to tuberculosis are held in clear view, and are used to measure individual opinions and recommendations as they are put forth, only those that rest on premises that have stood the most searching tests that the scientist and economist can apply will receive earnest attention. It is, therefore, of the highest importance that the main points in regard to tuberculosis, and especially those upon which repressive measures must depend, shall be presented as clearly and distributed as widely as possible. Although many of these points are established beyond controversy and are accepted by all who have studied the subject, they are still not as generally appreciated as they should be. This need not surprise us when we recall that but a few years have elapsed since Koch's epoch making discovery of the tubercle bacillus and the establishment of the identity of the human and animal tuberculosis. And when we recall the further fact that not a decade has passed since the discovery of tuberculin and that most of our knowledge as to the extent to which tuberculosis is distributed among animals has been obtained during this period, it no longer appears strange that the public fails to recognize facts that were unknown to the specialist such a short time ago.

The importance of the cattle tuberculosis problem is two-fold: First; tuberculosis in meat producing and dairy cattle constitutes a menace to public health, and, second, the cattle industry suffers seriously on account of the extensive prevalence of this disease.

As to the first point, there is no lack of observation to show that the products of animals in certain stages of tuberculosis contain tubercle bacilli, and it has been shown by the observation of numerous cases under natural conditions, as well as by definitely controlled experiments, that the ingestion of such material by animals may be followed by the development of tuberculosis. It is also known from accidental inoculations sustained by men that the tubercle germs from cattle may produce tuberculosis in a fatal form in man. Moreover, there are instances in which people
who have consumed the milk from tubercular cows have contracted tuberculosis when no other source of the disease was apparent, and all of the history pointed to infection from milk.

Most powerful evidence of the existence of this danger and the operation of this cause of mortality is furnished by the records of the General Registry office of England, as published by the last Royal Commission on Tuberculosis in 1898. It is shown by these records that the deaths from all forms of tubercular disease in England and Wales have diminished 59.1 per cent, in the last thirty-five years, a period of great sanitary advance in respect, especially, to habitations in towns and cities. The greater portion of this very gratifying diminution was in the lung form of tuberculosis or phthisis. On the other hand, the diminution in the intestinal form, or tabes mesenterica, has, in the same period, been but 8.5 per cent, and at one period of life, before the end of the first year, there has been an actual increase in this disease of not less than 27.7 per cent. If infants derive tubercular infection only from their associates and attendants, or, at any rate, from other persons, it is fair to expect the diminution in prevalence to be in proportion to that among their elders. As this is not the case, and as there is actually a large increase in mortality from tubercular disease during the period when milk constitutes the chief article of diet, this food is thus, in the opinion of the members of the Royal Tuberculosis Commission, placed under the strongest suspicion.

As to the direct injury to the cattle industry and the monetary loss caused by tuberculosis these, as the danger alluded to above, are in direct ratio to the prevalence of the disease. Unfortunately, no accurate statistics are available as to the general distribution of tuberculosis among cattle of the United States. We have, however, the reports on a vast number of tests of scattered herds, the slaughter house records of the Bureau of Animal Industry, and few reports from slaughter houses under local control, and the estimates of a number of veterinarians who have had long experience with this disease in large districts. It is not possible to go into the details of these reports in this summary but it may be said that tuberculosis prevails most extensively among cattle near the Atlantic sea-board and the old dairy districts. It becomes less prevalent towards the west and is almost unknown on the prairie farms of the far west, and among the range cattle of the plains and mountainous country beyond. The extent of prevalence in the old dairy sections of the east appears to be in direct proportion to the activity of cattle traffic. If it is the practice of herd owners to buy their cattle, or if, in breeding herds, there has been a considerable interchange of cattle with other herds, tuberculosis abounds. If, on the other hand, it is the practice, as in many large sections, to rear dairy cows on the farms on which they are used and the current of the cattle trade is outward rather than inward, tuberculosis does not exist or it is a rare disease.

To illustrate this point, I may refer to a large Jersey herd near Philadelphia. This herd was established about twenty-five years ago and consists of more than one hundred cattle. It is in a country in which there
is as much tuberculosis as in any county in Pennsylvania. The herd is increased by breeding and not by purchase, excepting a bull occasionally, and, as has been shown by a tuberculin test, it entirely free from tuberculosis.

In many of the interior valleys of Pennsylvania a large number of herds have been tested without finding a single tubercular cow. These valleys are breeding districts, their cattle are principally of stock that was brought in by the early settlers many years ago, and the trade in cattle is outward. In other sections of Pennsylvania and other eastern states, tuberculosis is very common; some herds have been almost completely exterminated by it and in certain restricted localities it exists on almost every farm. Notwithstanding the extent to which it prevails in some sections and the fact that it has brought ruin to many farmers, I do not think that the distribution among all the cattle of Pennsylvania exceeds about 2.5 per cent.

Tuberculosis has spread very rapidly among cattle in this country during recent years. Of this I am convinced by the statements of veterinarians, butchers and stockmen of many years' experience. While it is necessary to recognize the fact that much of this testimony is inaccurate it cannot be denied that much of it is of value and that practically all of it points in the same direction. Moreover, I have myself been able to trace the infection of numerous herds to a single source in localities recently infected. In one instance, the infection of seven herds in widely separated placed in Pennsylvania, including three districts in which tuberculosis was previously unknown, was traced to a famous herd of cattle that was broken up and sold at auction. It was afterwards ascertained that this herd was almost saturated with tuberculosis.

It is natural that tuberculosis should spread at a constantly increasing rate as the centers of infection multiply, unless active measures are taken to check it. As proof of this, we have the experience of the countries of Europe. The slaughter house records of France, Holland and Germany show that tuberculosis of cattle and swine has increased enormously in the past ten years in some places from 30 to 40 per cent of all cattle killed are tuberculous. Denmark is one of the few European countries where, thanks to the valuable original methods of Prof. Bang, the disease is actually being repressed.

Unless this cancer on our herds is to be permitted to develop until the annual losses occasioned by it are increased many fold and the conditions that now exist in Europe and in many parts of this country, become common, something must be done. As to who shall take whatever action is authorized, there can be no doubt that under present conditions the bulk of the work will fall upon state officials rather than upon those connected with the federal or with the local governments.

The federal government is doing very effective work in this connection by keeping tuberculous cattle out of the country and in assisting in the control of interstate shipments and in conducting careful meat inspection
in many places, but it has not yet taken active part in the suppression of tuberculosis in already infected herds. Nor have local governments taken up this work seriously other than New York City, Philadelphia, and, perhaps, a few other municipalities. Under the conditions prevailing and in view of the precedents already established, it is probable that this work be looked upon as state work for some time to come, although it is to be hoped that the Bureau of Animal Industry can eventually assume more of the responsibility for the examination of cattle, or at least of dairy cows and cattle for breeding purposes, shipped from one state to another.

Certain objections have been raised to public action in relation to tuberculosis and these may be formulated as follows:

A. Objections to all public measures.
   1. It is alleged that they are unnecessary.
   2. It is alleged that they cannot succeed.

B. Objection to certain measures.
   1. To the use of the tuberculin test on the alleged grounds,
      a. that it will injure healthy cattle;
      b. that it is not infallible, and,
      c. that it is too searching.
   2. To the payment of indemnity for animals condemned and destroyed.

A. 1. As to the first point, there are some writers and speakers who deny that tuberculosis is anywhere a wide-spread or even a serious disease among cattle. The tuberculosis question has now been discussed so much that such statements can be accounted for only by the assumption that their authors wilfully disregard knowledge that they may easily acquire and in this case, it is useless to discuss the subject with them.

Another objection, but a sincere one, that falls under the same heading, is based on the belief of some that tuberculosis of man and cattle are distinct disease, or, perhaps, such distinct varieties of the same disease that there is no danger that this affection may be transmitted from cattle to man. Quite recently this argument has been taken up in force by writers in agricultural papers as a result of the expression of an opinion before the legislative committee appointed to inquire into the tuberculosis question in New York State. This opinion is to the effect that there is no danger that tuberculosis of man may result from the ingestion of the milk of tuberculous cows, and is supplemented by the statements of several gentlemen who had owned tuberculous cows and had used the milk in their families, and otherwise, and had observed no bad results. If the matter were only one of opinion it would be sufficient to arrange the opposing opinions in two sets and weigh one set against the other, having due regard for the standing, attainments and experience of those responsible for them, somewhat after the manner of a French court-martial. If this were done, there can be no doubt that the weight of evidence, as is shown by the expressions at the recent tuberculosis congress in Paris and Berlin, would support the doctrine of transmissibility. But the question is not one
of opinion, but of fact, and the opinions count only as they have facts to support them. In this connection, we must remember that a positive observation records a fact and is worth innumerable negative observations. If a man should say, for example, that he and many of his friends had traveled without injury on railroads for years and that he did not believe in railroad accidents, there would be little consolation in this statement to the man whose child was killed in a railroad wreck, no matter how many endorsements the opinion might have. Thousands have been exposed to cholera and yellow fever without injury. Does this prove that these diseases are not contagious?

When it is said that if tuberculosis was carried by the milk of tubercular cows there would be far more tuberculosis among milk consumers than there is, we must bear in mind that the great majority of cows are not tubercular and that only a certain percentage of the tubercular ones furnish milk that contains tubercle bacilli. And we must not forget that tuberculosis is extremely prevalent among people and that while it kills from one-eighth to one-seventh of mankind it is even more prevalent than these figures indicate for tubercular lesions exist in many people that die from other causes. If there are those who hesitate to believe that tuberculous milk may cause tuberculosis on account of the alleged limited prevalence of this disease among people, how many people would have to become tubercular to convince them? At present, tuberculosis is the most widespread and fatal disease of man—a veritable scourge.

As to the identity of the tubercle bacilli from tubercular men and cattle, the observations on this phase of our subject cannot here be reviewed in detail, and it is perhaps sufficient to say, that they were declared to be the same in 1882 by Koch, their discoverer, and that since that time this view has been held by almost all bacteriologists, and no points of difference have been pointed out by any one who has studied these germs in any part of the world that are even as great as those observed between the germs of many diseases that are confined to but one species of animals. Such comparative observations and experiments as to virulence as have been made with tubercle bacilli from cattle and man indicate that, as a rule, the former are the more virulent. The germs of tuberculosis of cattle have been transmitted by either intentional or accidental inoculation to, and have produced fatal tuberculosis in horses, donkeys, swine, cats, dogs, sheep, goats, rabbits, guinea pigs and man. The milk from tubercular cows has been the cause of tuberculosis in numerous feeding experiments performed on calves, swine, dogs, cats, colts and other animals. The type of lesions produced in such cases have been observed in children and in others that have consumed milk from tubercular cows, and in many of these cases no other source of the disease was evident. To those who ask for further proof of the transmission of tuberculosis from cattle to man there can be put one convincing demonstration, and that could be obtained only by a deliberate feeding experiment on a person known to be free from tuberculosis and protected from all sources of infection excepting through the food, it is needless to say that this piece of evidence will not be adduced.
It is generally believed that usually people who contract tuberculosis are infected by way of the respiratory tract and that infection by food is rare except among infants and invalids. It is undoubtedly true that in a large measure the general health of a person determines his resistance to the attacks of the tubercle bacilli when introduced into the digestive or respiratory passages. It is natural, therefore, that as houses and workshops are improved in respect to lighting, ventilating, heating and cleanliness, and as the contagious nature of tuberculosis is recognized more and more, the disease should become less prevalent—and this has actually happened during the past twenty-five years and to marked degree. In the meantime, tuberculosis of cattle has been on the increase. Does this, as is frequently claimed, show the fallacy of the view that tuberculosis of cattle has some causal relation to tuberculosis of man? Evidently not, unless it is held that tuberculosis of cattle is the principal cause of tuberculosis of man. If the reserve fund of a bank is constantly falling, does this show that a particular depositor has reduced his patronage? Since there are more productive causes of tuberculosis in man than the milk from tubercular cows, is this a reason why this source of disease should not be removed?

2. In reference to the objection to all public action on the ground that it cannot be successful, it is well to consider the reasons upon which this allegation is based. It is claimed, for example, that tuberculosis is produced by bad conditions as to stabling and herd management or that these conditions are indispensable to its development and progress and that, therefore, the disease cannot be held in check until what the advocates of this view term "the root of the trouble" is cut off. That is, until farmers have clean, well lighted and ventilated barns and keep their cattle in a "natural" way it is useless to attempt to limit this disease. This view is carried so far by some that they hold that tuberculosis may originate de novo when the conditions as to stabling are bad.

This ground has been gone over so often since 1882 that it is useless to cover it at length here. As sufficient proof that these views will not stand a test, I have only to call your attention to the well known fact that many of the most extensively tuberculous herds have been kept in the best possible barns and subject to conditions that, in the light of our present knowledge, must be looked upon as perfect, with the exception that tubercular animals were not rigidly excluded by the application of the tuberculin test when the herds were established. As a recent notable example of such an incident I may cite the case of the Queen's dairy herd at Windsor. As a matter of fact, tuberculosis may spread under the best practicable stabling conditions.

On the other hand, the tuberculin test has been applied to a large number of herds in Pennsylvania that are kept under the worst conditions and has, in many instances, failed to disclose the presence of a single tubercular cow. As coming under such bad conditions, I may mention continuous stabulation for six months each year; close, dark and filthy stables and high and stimulating feeding on mill feeds and ensilage. Where herds
kept in this way are sound, it is because it has not happened that a tubercular cow has been added to them.

Another objection of this class is that no matter how thoroughly tuberculosis is eradicated among cattle it will soon return unless they are excluded from direct or indirect contact with tubercular people, tubercular dogs, cats, rats, swine, horses, etc., etc. As a matter of fact, though there are very many recorded instances in which tuberculosis has undoubtedly passed from cattle to other animals, and there is abundant proof that tubercular cattle are the chief source of tuberculosis in other animals which consumed their milk or tubercular tissues, I have not found one reported case in which it was even suspected that tubercular disease had passed in the opposite direction. While the theoretical possibility of such transmission cannot be denied, cattle are not exposed to infection from other animals, first, because they do not consume their products and, second, because they do not associate with them closely, as with their own kind, and are thus not exposed to more than a very few of the tubercle bacilli emanating from them. Again, the comparative rarity of tuberculosis in animals other than cattle, and swine that have been fed on the milk from tuberculous herds, reduces this alleged danger to such insignificant proportions that it may be safely disregarded in all cases excepting those in which there are obvious reasons for considering it.

As to the danger of transmission of tuberculosis from men to cattle, the recent experimental work of Dr. Theobald Smith and Dr. Langdon Frothingham and, in addition, numerous inoculations and feedings of calves with sputum from consumptives at the laboratory of the Pennsylvania State Live Stock Sanitary Board, have shown that tubercular sputum from man usually possesses but a low degree of virulence for cattle. Then, too, in some places, as Nantucket, Cape Cod and Saranac, there is much tuberculosis among people and little among cattle. This condition also prevailed in Japan until recently.

Indeed, there is as little danger that cattle may become infected with tuberculosis from other animals as that a flood may be caused in the Mississippi river by a discharge of water from a bayou fed from it.

B. 1. Objections to the use of tuberculin are becoming rarer and rarer. It was natural that there should have been much objection to its use in the beginning when its method of manufacture and properties were unknown and when special attempts were made to use it against the wishes of the owners of cattle. Reference to the records of thousands of animals that have been tested with tuberculin show that there is now no ground to fear that it will injure healthy cattle. That it is not infallible has not been claimed, so far as I am aware. There is nothing in the science of medicine that is infallible and all that is claimed for tuberculin is that it is exceedingly reliable and gives far more accurate results than have ever been obtained without it. Where an animal reacts in a characteristic manner to tuberculin that animal is tubercular in almost every instance. In the work of the State Live Stock Sanitary Board of Pennsylvania tubercular lesions
have not been found in six animals condemned by the use of tuberculin, and this out of 4,501 cattle destroyed. The errors, therefore, in this direction, are infinitesimal.

As to how many animals that are actually diseased are overlooked upon physical examination, and also fail to respond to the tuberculin test, there has been but little opportunity in this country to obtain knowledge. The fact that tuberculosis has been eradicated from so many herds by the use of tuberculin and that herds have remained free from tuberculosis during the several years that they have continued under observation, shows that not many infecting animals are allowed to remain in herds that have been well inspected. There are, however, a few cases, some of which have occurred under my own observation, in which, for some unknown reason, infecting cows that could not be detected clinically have failed to respond to the injection of tuberculin and have not been detected until after they have conveyed disease to some of their associates. For example, in one instance all of the reacting animals were removed from a herd and it was supposed that the herd had been placed on a healthy basis. Subsequent testing, after an interval of a year, showed the presence of several reacting cows. All of these were removed, and the herd was again tested after six months and more reacting cattle were found. At the last test, among others, a cow that had been in the herd for several years responded and was destroyed and was found to contain advanced lesions of tuberculosis. Since that time the herd has been twice tested and has been kept under the most careful observation and no more cases have appeared. Why this infecting cow failed twice to respond to tuberculin is unknown. The case is mentioned here to emphasize the fact that tuberculin is not infallible and that it must be used carefully and with judgment and that in herds extensively infected the test must be repeated. This case and its occasional counterparts cannot be used to support an argument against tuberculin because they are distinctly exceptional cases, and against such exceptional cases there may be arrayed hundreds in which the trustworthiness of the test is shown. No one can deny that tuberculin is the most accurate diagnostic known. It is not perfect, it is only the best.

A more popular objection to the use of tuberculin than that it fails to disclose the presence of existing diseases is that it is too searching and indicates, by producing a reaction, that animals are tubercular, when such animals are frequently infected to such a slight extent as to be of no consequence. It is true that a large number of early cases of tuberculosis are detected by the use of tuberculin. Many of these animals, it is alleged, would recover from tuberculosis, if not interfered with. Perhaps from one-fourth to one-third of the animals that are found to be tubercular by the use of tuberculin are not excreting tubercle bacilli at that time, and it may be that in the case of a few of them the lesions would become encysted and calcereous, constituting practical recovery. There is no reason to doubt that a large portion of the reacting cows that are not excreting tubercle bacilli when tested will, sooner or later, reach a point where they will become infecting.
If this disease is to be eradicated in a herd, it is not only important that the cows that are actually spreading tubercle bacilli shall be removed from contact with healthy cows, but also that the animals shall be removed that are almost sure to be capable of spreading disease in the future.

I have heard the remark made when a cow has been killed that showed slight lesions of tuberculosis that the condemnation of such an animal is unwarranted, because the disease is of such slight extent that the animal would recover or remain harmless for several years. Such statements express bold opinions for which there is no warrant. Cows that are most extensively diseased and in which the disease has pursued a rapid course were at first infected in but one small place and the lesions were as slight as in the cases so alluded to. No one can tell from the appearance of a fresh tubercle whether, if the natural progress of the disease had not been interfered with, it would have terminated in generalized tuberculosis or whether it would have progressed to a certain extent and then have remained stationary. If the outcome of the disease cannot be anticipated where the diseased part is thus exposed to the eye and can be subjected to examination in the laboratory, how much more difficult it is to prophesy what course the disease will follow in a reacting cow that is infected to an unknown extent and may harbor extensive lesions.

It is of the highest importance that tubercular animals shall be detected before they have reached the infecting state. When an animal in this condition is removed from the herd the expense is slight, but if this animal is allowed to remain in the herd until it has commenced to sow the seeds of disease among its associates, it becomes necessary not only to remove this individual, but also the others that have become infected from it.

2. The payment of indemnity for tubercular animals condemned and destroyed is sometimes objected to on the ground that such animals have no value and it is unjust to tax the public to pay for them. It is also held that a tubercular animal is a public nuisance and should be disposed of at the expense of the owner as some other public nuisances are allayed.

The claim that a tubercular cow has no value cannot be sustained excepting in the case of a cow in an advanced stage of disease. A tubercular cow unless extensively diseased can produce a healthy calf that will remain healthy if certain precautions are observed. Moreover, a tubercular cow produces milk that is perfectly wholesome after it is cooked, and the same may be said as to the flesh of a tubercular animal. Therefore, if the public demands that tubercular cattle shall be killed, it demands the confiscation of valuable private property for the welfare of the public, and even the state has no right to confiscate a man's lawful property without indemnity.

The payment of indemnity for tubercular animals is in the line of good business policy. If tuberculosis is to be suppressed among cattle, tubercular cattle must be discovered. If they are not discovered and reported by their owners, it will be necessary to employ an army of inspectors to hunt
for them. If the discovery of a tubercular cow would bring loss to its owner, attempts would be made to dispose of it as quickly as possible, thus distributing disease widely, or to conceal it. Such an inspection and method of control would be unpopular and its enforcement would be exceedingly difficult, if not impossible. It is far cheaper to make each herd owner an inspector of his own cattle, to discover and report suspicious cases among his own animals.

There is now consensus of opinion to the effect that some control should be exercised over tubercular cattle. The growth of public opinion on this question is interesting and instructive. At first there was violent opposition to any action on this question and it was denied that tuberculosis of cattle was a disease of any consequence. Then, when the tuberculin test came into use it was objected to most strenuously and all sorts of unfounded objections to it were made, and the most dismal prophesies as to the results of its use were published. At the same time there appeared a demand on the part of health boards and people in cities that their meat and milk should be protected from contamination with tubercle bacilli. Active controversies were instituted between the consuming public, represented by the daily papers and some sanitarians, on one side, and the producers, represented by the agricultural press, on the other. On one hand, the dangers from tuberculous meat and milk were set forth and were sometimes highly colored and exaggerated, while on the other hand they were minimized and facts concerning them were suppressed.

The Bureau of Animal Industry, the experiment stations and veterinarians during this controversy has been somewhat uncomfortable. They have been berated by some for not doing what was pointed out as their duty and at once examining, quarantining and destroying all of the tubercular cattle to be found. By the other party, they were accused of gross exaggeration and misrepresentation, of doing too much and interfering with old established customs and conditions and with private property and personal rights that were held to be of no consequence to the public. During this controversy, and before much accurate information was readily available upon this question, some writers stated so many things that are now known to be untrue that, even if they so desire, they find some difficulty in assuming an unbiased position at the present stage of the discussion. This is shown in a very amusing manner by the tendency of such writers to set up and knock down men of straw. They have ceased to say that tuberculosis of cattle is a disease of no importance and that it may be produced by tuberculin or that absorption or permanent loss of condition will follow the use of this diagnostic agent. They no longer say that the milk from the tubercular cows is harmless and may be used with impunity, no matter how extensive the disease.

That the attitude of some agricultural papers is not yet altogether fair is shown by their fondness for publishing misleading and unsubstantiated reports of alleged instances in which many healthy cattle have been condemned and killed, and other items calculated to discourage efforts to repress tuberculosis of cattle. Moreover, there is an inclination to publish
observations and opinions that tend to minimize the importance of this subject and to refrain from publishing carefully digested facts in regard to it. This is illustrated by a recent instance; the last royal commission on tuberculosis that was appointed by the English Parliament was a body of prominent men selected on account of their special ability to carefully weigh and consider this subject and to recommend the administrative measure necessary to repress tuberculosis. This commission called before it and obtained evidence from farmers, dairymen, butchers, cattle shippers and many of the most eminent veterinarians, pathologists, bacteriologists and the most experienced health officers in England. Nearly two years was devoted to this inquiry and it was conducted in a most exhaustive manner. The extensive and valuable report of this commission has scarcely been noticed by the agricultural papers of the United States. On the other hand, a certain professor in an agricultural college in England, a man who has no special knowledge of the diseases of animals, and does not claim to have, recently expressed his individual opinion in regard to tuberculosis in a most intemperate and unreasoning way and this statement has been printed and copied and reprinted and quoted by our own agricultural papers and writers to a remarkable extent.

As against this attitude of active and passive opposition to the acquirement of a fair knowledge of the facts in regard to this question, that has been manifested by some papers, there are some notable exceptions, and these papers deserve praise and confidence.

But the situation is gradually being made clear and at this time the most pressing question is not—shall tuberculosis of cattle be suppressed? but is—how shall tuberculosis of cattle be suppressed? Before deciding upon a plan it is necessary to review some of the facts in regard to the method by which tuberculosis is distributed among cattle and the methods that have been suggested to check this distribution.

In the first place, we must recognize that tuberculosis is a contagious disease that may be propagated by cohabitation of tubercular with healthy animals and by feeding the products of animals that are tubercular to a certain degree. Calves usually acquire tuberculosis by feeding upon the milk of tubercular cows. Older cattle usually acquire the disease by cohabitation. We must not disregard the fact that tuberculosis is sometimes congenital, although this method of transmission is rare and is likely to occur only from cows that are extensively diseased. It is necessary to at once recognize the inefficiency of suppressive measures directed wholly along the line of improved methods of breeding, housing and feeding. Tuberculosis is not a respector of any breed or cross, it is not produced and cannot be prevented by any method of feeding, and it occurs in stables that are of the best construction and among cattle that are handled in the most rational way.

It is also necessary to bear in mind that tuberculosis is frequently extensively developed in animals that show no external signs of disease and among animals that appear to be and are believed, until tested, to be in perfect health, and that such animals may excrete and scatter tubercle
bacilli. Most of the infectious material that is distributed by cattle comes from animals visibly diseased, but much tuberculous material, and enough to propagate the disease indefinitely, is excreted by animals that are not visibly diseased.

In view of these facts, we may conclude that if tuberculosis is to be suppressed among cattle, it is necessary to prevent the use of infectious food and contact with infected animals and objects, contaminated by them; and if these measures are to be enforced, their value will be in proportion to the thoroughness of separation (and this depends on the accuracy of the method of diagnosis that is employed), and upon the efficiency of the disinfection that follows the removal of infected animals.

Since the tuberculin test is by far the most accurate diagnostic that is at present available, it should enter into every plan for the suppression of tuberculosis.

If we accept the above as a basis upon which a plan for the suppression of tuberculosis should begin, and proceed from this to the formulation of a practical measure, we are at once confronted by several difficulties which may be classified as follows:

1. As to the selection of herds for inspection.
2. As to the treatment of reacting animals.
3. As to the prevention of the re-infection of inspected herds.
4. As to the expense.

Each of our states contains so many herds in which so many people, so much capital and so many interests are involved that the matter as to the selection of herds for examination should be carefully considered. All the herds in a state may be tested or all the herds in a certain district where tuberculosis is believed to prevail most extensively; or the herds for testing may be selected by physical examiners who examine all the herds in the state or in certain districts. Or, all herds supplying milk for shipment or for consumption as milk may be tested, or herds reported as probably diseased, may be tested.

The selection for compulsory examination of a part of the herds is sure to occasion friction and opposition. Many herds will be tested that do not need testing (useless expense) and many herds will be overlooked that should be examined.

The other method is to allow the herd owners themselves to select herds for examination. Every herd owner who appreciates the facts in regard to tuberculosis, will desire to rid his herd of this disease, provided the immediate loss is not greater than he can stand, and provided the conditions he must observe are not more onerous than the presence of the disease.

In regard to disposing of the reacting animals, this can be done by requiring that they shall be slaughtered at once or that all or that a portion of them, depending upon the stage of the disease, may be kept in quarantine for a limited or for an indefinite period. The method of treating
the animals after testing will depend upon their value and upon the char-
acter of dairying in the districts in which they are found and upon the con-
dition of public sentiment in that district.

When the animals are slaughtered the carcasses may be destroyed or
used for technical purposes, or, if killed in slaughter houses, the meat of
some may be used for food depending again on the stage of the disease. It
is only in advanced or generalized cases that tubercle bacilli enter the
blood and infect the meat. This condition can be recognized by competent
inspection and harmful meat kept from the market. Moreover, cooking
meat sterilizes it. The disposition of the carcasses of reacting animals
will depend upon the extent to which the public is informed on this ques-
tion.

The matter of expense is a local one and the work of each state must
be regulated in accordance with the funds that are available for this pur-
pose. Where reacting cattle are killed at once and paid for the expense is
very great. Where the carcasses that are suitable for food are sold for
this purpose the expense is materially reduced.

According to the method of selecting herds for treatment, the various
methods for controlling tuberculosis may be divided into those that are
compulsory in some respects and voluntary in others.

The most extreme example of the compulsory method is the one that
was instituted some years ago in Massachusetts. The general features of
this measure are well known, and it is only necessary to say here that it
did not succeed, and for the reason that it did not enlist the support of the
public. A somewhat similar but less rigorous method was adopted in
Belgium, in January, 1890, and this, also, has been materially modified,
because it became evident that it was too onerous and expensive. Massa-
chusetts and Belgium are both small, rich states, with comparatively
small dairy interests. The failure of these methods under such conditions
means that they can scarcely be expected to succeed elsewhere.

A method of compulsory control that was proposed by Professor
Siedamgrotzky at the meeting of the International Veterinary Congress
held in Baden Baden last month, is based on the pre-existence of a gen-
eral system of meat inspection covering all animals killed for food, and
the compulsory insurance of cattle against tuberculosis. Under this meth-
od herds, from which tubercular cattle are reported by meat inspectors
shall be examined and measures taken for the suppression of the disease
at the expense of the state. The tubercular cattle shall, when killed, be
used for food if suitable. If they are condemned, or parts are condemned,
75 per cent to 80 per cent of the loss shall be made good; one-third of this
amount being contributed from public funds, and two-thirds, or approxi-
mately one-half the entire loss, to be paid from the insurance funds. The
owner would then sustain one-fourth of the entire loss.

In France, the laws of 1888 and 1898 provide that animals discovered
to be tubercular shall be quarantined and sold only to be killed, and if the
meat is condemned the owners are reimbursed to the extent of one-half
the value, if the disease is generalized, and three-fourths of the value if the disease is localized.

As voluntary methods of control, we may consider those that are independent and not assisted directly by the state, and those that are encouraged and supported by the state. The first of these will succeed in proportion to the desire of herd owners that the disease shall be suppressed, and to their ability to undertake the measures that are necessary. It will succeed best in districts characterized by the greater intelligence and wealth of the rural population. It cannot be expected to succeed as a general measure. But when the system is supported by the state, its application will be wider. The extent to which any voluntary system will cover the herds of a state will depend largely on the proportion of the expense and loss that the state will bear. Every voluntary system must include, as one of its integral parts, a plan for distributing information on the general subject, and information as to the part that the state will assume of the burden of suppressing tuberculosis.

In Denmark, under the leadership of Professor Bang, a most successful struggle is being waged against tuberculosis. The plan there is largely voluntary. Upon application, tuberculous herds are examined and tested; Tuberculin and veterinary services are furnished by the state. Tubercular cattle must be destroyed if the udder is affected, or if the disease is advanced. The other cattle may be kept and used under regulations requiring them to be kept apart from healthy cattle, the removal of the calves from infected cows and the rearing of them on pasteurized milk, or milk from healthy cows. It is the almost universal practice in Denmark to pasteurize the cream used for making butter, and creameries are now required by law to heat skim milk before it is returned to the farm and to burn the separator sediment. In addition, the prompt destruction, with partial indemnity, or udder cases is now required.

In Norway a plan for controlling tuberculosis has been developed and operated by Dr. Malm, and this appears to work smoothly and to produce good results. It is similar to the Danish system.

In reference now to the Pennsylvania plan for suppressing tuberculosis: Pennsylvania has an area of 45,000 square miles, about 6,000,000 inhabitants and about 2,000,000 cattle. As no census has been taken for nine years, these figures must be approximated until after the census of 1900. The agriculture of Pennsylvania is mixed and the dairy industry is well developed. Many herds are devoted to the production of milk for shipment to Philadelphia, New York, Pittsburgh, Baltimore, and to other lesser cities. Much butter is made at the 600 creameries scattered over the state. The farmers in the eastern counties purchase many of their cows, about 20,000 each year, from outside of the state, and probably twice as many from the central, western and southern counties. There are also some districts in which many steers are fed. The steers come from the western part of the state, from the stock yards at Chicago, Buffalo and Pittsburgh, and from West Virginia. One county, Lancaster, feeds from 20,000 to 25,000 steers each winter.
As stated above there is much difference as to the extent which tuberculosis prevails in different parts of Pennsylvania. It is most common in the old dairy districts. Some herds are infected to the extent of 30 to 100 per cent, other herds contain few infected animals, and others—the great majority—are free from tuberculosis. Of all the cattle in the state the number of those infected appears to be between 2 and 3 per cent.

In 1895 a law was enacted creating the State Live Stock Sanitary Board and defining its duties. This board is organized to suppress, control, or eradicate dangerous, contagious or infectious diseases of animals; it is composed of the Governor, Secretary of Agriculture, Dairy and Food Commissioner and the State Veterinarian. The Governor is President, the Secretary of Agriculture is Vice-President, the Dairy and Food Commissioner is Treasurer and the State Veterinarian is Secretary of the Board. This Board has authority within certain limits to make its own rules and regulations and to enforce them. The Board has received a grant of $40,000 per year for the support of its field work in relation to all diseases of animals; anthrax, glanders, rabies, etc., as well as tuberculosis. In addition, it has a small grant used for the support of a laboratory for making the tuberculin, mallein and anthrax vaccine needed by the Board, and for research.

The work of the State Live Stock Sanitary Board did not begin until 1896. Up to that time the state has rendered herd owners no assistance in the suppression of tuberculosis, excepting in the case of a few herds examined under the authority of the Secretary of the State Board of Agriculture. It was evident at that time that the herd owners were generally interested in freeing their herds from infection and that if a satisfactory plan for co-operation were prepared, it would be accepted by many. It was evident that there was not money enough available to justify any scheme for the examination of all herds, and it was realized that if sufficient money were available the success that would probably attend such an effort would not justify either the expenditure or the interference with farming operations.

To examine all of the herds in one district, and to neglect those in others would be unjust and would be to excite opposition and invite defeat. While it was undoubtedly desirable to do away as promptly as possible with all advanced and udder cases, it was realized that to do this alone would be to permit the disease to continue indefinitely and that this would not reduce the distribution of tuberculosis. To purchase at any prices advanced and udder cases alone and not to free the herd from infection and disinfect the premises, would be to transform a work that should have permanent sanitary value into a free live stock insurance operation. If a herd owner is not himself interested in suppressing tuberculosis among his cattle, the work can only be done by the application of force and the use of a system of veterinary sanitary police that is not available in Pennsylvania.

Since so many herd owners did wish to eradicate tuberculosis, it was arranged that these should have help first and that no herds should be
tested with tuberculin excepting upon application from the owner. Application is made by signing a printed form which is also a contract under which the owner agrees to dispose of his reacting cattle in accordance with the rules of the Board, to disinfect, to correct faulty sanitary conditions and to do all that he can to keep his herd free from tuberculosis in the future. After test, the reacting animals are separated and the owner is permitted to keep alive those that show no clinical signs of tuberculosis if they are housed and cared for apart from the rest of the herd, if their calves are removed from the premises occupied by the cow as soon as born and if the milk will not be used without pasteurization. Or, the reacting animals may be killed at once after appraisal. The limit of appraisal for cattle is $25 for unregistered animals, and $50 for registered animals. The law provides that animals shall be appraised according to their actual value and condition at the time of the appraisal, but not to exceed these limits. An animal in an advanced state of disease is considered to have lost its value except for fertilizing purposes and is appraised accordingly.

It is interesting to note that, excepting in the rarest instances, farmers do not care to keep reacting cattle on the farm subject to the conditions it is necessary to impose. The extra time and expense necessary for their care and the fact that their presence is usually misunderstood by the neighborhood and that there is no market for heated milk, combine to make this practice unpopular. It is not followed, excepting once in a while in the case of a valuable cow that is in calf. The reacting cattle are usually killed in rendering works and made into fertilizer.

Our public complaisantly accepts and eats meat that is uninspected with the full knowledge that many diseased animals are killed for food but when a cow has been tested and declared tubercular an outcry is at once made against the sale of its meat, no matter how slight the lesion may be. This illogical but firmly rooted prejudice makes it impossible, at present, for the state to recover the meat value of a portion of these carcasses.

An attempt is always made to arrange for the destruction of tubercular animals in the neighborhood in which they are found. This makes it possible for interested persons to see the postmortem examinations and to gain information. This method of informing the public is supplemented by circulars and papers in agricultural reports.

Recently, the State Live Stock Sanitary Board has provided for the isolation and quarantine of such advanced and udder cases as are found. If their owners do not wish to have their entire herds tested, these animals may be appraised at a nominal price and destroyed, and the appraisal will be paid when the premises have been satisfactorily disinfected by the owner.

The demand for voluntary inspections has grown at a rapid rate and most rapidly in the sections in which the greatest number of inspections have been made. At this time each applicant is required to file a statement as to his reasons for desiring his herd tested and, since applications
are so very numerous and the funds are limited, inspection is not made unless there are satisfactory reasons to believe that the herd is infected.

In the beginning of this work about 25 per cent of the cattle in inspected herds were tubercular; now, although the method of selecting herds to test is more rigid and there are more infected herds reported to select from, only 11.6 per cent of the cattle in inspected herds are tubercular. These figures represent the conditions in the most extensively infected herds in the state. Up to this time the number of cattle tested under these regulations is 33,147, of which 4,561, or 13.7 per cent were tubercular. For these $102,909.62 have been paid, or an average of $22.56 per head.

In 1897 it became evident that some action should be taken to keep tubercular animals from coming into Pennsylvania, and such action was demanded by several representative agricultural organizations. Several states east of Pennsylvania had such protection and increased knowledge of tuberculosis, and a desire to free herds from it leads to the sale and distribution of many infected herds. Moreover, many farmers wanted to be able to purchase tested cows. Hence the law that went into operation January 1st, 1898, that requires all dairy cows and cattle for breeding purposes to be tested if shipped to Pennsylvania. Since that time, New Jersey, Illinois, and Kansas have enacted similar laws.

As knowledge in relation to the manner of warfare that is successful against tuberculosis becomes more and more widely distributed it is constantly applied without state aid by those who are able to do so, and the work of suppression is going on much faster than indicated by the statistics of the public work alone.

The operations have worked smoothly throughout, and instead of objections to the effect that too much is done, there is a strong desire on the part of herd owners that more work shall be undertaken in this direction. This is shown by the fact that there are more than three times as many applications for tests as can be responded to; and by the action of a prominent breeders' organization asking that herds believed to be tuberculous shall be examined, whether the owner so wishes or not, when a request to this effect is signed by three cattle owners in the neighborhood. All of the work has been facilitated by the general appreciation of the fact that it is conducted under the wing of the State Agricultural Department for the benefit of cattle owners.

The field work has been done by the general practitioners of the state and Pennsylvania is fortunate in having a body of veterinarians who have been able to assume the work of enforcing these measures in a way that has won and retained the confidence of the public.

With the establishment of a system of meat inspection under rational regulations, many economies could be effected and the work could progress faster without increased cost.

Since tuberculosis has been exterminated on so many farms and in so many districts, which are constantly growing more numerous, and since
the flow of tubercular cattle into the state has been cut off, are we not justified in looking forward to the time when the losses from this disease will be reduced to insignificant proportions?

**DISCUSSION.**

MR. RIDDLE—Is not tuberculosis aggravated by the confinement of animals in barns?

DR. PEARSON—I have known of cases where the disease appeared in cattle in pasture, and others where they were stabled part of the time.

DR. CLUTE—Do you have any trouble in making tests in hot weather?

DR. PEARSON—Sometimes cattle shipped into the state have to be detained several days on that account before they are tested, but that does not occur among cattle on the farm.

DR. CLUTE—There were four different cases last summer where we had to abandon the test on that account; the temperatures ran from 103 to 105.5 the first day, and perhaps next day would be normal.

THE CHAIR—How many preliminary temperatures do you take?

DR. PEARSON—Not so many as formerly. Not less than two, usually three.

DR. EISEMAN—I would like to ask what agent you use for disinfecting.

DR. PEARSON—Cabolic acid. We tried corrosive sublimate but it was not satisfactory. The objection to corrosive sublimate is that it does not penetrate as the carbolic acid does. In Denmark the use of corrosive sublimate has been entirely discontinued.

THE CHAIR—Do you follow that with an application of chloride of lime?

DR. PEARSON—Usually.

A DELEGATE—I would like to ask in regard to the compensation paid to owners of diseased cattle. You state that $25 is the limit for grades and $50 for pure bred cattle.

DR. PEARSON—This is only an arrangement we have made which proves entirely satisfactory. It can hardly be called an appraisement; it is more of an agreement than an appraisement.

A DELEGATE—About what does it cost per year to pay for reacting cattle?

DR. PEARSON—Between $25,000 and $30,000.

A DELEGATE—What is your average hour in the evening for injecting?

DR. PEARSON—Six or seven o'clock, after two or three preliminary temperatures, the following day continue taking temperatures until at least sixteen hours after injection.
MR. TILLMAN—I do not intend to controvert the fact that tuberculosis is a dangerous disease; dangerous to the animal and a source of great loss to the owners of stock and dangerous to the human family, but I want to ask this question: All of this investigation seems to proceed on the idea that the increase of tuberculosis in infants indicates that the disease had its origin in the food. I would like to ask if the increase in the density of population, warmer houses and closer confinement of infants in this age may not be the cause of the increase of the disease among infants. It seems to me that question should be disposed of before we assume that the milk consumed by infants is the cause of the disease. Cattle confined in stables are more liable to have the disease than those running in the pasture. Tuberculosis is more common in cattle housed than in those running on ranches or on the barrens. Before assuming that the milk consumed by infants is the cause of the disease, you should look and see if there is not another cause to take the place of that; whether the greatest density of population, and the increase in the comforts of home, the child going less in the open air, may not be the cause of the increase in the disease. After it leaves childhood and these conditions are changed in a measure, the disease decreases. Crowded up as they are in cities like London and Chicago, you would naturally expect to see an increase in the prevalence of the disease.

DR. PEARSON—I would say, in reply to that question, that these statistics that I have quoted, and that were quoted yesterday, were taken from the statistics of the General Registry office of England. It has been shown definitely, and it is a well known fact, that the buildings and houses and habitations generally in London, and also in the larger American cities are far better now than they were forty years ago. Laws have been enacted requiring area ways to be a certain width; the houses to have a certain amount of light, and the condition of the filthy tenament houses that existed years ago, where people were huddled together with whole families in a room, has been greatly improved. There is no reason to believe that the conditions are more favorable to the growth of tubercle bacilli in children than in adults. Now, if the conditions have been improved to such an extent that the disease has been restricted in a marked degree among adults, as has been found to be the case, we should naturally infer—and justly infer—that the same conditions would restrict the prevalence of the disease among infants, provided infants, derived the infection from association. The fact that there is a decrease in the prevalence of the disease among adults and an increase among infants, would indicate that the increase among infants is due to their food, and we know that tuberculosis has been developed in animals by feeding them milk from tuberculous cows.

MR. LOTT—In regard to dairy cows coming into the state subject to inspection; how do you manage cows coming into your market, ostensibly for feeding purposes, some of them going out for dairy purposes? Is there any restriction on that point?

DR. PEARSON—Our difficulty in that direction is not great. Our conditions are entirely different from the conditions that exist here. We have
no very large live stock market in Pennsylvania; the largest is at East Liberty, which is one of the largest markets in the east. We have an inspector constantly in these yards, and everything about which there is any doubt is inspected and tagged before it is allowed to leave.

**DR. PETERS**—Mr. President: I think whatever discussion is desired to be had on these papers might well be postponed until we have acted on the reports of committees.

**THE CHAIR**—Is the committee on Line and Open Season ready to report?

In response Mr. Niles, from the Committee on Line and Open Season, reported the following resolutions which were on motion, adopted:

Resolved. That this Association recommends the withdrawal of the quarantine regulations applying to cattle on account of Texas fever infection from November 1st to January 1st; Provided that cattle below the quarantine line destined for feeding in the states of Missouri, Kansas and Texas, and the territories of Oklahoma, New Mexico and Arizona, be inspected, and only allowed to proceed in case they are found free from ticks; And provided further, that all infested cattle in transit through the states and territories mentioned, be treated as are infested cattle during the quarantine season.

Resolved. That the interstate Association of Live Stock Sanitary Boards respectfully recommends to the Secretary of Agriculture the establishment for the ensuing year of the present quarantine line bounding the territory to be scheduled on account of danger from southern cattle fever with the exception that Cumberland, Cannon and Lincoln counties in Tennessee, be placed above the line, upon the State Sanitary authorities of that state furnishing to the Department satisfactory proof that said counties are free from infestation with *boophilus bovis*.

WHEREAS. There is no doubt that on the first of November, when the open season, under the present quarantine regulations, will commence, there are in the Southern Divisions of all Northern stock yards numerous young ticks (*boophilus bovis*) rendering it dangerous for native cattle to be yarded in said Southern Divisions prior to the occurrence of sufficient cold to destroy such ticks, therefore, be it

Resolved. That this Association respectfully petitions the Secretary of Agriculture to issue orders prohibiting the yarding of any native cattle in the southern divisions of stock yards north of the quarantine line at any season of the year.

**MR. RIDDLE**—There are some counties in Missouri, which, I think if Missouri was here to defend herself, she would ask to have put south of the line. The Department of Agriculture is familiar with these counties as is also Kansas, and I have it from Missouri people that they are making a special effort to free these counties from the infection, and if the Bureau of Animal Industry—which is an familiar with the conditions in these counties as Kansas is—I say, if they are willing to still allow them to remain
north of the line, Kansas will make no protest, knowing they are making their best efforts to make these counties clean. There are several counties in Texas under the same conditions. We are assured that Texas is doing her level best to make those counties clean, so we are willing to accept the line as it was last year.

Mr. Kleberg, from the committee on Resolutions, to which was referred the resolution on tuberculosis offered by Dr. Pearson on yesterday, reported the same back with the recommendation that it be adopted.

On motion of Dr. Reynolds, the report of the committee was concurred in and the resolution was adopted.

WHEREAS, The contagion of sheep scab has been and is now being wisely disseminated through channels of interstate commerce; and

WHEREAS, Said disease may be easily and cheaply cured by proper dipping; and

WHEREAS, The Secretary of Agriculture has recently promulgated regulations requiring the dipping of diseased sheep found in transit from one state to another; therefore be it

RESOLVED, That this convention expresses its appreciation of the efforts of the Department of Agriculture to repress and control sheep scab and recommends the rigid enforcement of such measures as may be considered necessary to secure this result.

On motion of Mr. Cameron, the Secretary was instructed to transmit promptly to the Secretary of Agriculture copies of the resolutions regarding the open season and the establishment of the quarantine line.

DR. NILES—I would like to ask Dr. Salmon if there is any likelihood of these resolutions being rejected by the Department of Agriculture, and whether it would be proper for the various states to give notice that the open season would be from November 1st to December 31st. My reason for asking this question is that a great number of stockmen who are interested in this matter are writing letters asking for information in that direction.

Dr. Austin Peters, Chairman of the Massachusetts Cattle Commission read the following paper, on

THE SUPPRESSION OF BOVINE TUBERCULOSIS IN MASSACHUSETTS.

Mr. Chairman and Gentlemen:

Mr. C. P. Johnson, Secretary of the Live Stock Commission of Illinois wrote to me some time ago suggesting that I make a few remarks at this meeting upon the suppression of Bovine Tuberculosis in Massachusetts.

I am of the opinion that it will be much easier to give you an account of the steps taken for the eradication of this disease in Massachusetts, than it will to give you an exact statement as to what has been accomplished;
that is it would be a very difficult matter to say exactly what proportion of
the cattle of our State were tuberculous on a tuberculin test, or upon a
physical examination prior to 1894, when the work of eradicating bovine
tuberculosis was first entered upon extensively, and the proportion of meat
stock in the State that would now react to tuberculin, or that today shows
physical evidence of disease, but it may be instructive to give you a brief
history of the work of the Massachusetts Cattle Commission with a short
account of its various vicissitudes, its ups and downs, its policy at different
times in the past and at present, and some of the difficulties it has had or
has to contend with, and some of the snags it has struck. Such a narrative
may prove instructive, and if heeded will no doubt be useful to public offici-
cials having sanitary laws to enforce where nothing has yet been done for
the suppression of this malady, or where steps are just being taken to em-
bark upon this enterprise.

The experience of the Cattle Commission of the old Bay State is valu-
able in showing what the farmers and dairymen of a locality will, or will
not submit to, and how far radical measures are expedient, what is suffi-
cient to protect the public health, and whether the public in general care
to go to any further extremes than those necessary simply to protect their
health.

Massachusetts, while not one of the great agricultural states, being
more a commercial and manufacturing commonwealth, at the same time
has a large number of farmers among her population whose herds and
flocks add materially to her wealth. We have over 200,000 head of neat
cattle in our State, the greater number of which are milch cows; hence it
is necessary that her live stock interests shall be protected, and for this
purpose there is a Board of Cattle Commissioners.

Massachusetts is a commission governed State, and its Cattle Com-
misson is one of its older commissions, and it is one of the oldest if not
the oldest of the Live Stock Sanitary Boards in any state in the Union. It
became necessary for the live stock interests of Massachusetts to be pro-
tected by law years before it was requisite for most of her sister states
and those that should have been protected failed to see the necessity for
such protection to their own subsequent loss, and at a great expense to the
national government in later years.

Forty years ago, in 1859, contagious pleuro-pneumonia was imported
into Massachusetts from Holland in a herd of Dutch cattle, and after a
struggle of five years it was finally stamped out in 1864, since which time
it has never again been allowed to make its appearance in our State.

Occasionally when I hear of the great achievements of the United
States Bureau of Animal Industry, and the credit it takes to itself for
eradicating contagious pleuro-pneumonia from this country, I am con-
strained to smile a faint smile to myself when I remember that Massa-
chusetts did this duty of her own volition years before the Bureau of Ani-
mal Industry was dreamed of, and that if New York and New Jersey had
been sufficiently fit for self-government at that time to do likewise, this
vast labor and expense would have been spared to the general government and contagious pleuro-pneumonia would never have obtained the foothold it did in Pennsylvania, Maryland, Virginia, and finally in some localities West of the Alleghenies, leading to the great outbreak here in the suburbs of Chicago in 1886, which I had an opportunity of seeing when here as a delegate to a Live Stock Growers Convention at that time.

After terminating its labors in eradicating contagious pleuro-pneumonia, the Massachusetts Cattle Commission was dismissed, but in 1867 an outbreak of Texas fever made it necessary for the governor to appoint a new commission and since that time it has had a continuous existence. For a number of years its duties were mainly confined to dealing with glandered horses, outbreaks of "hog cholera" (used here as a generic name for any supposed contagious swine disease) and keeping Texas fever out of the State during the summer months.

The attention of the Cattle Commission was first directed towards the problem of bovine tuberculosis, by Dr. J. F. Winchester, of Lawrence, who was appointed to the Board in October 1885, and served as a member until October, 1889. He noticed the alarming prevalence of this disease among our meat stock and while he was a member of the Commission a good deal of space in its annual reports was devoted to a consideration of this subject.

As the result of modern knowledge of tuberculosis, and constant agitation the Massachusetts Legislature in 1893 passed an act recognizing this malady as one of the contagious diseases of animals, and providing that animals suffering from it could be ordered killed by the Cattle Commission or any of its members without appraisal or payment. Tuberculosis was found to prevail, however, to such an extent among our herds that it proved a great hardship for cattle owners to have their animals killed without receiving anything for them, resulting in a change in the law in 1894, providing that cattle condemned as having tuberculosis shall be appraised and the owner paid half of the value for meat or milk purposes for each animal killed.

The Legislature also, in 1894, passed an act providing that the Board of Cattle Commissioners should thereafter consist of five members, instead of three, as formerly, it being represented to that body that there was so much work to be done in connection with eradicating bovine tuberculosis, that no three men (who had formerly been sufficient to perform all the duties on the Board) could do all the necessary labor, and that a larger Board was needed. This idea was correct to a certain extent, but no five men could do the work the reorganized Board had mapped out, and a small army of agents and assistants was required.

As far as doing the Executive business portion of such work is concerned a Board of three is better than one of five; even one man with the necessary employees could carry out the duties of the office, although at times the consensus of opinion of three men may be better than that of one but nothing is gained by having a larger body.
In the autumn of 1894, the Cattle Commission having investigated the use of tuberculin and decided upon its reliability as a diagnostic, announced that all the cattle in the State were to be tested, commencing on Cape Cod and working West, that all reacting animals were to be killed and under the law the owners were to receive half the animal's value. Under the provisions of this act 810 cattle were condemned and made into fertilizer or buried, as the law until 1898 provided that all diseased animals were unfit for human food, no matter how slight or localized the lesions might be. Since 1898 the Massachusetts Cattle Commission has had the power to make rules and regulations for the inspection of meat to conform with those of the United States Bureau of Animal Industry.

Our farmers very naturally objected to a law which gave the Cattle Commission the power to test animals that to all outward appearances were healthy, and then to kill those that reacted and pay but half the value, especially as it is very difficult to make the average farmer understand that a nodule the size of a pea in a mediastinal gland is a serious and dangerous disease, rendering the milk unfit for use and making the flesh dangerous as an article of food. The result was that in 1895, the law was so amended as to give owners of condemned cattle their full appraised value up to a limit not exceeding $60, and the passage of an act restricting the use of tuberculin. The use of tuberculin as a diagnostic agent for the detection of the disease known as tuberculosis in domestic animals shall be restricted to cattle brought into the Commonwealth from any point without its limits, and to all cattle at Brighton, Watertown and Somerville; provided, however, that tuberculin may be used as such diagnostic agent on any animal or animals in any other part of the State, upon the consent in writing of the owner or person in possession thereof, and upon any animals condemned as tuberculosis upon physical examination by a competent veterinary surgeon."

For carrying out the law in 1895, $150,000 was appropriated; about 8,100 cattle were tested, of which about 2,800 were killed and paid for. Of these 4,015 were reported as suspected of being tuberculous by the local inspectors, 1,795 were condemned or about 44 per cent, and 4,093 were tested at the request of owners who wished their entire herds tested, of which 1,081 were tuberculous, making about 26 per cent.

In 1896, $300,000 was appropriated for the use of the Commission; 8,969 cattle were examined, and 4,694 were condemned.

In 1897, $250,000 was appropriated; 9,991 cattle were examined and 5,435 were condemned.

By this time certain farmers discovered that a law which paid them full appraised value up to a limit not exceeding $60 was a very good one for them, and that by insisting on good prices they would sell old unprofitable cows to the State at a little better figure than to anyone else. The result was that some of them employed veterinarians at their own expense to test their cows and then reported the reacting animals to the Cattle Commission. In the winter and spring of 1897 about 3,400 animals were
tested in this way, and about 1,000 reacted and were taken by the cattle Commission. This right was so abused that an act had to be passed to stop it, after over $40,000 of the appropriation had been taken by men who had no idea of disinfecting their stables or buying tested cattle to replace those that were killed. This act is as follows:

"No person having animals tested with tuberculin shall be entitled to compensation from the treasury of the Commonwealth for any animals which react to the tuberculin test, unless such testing be done by the board of cattle commissioners, or by its authorized agents acting as such at the time of the test, and such testing shall be subject to the supervision and control of the board of cattle commissioners."

This work was done among a number of dairy farmers in the Eastern part of the State, in Middlesex and Essex counties, and gives a pretty fair idea of the condition of the average milkman's herd in Eastern Massachusetts.

In 1898 the Legislature made an appropriation of $20,000, then refused to give any more for the reason that it considered the Commission extravagant, and thought the dangers to the public health from bovine tuberculosis had been exaggerated. The House then voted to abolish the Commission, but this action could not be gotten through the Senate. The Governor then wrote the Legislature a special message, saying that something ought to be done; the House again voted to abolish the Commission and the Senate again refused to concur.

This year (1899) our laws relating to contagious diseases were recodified, the Commission reduced from five members to three, and $75,000 appropriated to carry out the law. The limit of value was reduced to $40. At present we are killing only cows that show well marked physical evidence of disease, or which have tuberculous udders. We are using very little tuberculin and then only on questionable cases, or where entire herds are tested; of the latter we have had very few. If an owner wishes his entire herd tested with a view to eradicating tuberculosis, the Commission does it only on condition that he will take what he can get for animals that pass as fit for beef, the State only paying for those that are unfit for food; that he will agree to thoroughly disinfect his barn and that he will buy only tested animals to take the places of those that are killed.

The Massachusetts Cattle Commission requires all cattle brought into the State to be tested with tuberculin, except beef cattle for immediate slaughter and calves under six months old. Here difficulties are encountered: At first at our great markets, Brighton and Watertown in 1894 and 1895, the cows were tested by the Commission upon arriving at the stock yards, but cattle amid strange surroundings, and excited by transportation and unfamiliar sights, are not fit to test, many will have rises in temperature not due to tuberculin. Out of 100 killed as reacting to tuberculin, 20 were found to have no lesions and this plan was abandoned. It would be too expensive to hold cattle in quarantine for a week and then test them; the only other plan was to allow the drovers to have them tested
by veterinarians in the states from whence they came, chiefly Maine, New Hampshire, and Vermont. Here the temptation to fraud is great, the drovers do not care to buy cows and have them react, and the farmers do not care to sell them to the drovers at their own risk, hence if the veterinarian doing the testing can be bribed or corrupted to fill out certificates without testing the animals it will be done.

A year ago last spring the Massachusetts Cattle Commission had a list printed giving the names of veterinarians whose tests it would accept. At that time the names of a number of men known to be doing dishonest work were dropped, and it was hoped that a reliable body of men to do the testing had been secured, but it did not take the drovers long to find out who was corruptible, and many of the cow dealers I fear are again bringing in cattle that have never been properly tested. The reason we have so few diseased cows brought in is not due to our requiring a test, but because the animals come from sections of the country where bovine tuberculosis has not yet acquired much of a foothold.

As animals do not always react after two or three tuberculin tests, it is possible to have a diseased cow tested honestly and fail to react because she has previously been tested by another veterinarian, or because the dealer has already given her one or two tuberculin hypodermic injections in order to lessen her chances of reaction.

The regulations of our Board requiring a tuberculin test are simply rules of our Commission and not law; perhaps statutes inflicting a heavy penalty on anyone who brings a tuberculous animal into a state would be more effective, and there is more certainty of cattle being properly tested if the State has quarantine stations where all dairy and breeding cattle can be held and tested by state officials, rather than relying upon veterinarians out of the State, many of whom, I am ashamed to say, as I am a veterinarian myself and feel a pride in my profession, are thoroughly dishonest and corrupt.

To sum up from our experiences in Massachusetts it can be said:

1. If tuberculosis prevails to any very great extent among the cattle of a community owners will not submit to having their cattle killed without compensation.

2. If the use of tuberculin is abused, farmers will insist that it shall be used under proper restrictions.

3. If owners are paid full compensation by the State, legislation may be necessary to prevent their selling diseased cattle to the State as a matter of speculation.

4. If the State pays full value for animals killed as tuberculous the taxpayers will not long tolerate killing cattle that are not sufficiently diseased to be a source of danger to the public health, especially if animals practically sound are to be made into commercial fertilizer.

5. In endeavoring to prevent the entrance of bovine tuberculosis into a state it is extremely difficult, if not impossible, to have animals honestly
tested with tuberculin outside the limits of the state; the safer way is for the state to have its own quarantine stations where the animals can be tested, if this work is to be undertaken.

In dealing with bovine tuberculosis it must be considered from two points of view. First, its danger to the public health; secondly, as a contagious disease among cattle causing annually heavy losses to our cattle owners. As a menace to the public health the danger should not be overestimated, as only cows badly diseased with general tuberculosis, or having tuberculous udders are likely to excrete tubercle bacilli in the milk, although some may be floating in the dust of the stable where badly diseased animals are kept, and perhaps a few tubercle bacilli may gain entrance to the milk from the air. Furthermore, there seems to be a question among recent investigators as to the identity of the bovine and human tubercle bacillus which may still farther lessen the danger; this question is, however still open to additional study.

Looking upon it as a contagious disease of animals it depends upon its prevalence in a given locality as to how radical the means to be taken for its suppression may be. Where very few cases are to be found in a community it is practicable to stamp it out; where it is widespread and there are many infected buildings as well as cattle, more gradual and conservative methods will have to be employed, always bearing in mind the importance of good sanitary surroundings, and raising young cattle that are healthy and with good constitutions to replace those that have gone before; remembering that the bovine population changes about once in twelve years in any event.

Do not think from what I have said that I underestimate the value of tuberculin, as I am well aware of its worth when properly used.

It has been a great pleasure to me to present my ideas to you gentlemen, and I thank you for your attention.

ELECTION OF OFFICERS.

The Chair announced the next order of business to be the election of officers.

Dr. Eisenman placed in nomination for the office of president, Mr. C. P. Johnson of Illinois.

On motion of Mr. Riddle, Mr. Johnson was elected president for the ensuing year by acclamation.

Dr. Pearson nominated Dr. E. P. Niles of Virginia as vice president for the ensuing year.

On motion, Dr. Niles was elected vice president for the ensuing year by acclamation.

Mr. Tillman nominated Mr. Mortimer Levering of Indiana for the office of secretary.
On motion of Mr. Lott, Mr. Levering was elected secretary for the ensuing year by acclamation.

Mr. Cameron nominated Mr. W. T. Tullis of Texas for the office of treasurer, and, on motion, Mr. Tullis was elected treasurer for the ensuing year by acclamation.

MR. TILLMAN—I notice from the minutes of the last meeting, at Omaha, that each of the states was assessed $5.00 for the payment of expenses. Nothing of that sort has been done by this convention.

THE CHAIR—That action applied only to the Omaha meeting, and it was taken in order to provide a fund for printing the proceedings of that meeting, that copies might be furnished to all of the states represented.

MR. LOTT—I move that each state represented at this meeting be assessed $5.00 for printing the proceedings of this year and of last year, and that the action of the Omaha meeting be confirmed in the assessment of $5.00 per year from now on.

MR. RIDDLE—I see an injustice in this motion. I believe only four states paid the assessment that was levied against them last year at Omaha and think the chances of recovering from the others is very small. So far as Kansas is concerned, we are willing to let that assessment go, but I believe that the proceedings at Omaha, and also those had here, can be printed in one volume, and the cost would not exceed what would be realized from a single assessment; therefore, I move as a substitute, that the assessment begin now, and that it apply to the expenses of this meeting and the printing of the proceedings of both meetings.

MR. PAINE—that is, make just one assessment for the two years.

MR. RIDDLE—Yes.

MR. CAMERON—I would like to ask, for information, whether, if a state has refused to pay its assessment, it would not be the fault of the officers of this Association?

THE CHAIR—I do not think any state has refused.

The question recurring upon the substitute offered by Mr. Riddle, it was adopted.

On motion of Mr. Riddle, it was ordered that if the funds realized from the assessment would permit, 500 copies of the proceedings be printed.

The next order of business being the selection of place and time for the next annual meeting of the Association:

Mr. Cameron presented the claims of Kansas City, Mo., and asked that the next meeting be held in that city.

Mr. Embry invited the Association to come to Louisville, Ky.

Dr. Niles asked that the meeting be held in Richmond, Va.
Dr. Pearson urged the selection of Philadelphia, Pa., as the place of meeting.

Dr. Peters offered the hospitality of Boston.

On motion of Mr. Paine, the roll of states was called, with the following result: Kansas City, 6; Louisville, 9.

On motion of Mr. Levering, the selection of Louisville was made unanimous.

The Chair announced that, under the resolution adopted at the time of organization of the Association, the time of meeting was fixed on the Tuesday after the first Monday in October, and if there was no action changing the time, that would be the date of the next meeting.

After some discussion, the date of meeting was left unchanged.

THE CHAIR—The programme, which tends to make these meetings interesting, entertaining and instructive, should be made up earlier than it was this year. I sent out circulars asking for suggestions from the various State Boards, but received no response, and it was devolved upon me as your President, to prepare such a programme as I could arrange for. I want to ask that within the next six months you communicate with me, or the Secretary, and make some suggestions as to questions of interest to be discussed, either in the form of papers or addresses; and if you would also suggest some one who is competent and willing to treat of the subjects in the best possible manner, it would be of great help in making up the programme.

In response to this request of the Chair, Mr. Embry suggested that Dr. Eisenman be requested to make a report at the next meeting of the Association on a test he (Mr. Embry) proposed to make in exposing native cattle to tick infested cattle fed upon distillery slop and stabled in distillery barns; and also by infesting native cattle, fed in distillery barns upon distillery slop, with young ticks, it being Mr. Embry's idea that native cattle so fed are immune to the disease.

On motion, a vote of thanks was tendered to Drs. Salmon, Gehrmann, Evans, Pearson and Peters, for their excellent addresses on the respective subjects handled; to the Union Stock Yards Co., the Chicago Live Stock Exchange, the Illinois State Board of Live Stock Commissioners, and the Press, for courtesies extended.

On motion of Mr. Lott, the convention adjourned sine die.

MORTIMER LEVERING, Secretary.  

C. P. JOHNSON, President.
SIXTY-SEVENTH
ANNUAL MEETING
October 13 to 18, 1963
WESTERN SKIES HOTEL
Albuquerque, New Mexico